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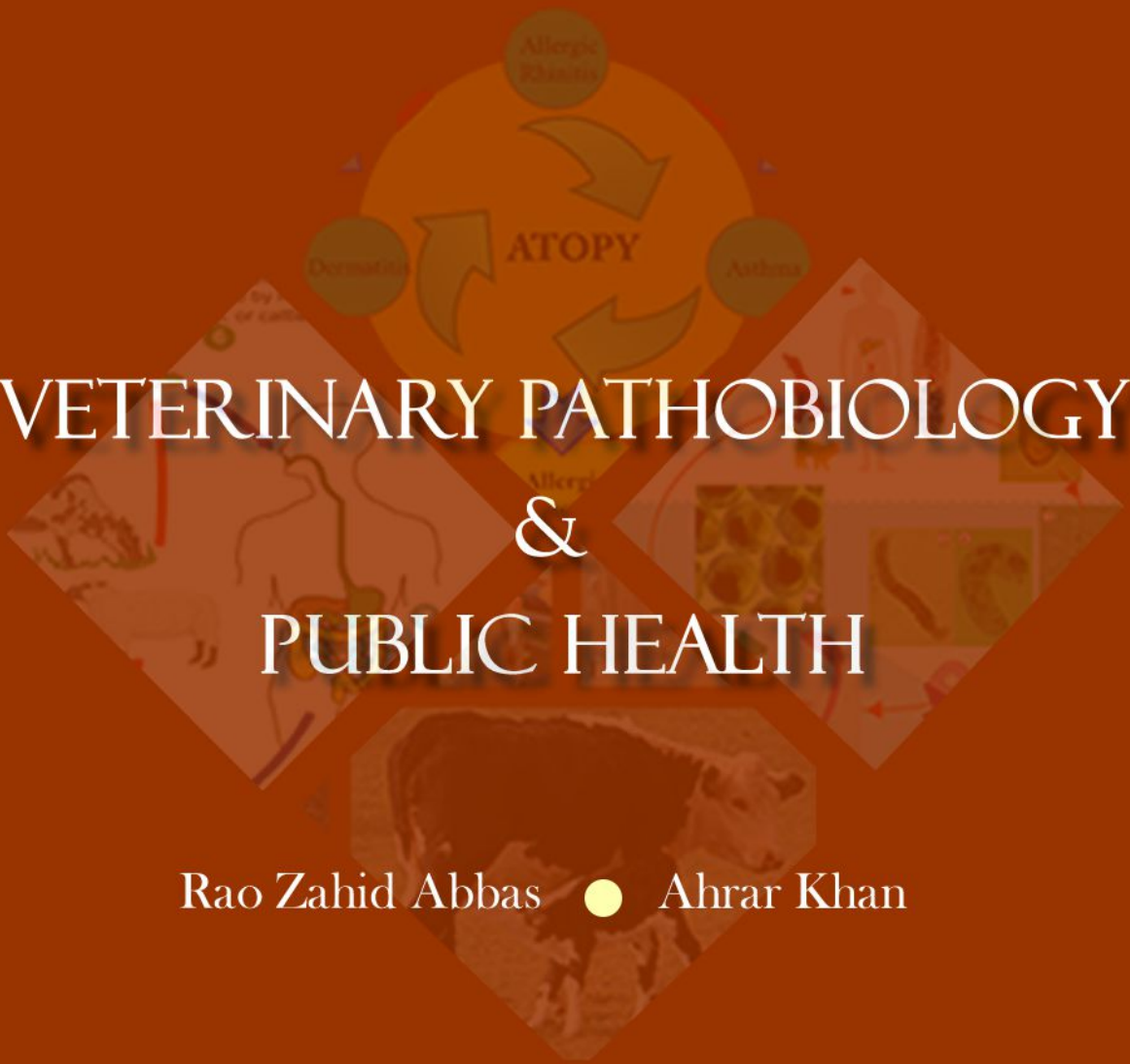

VETERINARY PATHOBIOLOGY & PUBLIC HEALTH

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VETERINARY PATHOBIOLOGY & PUBLIC HEALTH

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PREFACE

The well-being of public health and animals is pretty much intercalated. Its impossible to ensure human health, food security and food safety, and welfare without considering animal health. The approach of 'One-health' that encompasses human, animal and environmental health on the similar grounds of priority therefore becomes crucial. Its imperative to work with strong collaborations of animal, human research and development and finally its dissemination for improvement in the energy flowing across food chains.

The need to enhance the collaboration within human and animal health workers, researchers and academicians has moved the editors to develop this publication. The book takes into account the major threats of animal origin to the human health. It's a unique compilation of bacterial, viral and parasitic and vector-borne diseases of public health significance. The book highlights the food-borne diseases of small and large ruminants and fish. Concepts presented in the book could be a way forward in devising ways to improvise food safety from farm to fork. It is anticipated that this book would be of great use to a variety of readers. University students, graduates, practitioners, animal and human healthcare providers would definitely find this book of great importance. The language of book has been intentionally kept easier for a non-technical person to grasp the concepts on importance and interrelatedness of veterinary and public health from a global perspective. The editors wish to publish a series on the subject keeping in view the urgency to highlight these areas for awareness, research and development.

Editors

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Section A

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FASCIOLOSIS: A MAJOR GLOBAL FOODBORNE ZOONOSIS

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INTRODUCTION

Agriculture sector plays a major role in the economy of Pakistan. It contributes approximately 11.4% to gross domestic product. This sector hires about 45% of labor force of the country and contributes to other segments of the growth economy (Khan et al. 2018). The livestock sector holds a unique place in the National Growth Agenda and is a net source of international revenue (Saleem et al. 2018). Throughout the history, smallholders have used livestock to satisfy their everyday needs for milk, food protection, and cash earnings.

Additionally, livestock is seen as a means of jobs at the rural level (Sargison 2020). It contributes significantly to poverty alleviation and continues to improve the socioeconomic status of our rural commonalities. In 2013-14, livestock contributed roughly 11.9% to the national gross domestic product, adding 55.4% to agricultural value-added (Khan et al. 2020).

Parasitism is the major constraint to livestock production throughout the world (Das et al. 2018). The importance of worm diseases is magnified in underdeveloped countries, such as Pakistan, where 65.2% of the inhabitants live in rural areas and are economically dependent on livestock. Parasitism presents a significant challenge to the livestock economy of farmers (Abbas et al. 2014). Due to the broad spectrum of ultimate hosts, fasciolosis, also known as liver fluke disease, is very important among helminth pathogens (Chemale et al. 2010). Fasciolosis is a parasitic infection of the liver that affects both wild and domestic ruminants. It is caused by parasites of the genus Fasciola, which is found globally (Dawes and Hughes 1964). Economic losses occur due to cow deaths, feticide, stunted growth, decreased production of milk and meat, rejection of damaged liver and skinny cadaver, and animal treatment cost (Mehmood et al. 2017). Fasciolosis is responsible for a significant economic loss, triggered by *F. hepatica* in ruminants. The parasite has rapidly spread across Asia, Africa, and the Pacific. Though the disease is more prominent in young livestock, it may also affect older animals, impairing their fitness, growth rate, and production. Besides its common veterinary importance, fasciolosis caused by *Fasciola hepatica* and *Fasciola gigantica* has been a resurgent, affecting many human populations by spreading through zoonosis (Mas-Coma 2005).

The prevalence of Fasciolosis infestation is 49.01% in buffaloes. Between January and September, Fasciolosis was more widespread, and from October to December, it was least prevalent. Fecal tests showed a 65% prevalence of *F. hepatica*. In contrast, fecal egg counts showed that *F. gigantica* was more common in adult cattle than in calves (Khalil-ur-Rehman et al. 2009). The epidemiology and seasonal trend of flukes infection is determined by the availability of intermediate hosts and eating habits of final hosts. Life cycle of *F. hepatica* is complicated and consists of multiple stages that occur in the environment or the intermediate host (Mucheka et al. 2015). Metacercariae, the infectious stage, is encased and eaten by grazing herbivores. Temperature and rainfall are important factors affecting life cycle of *F. hepatica* and occurrence of infection (Karim et al. 2015). It has been identified in numerous studies that *F. hepatica* is endemic to cattle, buffaloes, dogs, goats and humans in Pakistan (Qureshi et al. 2005; Zafar et al. 2019).

Fasciolosis is a disease that can infect about 300M cattle 250M sheep globally (Mooney et al. 2009). The two liver flukes that are often reported to cause Fasciolosis in ruminants are *F. hepatica* and *F. gigantica* (Walker et al. 2008). Cattle that are infected usually have difficulties in gaining weight, and dairy cattle may have lower milk production and are more likely to develop metabolic diseases (Bekele and Getachew 2010). Fasciolosis costs the UK and Ireland more than £18 million annually. According to Mulcahy and Dalton (2001), the disease costs Sweden €52 million a year, or €299 per infected animal. Schweitzer determined that liver condemnations due to Fasciolosis cost Kenyan cattle slaughterers 0.26 million USD per year (Schweizer et al. 2007).

Fascioliasis causes substantial economic losses to pastoral agricultural communities and commercial animal farmers, estimated at US$ 2 billion per year, through the death of infected cattle, liver condemnation, and productivity losses associated with reduced feed conversion quality (Sanchez-Vazquez and Lewis 2013). Fascioliasis is widespread in tropical areas, affecting up to 90% of cattle, and is considered the most significant helminths parasitic disease (Sanchez-Vazquez and Lewis 2013). Notably, humans may also contract *Fasciola spp*. and the infection is referred to as human Fasciolosis. It is estimated that 2.4M people are sick with this disease in

more than 60 countries, and more than 180 million people are at risk worldwide (Rapsch et al. 2006). (Khalil-ur-Rehman et al. 2009) recorded a Fasciolosis infestation prevalence of 49.01% in buffaloes. Additionally, they found that the highest infestation occurred between January and September, while the lowest prevalence was between October and December. In contrast, Bhutto et al. (2012) found trematodes infestation in 4% of buffalo calves. *Fasciola hepatica* and, less often, *Fasciola gigantica* caused Fascioliasis. The large trematode's life cycle is illustrated in Figure 1.

Infected animals, such as humans, pigs, and buffalos, harbor adult flukes in their bile ducts, passing young Fasciola eggs in their feces. The next phase of the life cycle occurs in fresh water (Rapsch et al. 2006). After a few weeks, the eggs hatch into miracidium, which then infects a snail host. In the Snail, growth can be completed in 5 to 7 weeks under ideal conditions, and cercariae are then shed in the water. Cercariae lose their tails as they encyst to form metacercariae (infectious larvae) on water plants. Metacercarie, unlike cercariae, can live under damp conditions for extended periods because they have a hard-outer cyst wall (Zafar et al. 2019).

Evolution of Fasciola species in pre-domestication times

The uniquefeatures of Fasciolidae, with a total of just nine, largely geographically limited species scattered across three subfamilies, five monospecific genera, and only one multispecific genus indicate that this is an ancient tribe, with several members expected to have perished during its evolution (Mas-Coma et al. 2009). This evolution has resulted in the Fasciolids spreading across the Old World, with only one species, *F. magna*, having a Nearctic origin. In either case, the lack of Fasciolids in the Neotropical Region (all signs indicate that the presence of only *F. hepatica* in South America is a result of a recent human introduction) confirms the origin of Fasciolidae following the breakup of Gondwanaland into Africa and South America, approximately 90–100 million years ago (Dowd et al. 1994). The results of a recent phylogenetic analysis support the logical evolution of Fasciolidae members, including adult intestinal parasites with non-ramified caeca transmitted by planorbid snails, beginning in Africa and spreading to Asia and the rest of the Holarctic Region (Figure 2), with progressive adaptation to the liver microhabitat and transmission by lymnaeid snails (Lotfy et al. 2008). The origin of *F. hepatica* in Eurasia is widely known, owing to apparent preference of this Fsciolid for the lymnaeid *Galba truncatula*, which exhibits ecological characteristics associated with mild and cold climates (Mas-Coma et al. 2009). In terms of host origin, comparative evidence on infection, life span, egg shedding, and immunity suggest that sheep is more suited for *F. hepatica* than other host species. *F. hepatica* can live up to 11 years in sheep; adult flukes can produce a large number of eggs. However, there is no indication that sheep or goats develop immunity to *F. hepatica*. The disease is self-limiting in cattle. With the majority of flukes being removed within 9–12 months, egg development is brief, with high egg production lasting for just a few weeks, and resistance is gained during primary infection (Bekele and Getachew 2010; Mas-Coma 2005). These data indicate that *F. hepatica* originated in Eurasian ovicaprines, most likely in Ovis animals.

For *F. gigantica,* cattle tends to be more suited than sheep in terms of host origin, as it is more contagious and lasts longer in the former species. Resistance to *F. gigantica* has been found in sheep and *F. gigantica* has also been observed in goats (Anderson et al. 1999). Although the majority of *F. gigantica* adults survive less than a year in cattle, others may persist for at least 3–4 years (Schweizer et al. 2007), and faecal egg counts are up to 80% lower in buffaloes than in cattle with the exact infection dosage (Mehmood et al. 2017). These findings indicate that *F. gigantica* originated in African ruminant communities distinct from ovicaprines and somewhat resembling bovines (Ahmad et al. 2021).

Life cycle

Liver flukes infect a wide range of mammalian hosts and have a complicated life cycle (Valero et al. 2009). Humans are the accidental host of the genus Fasciola (LaPook et al. 2000). Metacercariae is the contagious type of this parasite, which burrows through the small intestinal wall and settles for a short time in the peritoneal cavity. Numerous liver fluke species are drawn to the biliary tree, whether intrahepatic or extrahepatic (Nyindo and Lukambagire 2015). The hepatic stage, which in *F. hepatica* is predicted to last for approximately 6–7 weeks (Jones et al. 2008), is characterized by mechanical penetration of the parenchyma from the liver capsule, with eventual entrance into the bile duct; this is the biliary stage. After sexual reproduction, parasites mature and lay eggs (Nyindo and Lukambagire 2015). In humans, metacercariae attain maturity within 3–4 months. Each adult worm produces an unusual number of eggs per day, depending on its definitive host. Sheep, pigs and black rats, according to reports, can extrude up to 25,000, 12,000 and 2,150 eggs, respectively (Youssef and Uga 2014). An individual liver fluke has been reported to extrude approximately 40,000 eggs per day (Mucheka et al. 2015). These immature eggs are carried to the small intestine by bile medium, where they are mixed with feces. They are excreted in the pasture and undergo embryonic growth in definitive ruminant hosts under optimum humidity and temperature. Since aquatic bodies play a critical role in the development of liver fluke larvae, hatching is determined by environmental factors such as light, temperature, and humidity (Tavil et al. 2014). Via constructive chemotactic and phototactic motions, the emerging free-swimming ciliated miracidia are designed to locate an essential Limnaeid snail intermediate host in less than 24 hours (Mucheka et al. 2015). They mature into sporocysts by mechanically penetrating the body wall and tissues of their snail hosts with their sharp stylets and proteolytic enzymes (Tavil et al. 2014). Mother rediae are produced by the further metamorphosis of the sporocysts,

which grow into daughter rediae. In the snail host, these rediae metamorphosize into cercariae, which can passively infect suitable vertebrate hosts and humans, who drink infested water (Nyindo and Lukambagire 2015). Relative humidity of more than 65%, rainfall more than 100 mm annually, and an atmospheric temperature of 25-30°C have been identified as favorable conditions for cercariae growth and shedding (Gu et al. 2012).

Epidemiology of fascioliasis

Fasciolosis is widespread (Figure 1) in a variety of Asian countries, including the Middle East (Saudi Arabia, Russia, Pakistan, Thailand, Iraq, Iran, China, Iraq, Turkey, Vietnam, Japan, Korea, Iran, Philippines, Cambodia, Bangladesh, and Nepal) (Mehmood et al. 2017). Prevalence of Fasciolosis has been identified in thirteen countries and forty-one trials, ranging from 0.71-69.2% in cattle to 0.0-47.0% in goats (Table 1 and Figure 1). Table 1 shows the prevalence of Fasciolosis in Asian countries from 2000 to 2015. Iran and Pakistan have the most significant number of records among Asian countries. The bulk of experiments containing a greater number of animals reported a lower prevalence in comparison to those involving a short period and a smaller number of animals. Although the prevalence of Fasciolosis in Bangladesh has been reported to range between 14.28 and 21.54% in goats (Mehmood et al. 2017; Sanchez-Vazquez and Lewis 2013), and no data in other output species are known, the higher prevalence of parasite in this country can be due to the moist conditions and high rainfall, which are ideal for survival and reproduction of snails (Ahmed et al. 2007). According to a regional information method for assessing Fasciolosis, the prevalence of parasites in cattle from Cambodia has been estimated to be up to 20% (Dorny et al. 2011). Fasciolosis may affect 28% of cattle in Cambodia, areas with high and average risk concentrated in southern and central Cambodia (Dorny et al. 2011). According to a study conducted on sheep in China, the overall prevalence of liver fluke was estimated to be 28.5%, indicating that management strategies should be applied (Wang et al. 2006). In India, various figures have been recorded for sheep (2.78-8.98%), goats (2.35-15%), cattle (10.79%), and buffaloes (13.9%) (Mehmood et al. 2017). The high prevalence of these parasites may be attributed to suitable weather conditions for snails, the abundance of low-lying, well-irrigated marshy land and pastures near water sources, which are ideal for breeding of intermediate hosts. In Iran, prevalence of Fasciolosis has been reported in goats (0.20-4.4%), cattle (0.71-81.5%) and sheep (0.35-31.25%). There is a major seasonal difference in the prevalence of *Fasciola spp* (Bhutto et al. 2012). Fluke egg hatching and snail replication involve high rainfall and cool temperatures, occurring in spring and autumn. Fasciolosis has been reported in Iraq with a rate of 0.36% in sheep, 0.14% in goats, 1.27% in cattle, and 2.08% in buffaloes (Kadir et al. 2012), although it has been reported at a rate of up to 2.00% in Japan. It infects 1.10% in the Korean goat population, although it was the largest in buffalo and goat populations in Nepal compared to all other Asian countries (Mehmood et al. 2017). Numerous tests have been performed in Pakistan, and the average prevalence of Fasciolosis in sheep is 6.50-29.99%, in goats it is 0.66-28.75%, in cattle it is 19.30-20.42%, and in buffaloes it is 14.71-30.50% (Mas-Coma et al. 2009). The causes for the rise in Fasciolosis infection rates may be the farmers' socioeconomic status or the creation of tolerance due to the inappropriate use of anti-parasitic drugs at insufficient doses for a prolonged period. In Saudi Arabia, Fasciolosis affects 13.50% of sheep and 52.90% of cattle (Sanad and Al-Megrin 2005). The higher incidence in cattle may be attributed to local farmers' lack of awareness about control measures. Numerous experiments conducted in Turkey indicate that Fasciolosis is present in up to 16.20% of sheep, 0.80% of goats, and 69.20% of cattle. Owing to less swampy regions and global warming, Turkey has a lower incidence of Fasciolosis than the nearby countries (Mehmood et al. 2017).

The transmission pattern of Fascioliasis

It is worth noting that there is remarkable variability of human Fascioliasis in epidemiological scenarios and dissemination rates worldwide (Ahmad et al. 2021). It may be inferred that well-known circumstances and trends do not always sufficiently account for disease attributes in a given region (Mehmood et al. 2017). Thus, when tackling an unstudied endemic area, the scenarios and dynamics of human infection outlined above must always be regarded as starting points. Only after an accurate evaluation of the epidemiology and transmission characteristics of the new region has been undertaken, suitable protection mechanisms for the zone in question can be planned (Nyindo and Lukambagire 2015). It is critical to emphasize on the relationship between the lymnaeid vector species and the propagation pattern in question. Lymnaeids exhibit marked ecological and behavioral variation among species. Factors such as water selection temperature thresholds, habitat type, population dynamics, seasonality, and liver fluke infection susceptibility are critical for Fascioliasis (Mas-Coma 2005). All of these facts suggest that lymnaeids can serve as excellent disease markers, help differentiate between the various human Fascioliasis scenarios and trends, and thus as determinants for developing effective control strategies (Kadir et al. 2012).

A very high-altitude trend is linked with only *F. hepatica* transmission in Andean countries through imported Galba truncatula; within this group, two sub-patterns can be differentiated based on seasonal and psychological characteristics (Mas-Coma 2005). The altiplanic pattern is characterized by year-round transmission (e.g. in the Northern Bolivian Altiplano and the Puno Altiplano). The valley pattern is seasonal and varies in severity due to altitude as in the Peruvian valleys of Mantaro and Cajamarca (Mehmood et al. 2017). A Caribbean insular trend, with smaller yet more regular outbreaks in human hypo -endemic regions and lymnaeid species except

Table 1: Global prevalence range (%) of Fasciolosis in ruminants reported in different continents from 2000 to 2020 (Mehmood et al. 2017; Opio et al. 2021).

Sr. No.	Countries	Continent	Studies	Prevalence in different species			
				Goats	Sheep	Cattle	Buffaloes
1	Egypt, Kenya, Chad, Ethiopia, Nigeria, Sudan, Tunisia, Uganda, Zimbabwe, Tanzania, Zambia	Africa	31	0.28-68.4	0.19-73.7	1.2-91.0	9.73-33.7
2	Colombia, Argentina, Peru, Brazil, Mexico	America	10	24.5-100	8.87-100	3.0-66.7	11.4-24.4
3	India, Bangladesh, China, Iraq, Cambodia, Pakistan, Nepal, Iran, Japan, Korea, Vietnam, Saudi Arabia, Turkey	Asia	41	0.0-47.0	0.35-31.4	0.71-69.2	2.08-68.0
4	Australia, Papua New Guinea	Australia/Oceania region	03	(18.2)	5.5-52.2	26.5-81.0	--
5	England, Italy, Belgium, Denmark, Spain, Germany, Ireland, Switzerland, Poland, Sweden, Wales	Europe	23	0.0-0.8	3-83.3	0.12-86.0	--

(): just single study no range is available.

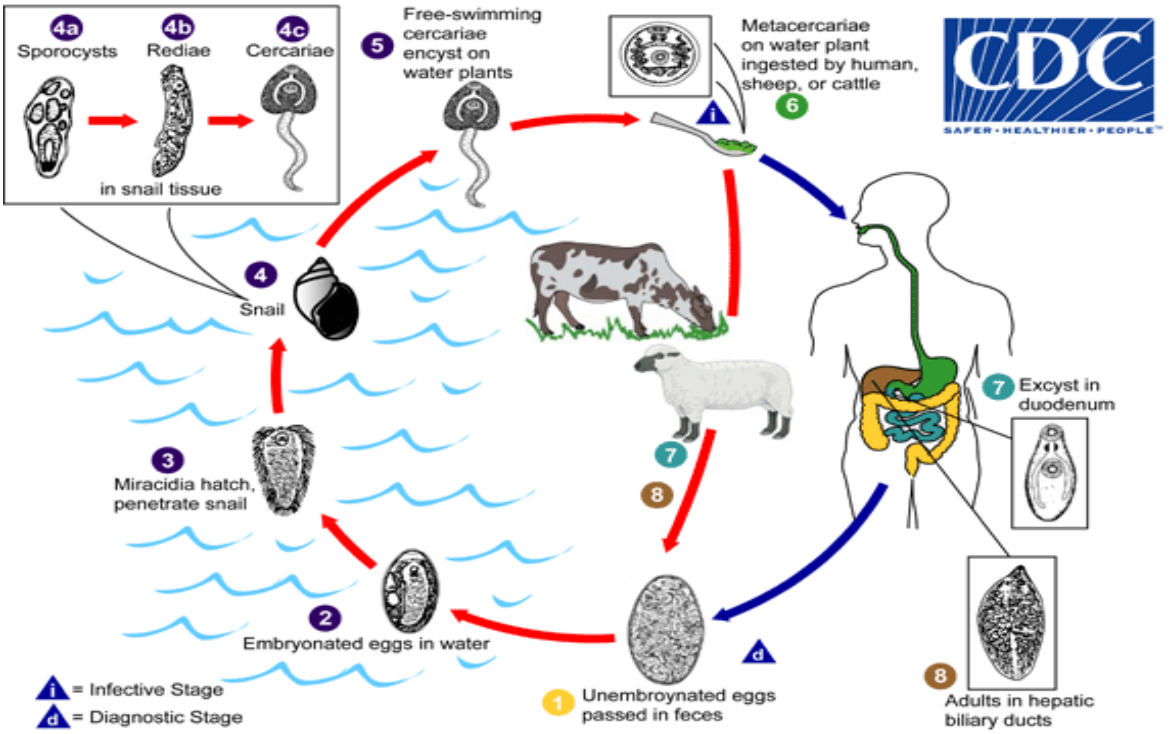

Figure 1: The life cycle of large liver fluke (*F. hepatica*) (Hurtrez-Boussès et al. 2001).

primary vector species, is involved in transmission, as in the Pinar del Rio Province in Cuba. A trend characteristic of Afro-Mediterranean lowlands involves alternating *F. hepatica* and *F. gigantica*, many Galba fossaria and Radix lymnaeids, as well as secondary transmitting Pseudosucinea (Ahmad et al. 2021). A trend was seen in surrounding areas of Caspian Sea, including human hypo-endemic zones, in which significant epidemics occasionally reach 10,000 people, including *F. hepatica* and *F. gigantica*, as well as many Galba fossaria, Radix, and stagnicoline lymnaeids (Iran) (Lotfy et al. 2008).

Diagnosis and treatment

In endemic areas, if the clinical picture is highly indicative, it is often unremarkable. As a result, coprological procedures, such as looking for larvae, or examining adult worms vomited or passed out in feces are used for diagnosis (Le et al. 2008). In the GenBank database, two sequences corresponding to Fasciola buski's 18S rRNA gene are available. Recently, Vietnamese child raise sequence of a fluke was used to verify the diagnosis. When the alleles were compared, only two nucleotide substitutions were detected (Nguyen et al. 2009). A number of drugs have been evaluated for the treatment of the disease in different ways. Tetrachloroethylene was discovered as an effective antibiotic (Karim et al. 2015) and became the preferred drug. Thiabendazole, levamisole and mebendazole are ineffective (Kelly et al. 1977). In the later studies, praziquantel was found to be effective, even in severe cases, resulting in prescribing a single 15 mg/kg dosage as the preferred therapy (Coles 1986), even though this drug was unable to save the life (Maqbool et al. 2002). In experiments on pigs, relative efficacy of triclabendazole, rafoxanide and oxyclozanide for the treatment of Fascioliasis was investigated. It is worth mentioning that triclabendazole has a high degree of efficacy (97.12%), followed by oxyclozanide (93.27%) and rafoxanide (83.17%), all of which are completely safe (Chhabra and Singla 2009).

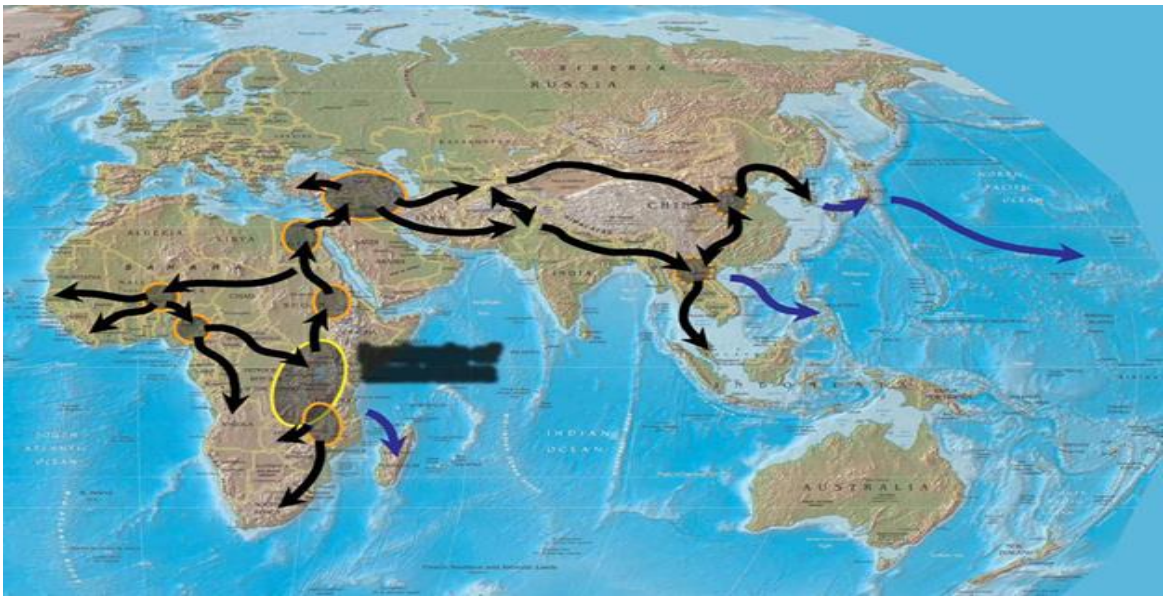

Figure 2: Geographical spread routes followed by *F. gigantica* in the post-domestication period (Mas-Coma et al. 2009).

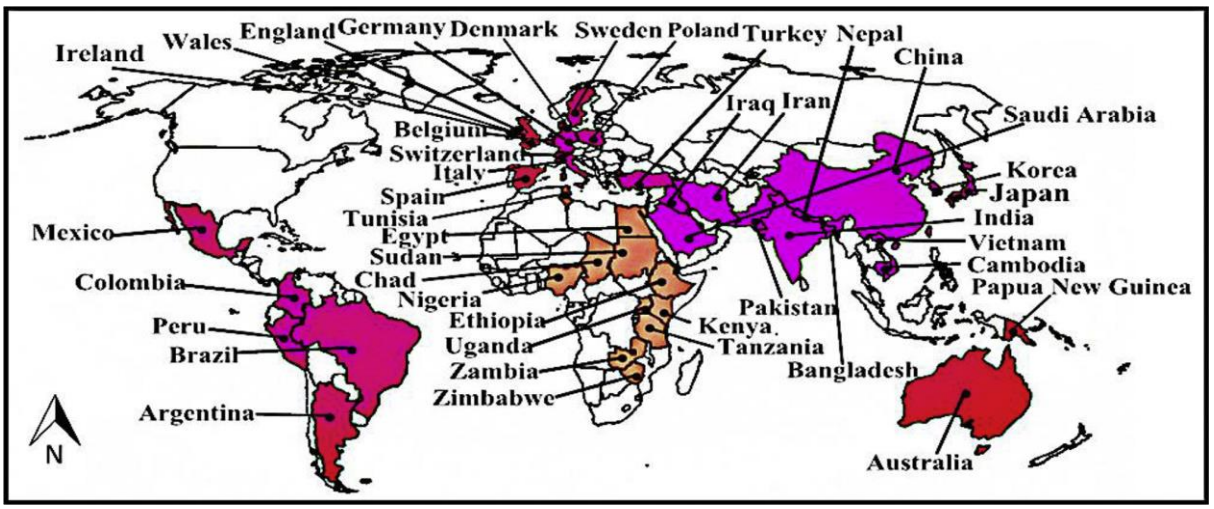

Figure 3: Global prevalence of Fasciolosis in ruminants reported (2000-2015) in different countries (Mehmood et al. 2017).

A neglected disease

Fascioliasis caused by certain species of macroscopic and leaf-like digenetic liver flukes of the genus Fasciola is a medically necessary, primitive foodborne neglected zoonotic disease (Hugh-Jones et al. 1995). In 1379, a French scientist, Jehan De Brie identified the first known parasite, *F. hepatica* (ME et al. 1995). A pathologist and resident physician in Calcutta, Professor James McConnell, in 1874, discovered a Chinese human liver fluke called *Clonorchis Sinensis* after an autopsy on the body of a twenty-year-old carpenter (Nyindo and Lukambagire 2015). At the moment, alleged hybrids between *F. hepatica* and *F. gigantica* are being studied to determine their actual taxonomic status (Jones et al. 2008).

This is because an estimated 2.4M people in more than 70 countries worldwide suffer from Fascioliasis. These trematodes infest every continent, and 180M people are at risk (ME et al. 1995). Moreover, it is estimated that *F. hepatica* infects over 250M sheep and 300M cattle worldwide, along with *F. gigantica*, causing an estimated US $ 3B in economic damage per year (Hugh-Jones et al. 1995). Recently, the global incidence of human Fascioliasis has increased significantly (Mehmood et al. 2017), with a close link to a high infection rate in definitive ruminant hosts. The transmission of parasite is carried out by a diverse population of universal aquatic snails in Limnaeidae. For example, *Hinkleyia caperata, Austropeplea tomentosa, Galba truncatula, Radix rubiginosa, Stagnicola corvus,* and *Pseudosuccinea columella* have been recorded as endemic to Australia, North America, Europe, Africa, Asia, and South America, respectively (Vázquez et al. 2018). Fascioliasis caused by *F. gigantica* is endemic in tropical and subtropical lowlands. As a result, more disease cases are being recorded in a more significant portion of Sub-Saharan Africa (SSA), where suitable snail intermediate hosts occur naturally (Kelly et al. 2019).

Due to the high prevalence of Fasciolid infection in humans, this disease has been underestimated. Between

the 1950s and 1990s, the disease gained attention in France due to the hundreds of thousands of patients hospitalized during that period (Corvo et al. 2013). Fascioliasis was one of the most untreated illnesses on a global scale. Although the situation has dramatically changed since the 1990s, the often-used term sheet and cow disease' continues. This presents challenges in convincing political and health authorities of vital existence of human infection in many countries and the importance of introducing prevention measures, expert research and health literature (Jones et al. 2008). Thus, Fascioliasis receives no coverage in textbooks covering various diseases, such as tropical or human parasitic diseases. Researchers make fewer attempts to update knowledge on secondary infections than on primary ones. At times, research papers published in high-impact technical journals include inaccuracies, misunderstandings, or mistakes (Le et al. 2008).

Immediate concern about Fascioliasis

Global understanding of zoonoses and a one-health solution are by far the most systematic approaches for the control of Fascioliasis (Phiri et al. 2005). Sustaining actions, including trade and travel restrictions, are expected to prevent infected animals transmission between countries. Additionally, enhanced food and water hygiene systems are critical parts of the monitoring scheme (Hammami et al. 2007). Stakeholder engagement and political support for these policies are essential for their successful implementation. Complete triclabendazole resistance cases (Islam and Ripa 2015) should be thoroughly reviewed to find out alternate treatment or solutions.

Additionally, vaccine development should be a focus of future studies. Multiple trematodes or intestinal parasite infections complicate the early diagnosis of Fascioliasis (Ahmed et al. 2007). Delayed and missing diagnosis, principally among the young ones, magnifies adverse effects of the disease (Karim et al. 2015). While Fascioliasis has been recognized as a disease of human importance, it is mainly considered as an animal disease, most notably of cattle and sheep. Lack of knowledge is a significant impediment to effectively managing human Fascioliasis (Bekele and Getachew 2010). Unfortunately, Fascioliasis in humans is not a recognized and reportable disorder in a large proportion of the world's least developed countries, which are plagued by hunger and infectious diseases. Sensitization and awareness are critical first steps in any proposed intervention strategy. Fortunately, there is no death identified due to human Fascioliasis infection to date. As a result, the disease is predictably assigned a low emergency health priority, making it one of the most underestimated tropical diseases (Mehmood et al. 2017).

Additionally, a slightly higher prevalence in school-aged girls has been reported. Thus, the morbidity and Disability Modified Life Years (DALY) effects of the disease are of great significance (Moje et al. 2015). The morbidity associated with chronic disease remarkably leads to reduced life expectancy, poor quality of life and economic performance. The adult worms can survive for more than ten years in a suitable host (Mas-Coma et al. 2009).

Fascioliasis as an emerging zoonosis

The World Health Organization has stressed the critical nature of foodborne fluke infections and the critical nature of controlling measures (ME et al. 1995). Plant-borne trematodes were newly added to the Institute of Food Technologists' Specialist Council on Food Safety and Nutrition (Hugh-Jones et al. 1995). In November 2004, the Third Global Meeting of the Partners for Parasite Control at the WHO Headquarters in Geneva, Fascioliasis and other foodborne trematode diseases were added to the significant list of helminthiases that have a direct effect on human health (Nyindo and Lukambagire 2015). Additionally, it appears that current climate and global patterns affect some snail-borne helminthiases that depend on the environment for their transmission. Fascioliasis is an excellent example of a parasitic disease emerging/re-emerging in various countries due to a mixture of natural and human-made changes (Nguyen et al. 2009).

Prevention and control

Regulations can be made for handling livestock, preventing augmentation, and adopting new pig farming techniques (Schweizer et al. 2007). Individual avoidance is straightforward: prevent use of foods obtained from freshwater plants. However, application of such regulation is incredibly difficult when century-old rituals are involved (Graczyk and Fried 2007). Infections are most likely to spread within families, since food storage, and eating practices are passed on over generations (Hurtrez-Boussès et al. 2001). Additionally, due to their low cost and availability, aquatic plants are a common food source (Hugh-Jones et al. 1995). Techniques for preventing contamination include preventing the use of human feces as waste, abandon defecation, and washing pig excreta into nearby water bodies (Le et al. 2008). Desiccation and direct solar radiation destroy metacercariae. The prevention approach should be focused on sustained outreach, emphasizing the importance of extensively cooking freshwater plants, immersing fruit and plants in boiling water for a few minutes, and use of boiled water in areas without access to purified water (Jones et al. 2008).

Future challenges

There have been significant advancements in the diagnosis, surveillance, and treatment of Fascioliasis. Nonetheless, under-developed countries, primarily low-income populations, cannot benefit from these advancements due to poverty. With pathogens, civil strife, and competition for scarce capital, it is unsurprising that these areas have too few cases of human

Fascioliasis. Control initiatives should continue with extensive education and sensitization campaigns about the effects and magnitude of Fascioliasis in animals and humans. The new "One Health Integrated Global Approach to Disease" is the most systematic and participatory response available, not only for human Fascioliasis but also for most zoonotic diseases in general. A classic indicator of the challenges it discusses is the latest movement to "Go Eco" as a sustainable alternative to the current artificial lifestyle. This has resulted in extraordinary growth in the intake of fresh, raw/green vegetables and fruits. This is contradicted by a lack of funding for water protection, fertilizer-pesticide controls, and waste management. The use of unmonitored and unprocessed fresh vegetables has aided in the spread of plants/foodborne liver flukes such as Fascioliasis and a variety of other health problems. Clinical trials to assess confirmed cases of Fasciola bithionol and triclabendazole resistance are urgently needed. Further production of chemotherapeutic agents, such as Mirazid and nitazoxanide extracted from myrrh, and other innovative interference directed at the snail, could provide much-needed alternative chemotherapy. Aggressive monitoring for disease hotspots and control techniques run at animal reservoirs allow early intervention. Simultaneously, increased water and food safety, in conjunction with vaccine production, is crucial for human Fascioliasis prevention strategies. All of this must be followed by vigorous understanding, sensitization, and political support to optimize these measures to be effective.

Conclusion

Fascioliasis has been recognized as a significant foodborne pathogen with zoonotic and veterinary implications. The advent of drug resistance, the creation of new parasite strains through hybridization and climate change are the major threats that can alter the epidemiology of the disease in the immediate future. To this end, researchers must accelerate their efforts to develop effective vaccinations that provide optimum protection for farm animals and humans while still substantially contributing to the global eradication of the disease by minimizing its occurrence and severity. The governments of tropical and subtropical countries should make deliberate efforts to raise support for research workers.

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PARASITIC ZOONOSES AND CAMEL

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INTRODUCTION

With a total population of over 37 million heads, camels serve as an important source of milk and meat around the globe, especially in Asia and Africa. The single-humped camels, renowned as dromedary (*Camelus dromedarius*), are approximately 95% of the total population of camelids and are present in 47 countries (Food Agriculture Organization of The United Nations 2019), where they are playing a pivotal economic role. As the camels are an important food source in arid and semi-arid zones, the term used for camelids has been transformed from "ship of the desert" to "food security livestock" species. One evidence is that between the years 2008 and 2018, the camel world population increased by 21% compared to 4, 5, 9, and 15% for pigs, cattle, sheep, and goats, respectively (Food and Agriculture Organization of The United Nations 2019). However, despite being extremely resistant to harsh environmental conditions, dromedaries can get infected with several zoonotic pathogens, thus posing a public health risk (Sazmand et al. 2019b; Zhu et al. 2019).
Almost 65% of the published research articles on zoonotic pathogens of camels during 1970-2018 focused on Rift Valley fever, brucellosis, hydatidosis and Middle East respiratory syndrome (MERS) (Zhu et al. 2019). Despite, Echinococcosis in camels, which is the most studied zoonotic parasitic infection, some other parasites like *Linguatula serrate*, *Trichinella* spp., *Fasciola* spp., *Cryptosporidium* spp. and *Toxoplasma gondii* originating from camels cannot be ignored in view of public health significance (Zhu et al. 2019). Relatively a few parasites of camelids are considered as host specific (Schuster 2018), whereas most of the parasites infecting camels are i) non–zoonotic but with a large host range, or, ii) of zoonotic concern. Transmission routes of zoonotic parasites includes faecal contamination (*Enterocytozoon* spp., *Blastocystis* spp., *Balantidium coli*, *Giardia duodenalis*, *Cryptosporidium* spp.) or eating of undercooked meat or drinking raw infected milk (*e.g.*, *Linguatula serrate, Trichinella* spp. and *Toxoplasma gondii*). In addition, camels act as reservoir for vector-borne protozoan parasites e.g. *Trypanosoma evansi,* gastropod-borne trematodes (e.g. *Schistosoma* spp., *Dicrocoelium dendriticum* and *Fasciola* spp.) or larvae of zoonotic cestodes, e.g. *Echinococcus granulosus* (sensu lato) (Sazmand and Joachim 2017; Sazmand et al. 2019b). Moreover, camels are the source blood for several haematophagous ectoparasites, such as ticks and fleas, which ultimately transmit zoonotic viral and bacterial pathogens *e.g.*, Crimean–Congo hemorrhagic fever virus, *Coxiella burnetii*, *Rickettsia* spp., *Bartonella* spp. and *Yersinia pestis* (Wernery et al. 2014; Sazmand et al. 2019a;). In this chapter most important parasitic zoonoses related to camels are presented.

Toxoplasmosis

Toxoplasmosis is caused by *Toxoplasma gondii,* which is an intracellular protozoan that infects many domesticated and wild animals, as well as humans (Donahoe et al. 2015). *Toxoplasma gondii* was first discovered in 1908 and its life cycle was completely explained in the 1970s (Dubey and Frenkel 1972). Due to wide range of host species, *T. gondii* is one of the most important zoonotic parasites of the world (Djurković-Djaković et al. 2019). About one third of the world's population is under the threat of this food and water borne parasite (Bahia-Oliveira et al. 2017). Previous studies have shown that 32,700 disability-adjusted life years (DALYs) of Toxoplasmosis are being reported annually in USA with 86,700 confirmed patients and 330 deaths (Scallan et al. 2015). However, its economic significance in developing and underdeveloped countries may be estimated to be much higher due to low level of food hygiene conditions.
The only reported definitive hosts for this parasite are the members of *Felidae* family (especially cats). Faeces of cats have unsporulated oocysts. Although these oocysts usually shed in feces for a few weeks, their large number makes them significant. These oocysts usually sporulate in 1–5 days in the environment and finally become infective. The intermediate hosts, including rodents and birds, become infected following the ingestion of soil, plant material or water contaminated with oocysts. Shortly after the ingestion, these oocysts transform into tachyzoites. The tachyzoites are settled in muscle and neural tissues and transform into bradyzoites. The cats get infected while consuming these intermediate hosts

sheltering tissue cyst bradyzoites or by ingesting the sporulated oocysts directly. Camels however, can only get infected by ingesting the sporulated oocysts. Humans can become infected by any of the following routes:

- Eating the tissue cysts due to undercooked meat.
- Utilizing the food or water which is contaminated with feces of definitive host.
- Organ transplantation or blood transfusion.
- Vertical transmission to the offspring.

In humans, the cysts commonly harbor in muscles, eyes, brain and myocardium and may survive throughout the life (https://www.cdc.gov/parasites/ toxoplasmosis/ biology.html). The camel-rearing nomads in Asia and Africa usually like to eat raw camel liver (Saeed et al. 2005; Gebremedhin et al. 2014), resulting in providing the favorable conditions for transmission of *T. gondii* infection from camel to human population (Belluco et al. 2016). Additionally, the use of camel milk is becoming more popular these days, as it contains higher amount of iron and vitamin C for the treatment of several diseases including tuberculosis and type-1 diabetes (Boughattas 2017). However, consumption of raw camel milk can also be a source of *T. gondii* infection (Medani and Mohamed 2016). Several studies have shown the presence of all the three clonal lineages (Types I, II and III) in camel milk and meat (Tavakoli et al. 2018). These three types have also been reported in infected humans (Ajzenberg et al. 2009). As the conventional labeling of *T. gondii* isolates cannot sufficiently explain the diversity of prevailing genotypes (Shwab et al. 2014), multilocus targeting PCR-RFLP is necessary for understanding the transmission dynamics of Toxoplasmosis in association with their dairy products and meat. Serological tests are commonly used to study the seroprevalence of *T. gondii* infection in camels (Hamidinejat et al. 2013; Fatima et al. 2019).

Trypanosomiasis

Several *Trypanosoma* species, including *T. evansi*, *T. vivax*, *T. congolense* and *T. brucei,* infect camels (Roettcher et al. 1987; Dirie et al. 1989; Birhanu et al. 2015), however *Trypanosoma evansi,* the causative agent of "Surra", is the most prevalent parasite (Desquesnes et al. 2013). This flagellated haemoprotozoan parasite is the first pathogenic mammalian trypanosome described in the World by Griffith Evans in equids in the Dera Ismail Khan district in Pakistan (Evans 1880). Due to semi loss of *T. evansi* mitochondrial DNA, that occurred while its separation from *T. brucei* (Lai et al. 2008), this parasite can be transmitted mechanically by biting flies, hence its global distribution is beyond the limits. *Trypanosoma evansi* infects many domestic, as well as wild mammals in Asia, Africa and South America (Aregawi et al. 2019). The recent outbreaks of *Trypanosoma* infection amongst camels in France, mainland Spain and the Canary Islands depicted that this parasite can spread anywhere in the world (Gutierrez et al. 2010). In one-humped camels, this infection can cause higher morbidity and severe decline in productivity and even mortality (Sazmand et al. 2011; Sazmand et al. 2016). Camels are more prone to *Trypanosoma evansi* infection as compared to other animals, including small ruminants, equids, dogs, cattle and buffaloes (Aregawi et al. 2019), but in contrary to other animal species, its economic burden in camels has not been evaluated yet (Reid 2002). Zoonotic importance of *T. evansi* infection has been reported from India, Sri Lanka, Egypt, Indonesia and Vietnam (Joshi et al. 2005; Truc et al. 2013; Van Vinh Chau et al. 2016; Sawitri et al. 2019). For many years, it was assumed that human's susceptibility to this parasite may be linked to improper amount of trypanocide apolipoprotein L1 (APOL1), which is a trypanocidal component present in human serum (Vanhollebeke et al. 2006). However, its report in a diseased person with no history of previous immunity related complication, 2 wild–type APOL1 alleles and a normal serum APOL1 concentration concluded that the parasite is zoonotic in nature (Van Vinh Chau et al. 2016).

Cryptosporidiosis

The causative agent of Cryptosporidiosis is *Cryptosporidium* spp. (Phylum Protozoa; Subphylum Sporozoa; Class Gregarinomorphea; Subclass Cryptogregaria; Order Cryptogregarida; Family Cryptosporidiidae), which infect almost all vertebrates including camels. Various enteric protozoan parasites, such as *Eimeria* spp., *Cystoisospora orlovi* and *Sarcosystis* species, develop exclusively in camels (Dubey and Schuster 2018). However, a number of scientific studies reported the camel infection via different important zoonotic parasites, including *Cryptosporidium* spp. (Zahedi et al. 2016). Transmission of Cryptosporidiosis is associated with ingestion of contaminated food and water, with more than 8 million people get infected with this disease annually (Ryan et al. 2018). Currently, 48 *Cryptosporidium* species are considered valid (Ježková et al., 2021). In the human, more than 20 species and genotypes have been reported and its clinical manifestations are associated with gastrointestinal problems (Ježková et al. 2020). The imported subtype of *Cryptosporidium parvum* IIaA17G2R1, *C. hominis, C. andersoni*, *Cryptosporidium* rat genotype IV and *Cryptosporidium* camel genotype have been reported in dromedary camels through PCR and sequencing. The subtype IIaA17G2R1 of *C. parvum* has zoonotic importance and reported from all over the World in humans and animals (Gu et al. 2016; Baroudi et al. 2018; Zahedi et al. 2018; El-Alfy et al. 2019;). Although, no direct transmission of *C. parvum* and *C. andersoni* to humans has been reported in contrast to other livestock such as cattle (Lal et al. 2016), however, there is only one study from Iran which revealed the zoonotic association with camels, where 24% of the farmers were found sero-positive for *Cryptosporidium* spp. (Sazmand et al. 2012).

Echinococcosis

Genus *Echinococcus* of family *Taeniidae* causes Echinococcosis of zoonotic importance. *Echinococcus granulosus* sensu lato causes Cystic Echinococcosis (CE),

first reported from Sudan in 1908 in the annual reports of the Ministry of Animal Resources, Khartoum, Sudan (Craig et al. 1996). *Echinococcus granulosus* sensu lato has 10 genotypes (G1-10). Among these, most important ones are *E. granulosus* (sensu stricto) (genotype G1-G3 and their micro-variant), *E. ortleppi* (G5) and *E. intermedius* (G6–G7) in camels (McManus and Thompson 2003; Deplazes et al. 2017; Ebrahimipour et al. 2017; Dehghani et al., 2020). Canines are the definitive hosts, while vertebrates (domestic and wild ruminants, horses, pigs, camels and member of cervid family) are the intermediate host for Echinococcosis. The disease is distributed in endemic areas of North Africa, Middle East, South and Central America, Asia and Europe. This parasite resides in the gastrointestinal tract of the final host and visceral organs of the intermediate host. The parasite forms hydatid cysts in visceral organs of the host and the disease is called as hydatidosis (Craig et al. 1996). Life cycle of this parasite starts with the release of gravid proglottids from the final host with faeces in the environment, which are ingested by intermediate hosts (ruminants, wild animals and humans). Eggs hatch in the small intestine of the intermediate host to release six-hooked oncospheres. These oncospheres penetrate the intestinal mucosa to enter the circulatory system and reach various visceral organs, especially lungs and liver. The oncospheres develop into hydatid cysts, which gradually enlarge to produce protoscolices and daughter cysts. Hydatid cysts are fluid-filled bladder like structures, which are lined by delicate parasitic membrane and often encapsulated by host fibrous tissue. There is a glycoprotein layer between organ encapsulated and the cyst to protect it from host immune response. The lining of the cyst is called as germinal epithelium, from where buds are formed which grow into brood capsule (Derbel et al. 2012). Over the time, hundreds to thousands of brood capsules are produced, which contain several inverted scolices. When a cyst ruptures, it releases protoscolices to develop into secondary cysts. Larval stage (metacestode) mostly develops in lungs and liver (Ohiolei et al. 2020). The definitive hosts ingest the infected offal of intermediate host having protoscolices which evaginate to attach with intestinal mucosa and develop into adult stage within 32-80 days (Al-Khalidi et al. 2020). At this stage, only one gravid segment is shed into the environment by each worm in a week to be the source of infection for intermediate host. Humans are the aberrant intermediate hosts and become infected by ingestion of parasite eggs (Eckert and Deplazes 2004). The most common route for transmission of Cystic Echinococcosis to human, ruminants and wild animals is by consumption of water, soil or food contaminated by stools of infected dogs (Khan et al. 2020). Other sources for infection are emigrant population, none industrial abattoirs and home slaughtering of infected animals (Seimenis 2003).

The disease has high incidence in areas where there is close association among farm animals, humans and dogs. The prevalence of hydatid cyst in lungs, liver and both organs has been reported as 77.5, 3.2 and 19.4%, respectively in Pakistani camels by Anwar and Khan (1998). Estimated prevalence of Cystic Echinococcosis in camel is 8-36% in different endemic countries (Deplazes et al. 2017; Ibrahim 2010). Variation in the prevalence of Echinococcosis has been reported such as 100% of *E. intermedius* (Cardona and Carmena 2013) and 17.0–88.4% of *E. granulosus* in camels (Rostami et al., 2015). It is reported that prevalence of 11 and 88% of human Cystic Echinococcosis is due to *E. intermedius* and *E. granulosus* sensu stricto, respectively (Rojas et al., 2014). As camel is the source of milk, meat and having close contact with humans, it is the major source of disease transmission in humans. Therefore, camel strains of *E. granulosus* and *E. intermedius* have been detected from human infections (Sadjjadi et al., 2013). Hence, camels are the most important intermediate hosts of Echinococcosis in endemic areas of different countires, where they play an important role in the transmission of *E. granulosus* to humans. Yet, nature and variation of *Echinococcus* in the camel is not properly understood (Laurimäe et al., 2018). It is estimated that hydatid disease results in economic losses of US$165.72 per hundred infected camels (Latif et al. 2010).

The *E. granulosus* (hydatidosis) is suggested to be diagnosed by identification of cyst-like mass with history of sheepdog exposure to endemic areas (Eckert and Deplazes 2004). As adults shed a gravid segment in a week, in most cases gravid segment cannot be found in the faeces of the final host. Therefore, arecoline purges are used to flush out the adult worms for diagnosis of the parasite (Varcasia et al. 2004). The anterior portion of worm is buried inside the intestinal mucosa, therefore it is very hard to observe the worm. Hence, microscopic examination of intestinal scraping is required for the detection of these worms. Several advanced imaging diagnostic techniques for Cystic Echinococcosis are CT scans, ultrasonography, and MRIs. The Cystic Echinococcosis must be differentiated from malignant and benign neoplasms, abscesses, cavitary tuberculosis, mycoses and benign cysts (Stojkovic et al. 2012). After identification of cysts by using the above technique, serological test is necessary for confirmatory diagnosis. Serologic tests, including indirect hemagglutination test and enzyme-linked immunosorbent assay (ELISA), are highly sensitive methods (Auer et al., 1988).

The treatment methods vary with cyst characteristics such as type, size, location and post-operative complications. In the past, surgery was the only option for treatment of hydrated cysts. It is more difficult to treat Alveolar Echinococcosis than Cystic Echinococcosis, the former usually requires long-term chemotherapy and radical surgery, or both at same time. Liver cysts greater than 7.5 cm are more likely to possess biliary communication; surgical removal may be the best option (Greco et al. 2019). Cyst puncture, chemotherapy, and PAIR (percutaneous aspiration, chemicals injection and re-aspiration) are being used instead of surgery. Few cysts do not show any symptoms (inactive) and often go away un-treated. Benzimidazoles remain effective treatment in some patients. Albendazole (10-15 mg/kg) remains effective in patients having multiple but small cysts in

several organs. The use of mebendazole (40-50 mg/kg) continuously for several months has been found highly effective. Additionally, the use of both methods (chemotherapy and surgery) has been found very effective (Velasco-Tirado et al. 2018).

Control of Echinococcosis is possible by preventing transmission of the parasites (Craig et al. 1996). The measures include:

1. Prevent the dogs to feed on hydatid cyst infected carcass.
2. Control the population of stray dogs.
3. Do not consume contaminated water or food (meat, vegetables and fruit).

Schistosomiasis

Schistosomiasis is the infectious disease of human and vertebrate hosts (McManus et al. 2018). Mammalian hosts consist of humans, camels, dogs and mice (Parsani et al. 2008). The parasites reside in the vascular system (mesenteric and hepatic veins) of vertebrate hosts. Snail is the intermediate host for *Schistosoma* and vertebrates are the final hosts. Four S*chistosoma* species infect camels including *S. bovis*, *S. mattheei*, *S. indicum* and *S. turkestanica*. Except *S. indicum*, all these species have been reported in humans (Cox 2015; Sazmand et al., 2019b). The *S. spindale* has been reported in Egyptian camels (El-Khabaz et al. 2019). The disease is more common in South America, Middle East, Africa and Asia. Beside camels, more than 230 million people are affected with this disease around the globe (Colley et al. 2014). According to an estimate, more than 700 million people from more than 70 countries reside in the common disease areas. Life cycle of *Schistosoma* occurs in two hosts (mammals and snails). Asexual reproduction takes place in the intermediate host (snails). The development of miracidia to sporocysts occurs in the snail, the sporocysts multiply and grow into cercariae. In the final hosts (mammalian), parasites mature, mate, and reproduce to lay eggs (Viana et al. 2018). Worm eggs are released into external environment through urine or faeces of mammal hosts. These eggs transform into miracidia in freshwater, which hatch to float in water and reach the snail (Shuja et al. 2018). Daughter sporocysts either produce cercaria (cercariogenous sporocysts) or more sporocysts (Mouahid et al. 2018). Infected snails can shed hundreds of cercariae in a day (Braun et al. 2018). The parasitic larvae are released into freshwater by snails, which penetrate the skin of mammals. The disease spreads by the people having Schistosomiasis, who contaminate the freshwater with their excreta containing parasite eggs (Bekana et al., 2019). Agricultural and fishing population is more prone to Schistosomiasis.

Clinically, Schistosomiasis consists of acute and chronic forms, with incubation period of 14-84 days. Symptoms of acute disease condition include fever, headache, rashes, myalgia respiratory symptoms, chills, dermatitis and muscle aches (Sahba and Malek 1979). While, in chronic Schistosomiasis, symptoms include abdominal pain, hypertension of abdominal blood vessels, enlarged liver and spleen, blood in the urine or stool, fibrosis of urinary bladder and ureters, kidney damage and problems in passing urine (Resources for health professionals: parasites–schistosomiasis; https://www.cdc. gov/parasites /schistosomiasis/health_professionals/index.html#tx).

Prevalence of *S. spindale* in Egyptian camel was reported as 0.8% (El-Khabaz et al. 2019). In north–west part of Thar Desert in India, prevalence was 1.45 % (Singh et al., 2013). Similar species of *Schistosoma* cause disease in camels and humans. Hence, *Schistosoma* is zoonotically important. It is estimated that 779 million people are at the risk of infection, among them 85% are in Africa. Approximately, 207 million people from 74 countries are infected with Schistosomiasis, and 120 million of these infected people develop clinical signs of the disease (Bajiro et al. 2016).

Schistosoma eggs can be found in faecal, stool and urine which are helpful for the disease diagnosis. Several molecular techniques, such as polymerase chain reaction, nested PCR and real time PCR, are being used for molecular diagnosis of the disease. Immunological techniques, such as antibodies and/or antigens detection like immunofluorescence test (IFAT), enzyme linked immunosorbent assay (ELISA) and immunoblotting of blood or urine samples, are used for detection of the infection (Utzinger et al. 2015). The drug of choice against all species of Schistosomes is praziquantel @40mg/kg. Recovery rate of 65-90% has been found after a single dose with praziquantel. If the parasite is not killed by the drug, its efficiency of egg production is reduced by 90% (Keiser et al. 2014).

The preventive measures of Schistosomiasis are: to avoid the paddling, washing and swimming in fresh water. Preference should be given to only swim in the chlorinated or sea swimming pools. Preferably, use filtered or boiled water before drinking (Inobaya et al. 2014).

Fascioliasis

This is a food and water borne zoonotically important disease, mainly caused by *Fasciola hepatica* and *Fasciola gigantica*. The target site of *Fasciola* is bile duct and liver (Hanna 2015). *Galba truncatula* (snail) is the intermediate host of *Fasciola* species. *F. hepatica* is prevalent in almost all parts of the world, including Europe, Africa, Middle East, Asia, Oceania and parts of Latin America, while *F. gigantica* is prevalent in relatively fewer localities.

Individuals usually get infection after taking raw watercress or any water plant. After ingestion, the young worms penetrate through the intestinal mucosa, abdominal cavity, the liver and finally reach the bile duct. The parasites become adult flukes for egg production (Torgerson and Claxton 1999). Un-embryonated eggs are released into biliary ducts, and are passed in faeces/stool of humans (incidental hosts) or herbivores (definitive hosts). Embryonated eggs release miracidia in water, which swim to reach and invade the snail. In the intermediate host, the parasites undergo several developmental stages (sporocysts, rediae, and cercariae). The cercariae are released and encyst as metacercariae on

aquatic vegetation to be exposed to the final host. Transmission occurs by ingestion of raw and freshwater vegetation containing encysted metacercariae of flukes. Usually, the invasive phase lasts for several weeks (Moazeni and Ahmadi 2016). The common symptoms are intermittent fever, abdominal pain, hepatomegaly, malaise and muscle wasting (Kaya et al. 2011). Both *Fasciola* species have been found in camels, with prevalence ranges from 3.3 to 15% (Banaja and Ghandour 1994; El-Khabaz et al. 2019). In Pakistan, prevalence of Fasciolosis is recorded as 30.73% in camels (Ijaz et al., 2018). Fascioliasis is considered as a highly neglected tropical disease, which infects an estimated population of 35-72 million people around the world. Microscopic examination of faeces or stool is used to observe the presence of parasites. For this purpose, more than one specimen should be examined to find the presence of parasites. The eggs are not shed immediately after infection but it needs several months. Molecular (PCR, nPCR and RT-PCR) and immunological (ELISA, IFAT, immunoblotting) methods are more sensitive for the diagnosis of *Fasciola*. The drug of choice for Fascioliasis is triclabendazole, which is administered orally in two doses (Gandhi et al. 2019). Most of infected people respond very well to the treatment. To date, no vaccine is available for humans and animals against *Fasciola* infection. Strict control measures for the sale and growth of watercress and other edible water plants are important.

Blastocystis

A complex and diverse group of heterotrophic and photosynthetic protozoa belong to phylum Stramenopila and the disease caused by its members is called intestinal Blastocystosis. This parasite usually colonizes asymptomatically in lower digestive tract of humans in different infective forms e.g., vacuolar, granular, amoeboid and cyctic (Parija and Jeremiah 2013; Besteiro 2014). It comprises 17 different sub-species (ST1-ST17) infecting 1 to 2 billion people, with the prevalence rates of 15 and 100% in developed and poorly developed countries, respectively. Blastocysts subtypes ST1-ST9 are mostly associated with human infection, however sub-types ST1, ST3, ST5, ST14 and ST15 and their mix infection are reported from camels in different parts of the World. Three sub-types i.e., ST1, ST3 and ST5 have zoonotic potential (Scanlan and Stensvold 2013; Lepczyńska et al. 2017; Mokhtar and Youssef 2018; Sazmand et al. 2019a). Transmission of this parasite occurs through oral-fecal route by ingesting contaminated food, drinking water or through direct contact. Cystic form of Blastocyst can survive up to 19 days in water under normal temperature. Infection of Blastocystis can be characterized by diarrhea, abdominal pain and irritable bowel syndrome (Garcia 2017; Toro Monjaraz et al. 2018). Blastocysts subtypes, immune level of the host and virulence of the sub-type might be the factors responsible for differences in clinical presentation (Stensvold 2013).

Diagnosis of Blastocystis is mostly made by microscopic examination of the stool, as the organism appears in stool samples in its amoebic, granular, vacuolar and cystic form. The size of *B. hominis* present in the diarrheal fluid ranges from 6 to 40 µm and can be easily seen under microscope. Molecular and *in-vitro* culture techniques are other methods used for the identification of Blastocystis but these methods are not routinely used for diagnosis (Garcia 2006; Tan and Suresh 2006). Infection of Blastocystosis may be self-limiting and the infection should be treated symptomatically and according to the presence of other pathogens. The drug of choice for Blastocystosis is metronidazole, while trimethoprim-sulfamethoxazole, nitazoxanide, and paromomycin-metronidazole may also be used in combination as an alternative, depending upon the severity of the infection (Mirza et al. 2011; Sekar and Shanthi 2013).

Trichinellosis

The disease caused by parasitic nematode *Trichinella spiralis* (Phylum Nematoda; Family Trichinellidae; Genus *Trichinella*) is called Trichinellosis which is worldwide. *Trichinella spiralis* life cycle's is synanthropic in pig, rat, horse, camel, dog, fox, bear, humans; muscle cysts with capsule are present (Mitreva et al. 2011). Nine species and three genotypes (*T. spiralis* (T1), *T. native* (T2), *T. britovi* (T3), *T. pseudospiralis* (T4), *T. murrelli* (T5), *T. T6* (T6), *T. nelson* (T7), *T. T8* (T8), *T. T9* (T9), *T. papuae* (T10), *T. zimbabwensis* (T11), *T. patagoniensis* (T12)) of *Trichinella* have been documented till now; the first four (T1 – T4) with high pathogenicity to humans (Rostami et al. 2017), are found in the muscles of camels causing Trichinellosis in this species. Trichinellosis is cosmopolitan food borne zoonotic disease and approximately 0.1 million cases are reported from all over the world annually. (https://www.cdc.gov/parasites/trichinellosis/epi.html).

The disease is more common in pork and is a food borne pathogen (Söderberg et al. 2021). Humans acquire infection by eating raw or undercooked meat of infected camels, birds, horses and pork (Bommer et al. 1980; Arbaji et al. 2005; Devleesschauwer et al. 2015). Life cycle of *Trichinella spiralis* consists of two phases i.e., intestinal phase and muscular phase. Infection starts by the ingestion of meat contaminated with encysted larvae. Larvae are liberated in small intestine under the influence of pepsin and gastric juices, penetrate the mucosa of small intestine and moult to adult parasites. After the period of 1-week, adult female releases larvae that migrate to striated muscles and encysted again by the host (Rostami et al. 2017; https://www.cdc.gov/parasites/trichinellosis/biology.html). Clinical manifestation of Trichinellosis depends upon the phase of infection. In intestinal phase, the most common symptoms are diarrhea and abdominal pain, while fever, myalgia, facial oedema and myocarditis are obvious signs in muscular phase (Pozio et al. 2003; Rostami et al. 2017).

For the diagnosis of the *Trichinella* spp., routine examination of infected animal is mandatory. In humans, diagnosis is made by *Trichinella* antibody test and in some cases infected muscles biopsy is recommended. In camels, it is also diagnosed through ELISA by anti-

Trichinella IgG. *Trichinella zimbabwensis* has also been identified through 5.8S ribosomal RNA.

Albendazole @400 mg/kg twice up to 8 days and mebendazole 200 mg/kg thrice for three days are recommended. By adopting good hygienic conditions and avoiding improperly cooked or raw meat can also protect from the infection (Nöckler et al. 2000; https://www.cdc.gov/parasites/trichinellosis/health_professionals/index.html#tx.).

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ARTHROPOD ALLERGY AND PUBLIC HEALTH

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INTRODUCTION

Allergic disease, or simply allergy, is a common disorder which affects approximately 40% of global population (Johansson et al. 2000). It is an allergen-mediated hypersensitivity response, involving diverse immunological mechanisms initiated by specific antibodies or cells (Halliwell et al. 2006). Austrian physician Clemens von Pirquet introduced the term "allergy" almost a century ago in a German medical journal to describe the "altered biological reactivity" of the immune response against allergens in the host (Von Pirquet 1906). With the advent of, and advances in molecular biology, the science of allergy has transformed into a major branch of highly sophisticated human and veterinary medicine (Noli et al. 2013).

In immunological terms, an allergen is an antigen. The allergen itself is a non-toxic, non-invasive protein with the potential to initiate immunogenic type-I hypersensitivity reaction by specific immunoglobulin E responses when inhaled, ingested or injected (Goldsby et al. 2003). Most allergens in their natural state are soluble proteins, which chiefly exhibit proteolysis amongst other types of enzymatic activities (Morgan and Arlian 2006; Jeong et al. 2010). The allergic reaction is induced by production of IgE and cross linking of the high-affinity IgE receptor (*FceRI*) on basophil and mast cell surfaces (Kinet 1990; Aalberse 2000). The allergic potential of an allergen is determined by its critical physiochemical properties, such as membranous, mucosal and epithelial permeability, solubility and stability under varying pH and temperature conditions (Christensen et al. 2008). An allergic reaction is dependent upon the amount of allergen exposure and the consequent aggregation of the receptors (Marshall et al. 1986). Allergy, being an immune-mediated disease, is complex and multifactorial in nature. Evidence suggests that allergic individuals have genetic predisposition (Vercelli 2008; Tan et al. 2012; Kanchan et al. 2021). Key factors affecting the outcome of allergy are a combination of underlying genetic vulnerability and triggering factors, such as immunological dysregulation and environmental influences (Jabbar-Lopez et al. 2020; Kanchan et al. 2021).

The term atopy is derived from the Greek word "atopos", meaning out of place, and is typically associated with IgE-mediated diseases (Kay 2001). Asthma, atopic eczema, dermatitis and allergic rhinitis are the most frequent manifestations of atopy and are amongst the most common global causes of chronic public health problems. Combination of these diseases is called the atopic triad, as shown in Figure 1 (Devereux and Seaton 2005; Vaillant et al. 2020). The immunopathological hallmark of atopy is type 2 helper cell (Th2) infiltration. An exaggerated immunological response elevates serum IgE levels and induces cytokine production by Th2 cells (Lauzon-Joset et al. 2020; Lee et al. 2021). The concentration and duration of allergen exposure, along with the avidity of allergen-specific interactions, determine the influence of Th1 and Th2 cells in the affected tissues (Constant and Bottomly 1997; Rogers and Croft 1999). Atopic allergic diseases are hereditary and often have a family history (Kay 2001). Several loci have been linked to atopy through candidate gene approach. Polymorphisms in the IgE receptor gene (*FceRI-β*) are reportedly associated with severe atopy (Cookson 1999). However, the clinical significance of genetic investigation is unclear, because inheritance of susceptibility genotype does not guarantee allergic phenotype, the environment and lifestyle choices strongly influence disease onset and severity (Noli et al. 2013).

Pakistan is a developing country with a very high burden of both communicable and non-communicable diseases (Sultan and Khan 2013). Although researchers, physicians and healthcare professionals in Pakistan are primarily focusing on infectious diseases, allergenic diseases are mostly ignored. Allergy is a neglected disease in Pakistan and little or incomplete data exists on its prevalence. Patients with allergy symptoms ignore them and neither report nor seek effective treatment (Greiner et al. 2011).

Molecular Mechanisms and Pathophysiology

Allergen molecules have specific epitopes which upon exposure in a susceptible individual are recognized as antigens by the immune system. B-cell surface receptors bind to these antigens, signaling endosomes for endocytosis and subsequent degradation by proteases enzymes into immunogenic peptides (Janeway et al. 2001). MHC bearing Golgi bodies fuses with proteolytic enzymes to form peptide-loading vesicles, which bind with the immunodominant peptide. The endosome moves to the cell surface, transforming the B cell into an antigen presenting cell (APC). Helper T-cells (Th1 and Th2) recognize and bind to these B-cell APCs, activating both the T and B-cells (Kinet 1990; Aalberse 2000). A cytokine cascade of interleukin (IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13) is initiated by the activated Th2, enhancing B-cell growth, proliferation and differentiation into IgE producing plasma cells and memory B-cells. Immune cells such as leukocytes (eosinophils, basophils and neutrophils), mast cells and lymphocytes (T and B-cells) are also attracted to the site of allergic reaction (Romagnani 2000).

The B-cell secreted IgE antibodies circulate in the blood and bind to the surface of acute inflammatory immune cells through high-affinity IgE-specific receptors (fc receptors FcεRI). These IgE antibody-coated cells become sensitized to this specific allergen. The main effector cells involved in acute allergic reactions are basophils and mast cells, chiefly located in the eyes, nose and gut mucosa. Figure 2 illustrates the sequence of type-I hypersensitivity reaction upon further allergen exposure. Sensitized cells are activated by forming a cross-linked complex between the IgE antibodies and fc receptors on their surface. Activated cells release powerful soluble inflammatory chemical mediators (histamine, prostaglandins, chondroitin sulfate, heparin, leukotrienes, interleukins, and cytokines), protease enzymes (cathepsins, chymase, tryptase and carboxypeptidase), lipid mediators (thromboxanes, prostaglandins and leukotrienes) and cytokines (IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and TNF-fi) through a process called degranulation (Kinet 1990). As a result surrounding tissue undergoes physiological effects, such as nerve stimulation (itchiness and sneezing), mucosal secretion (rhinorrhea and epiphora), smooth muscle contraction (wheeze and dyspnea) and vasodilation (edema and erythema). Allergen concentration and host susceptibility can lead to classical anaphylaxis (Janeway et al. 2001; Arlian 2002).

Arthropod Allergy

Over a million species of arthropods have been described, all of which are important to humans due to their economic, ecological or pathological significance. Arthropods of clinical relevance include vectors, parasites and allergen producers (Yong and Jeong 2009). Arthropod proteins of virtually all species may be potent sources of IgE-mediated allergy in susceptible human populations (Kagen 1990; Kim and Hong 2007). Arthropod allergy and characteristics of their allergens have been extensively studied as a major research discipline in parasitology and medical arthropodology.

In recent decades, a rise in arthropod allergy has been reported which is mostly attributed to household arthropods. This trend may be a consequence of modern human lifestyle and extended periods of indoor activities. Economics, lifestyle choices and environment encourage the growth and multiplication of certain arthropods, such as cockroaches and mites, which results in increased human exposure, leading to sensitization or clinical manifestations (Kim and Hong 2007). Arthropod allergies are reported due to ingestion (shellfish allergy caused by crustaceans), inhalation (aeroallergens of mites, roaches and insects), contact (silk proteins) or injection (hymenopteran and formicidae stings) (Arlian 2002). Types of arthropod allergens are illustrated in Figure 3.

Several allergens identified from arthropods are classified, based on their biological functions and molecular structures (Gaffin and Phipatanakul 2009). More than half of major allergens are lipid and fatty acid binding proteins, calycins and lipocalins found in arthropod fluids and secretions (Mantyjarvi et al. 2000; Trompette et al. 2009).

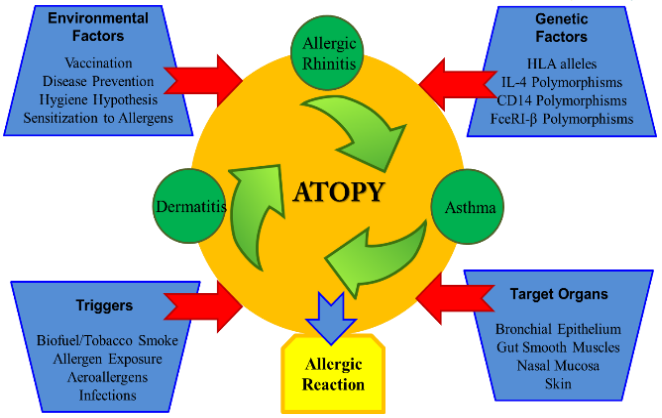

Fig. 1: The atopic triad: Asthma, atopic eczema/dermatitis and allergic rhinitis.

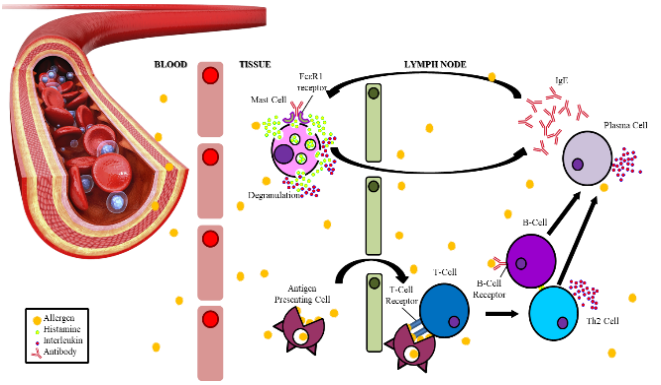

Fig. 2: Acute phase hypersensitivity type-I reaction against an allergen.

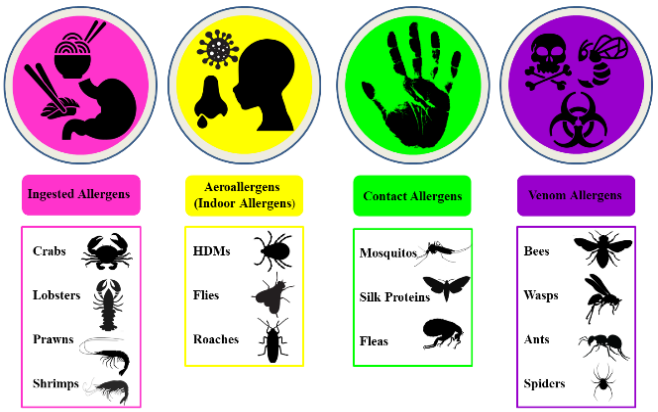

Fig. 3: Types of arthropod allergens.

Evidence suggests that protease activity is also an important allergenic property and is implicated in increased IgE production by direct epithelium damage (Jeong et al. 2006; Chapman et al. 2007). None of the known cockroach allergens exhibit active proteolytic activity, however allergic inflammation is associated with serine proteases found in cockroach extracts (Pomes et al. 2007; Jeong et al. 2008). House dust mite (HDM) allergens are identified as chitinases and have demonstrated an important role in development and mediation of Th2 cell-driven inflammation in asthma (O'Neil et al. 2006). Tropomyosin is a heat stable allergen found in edible arthropods, such as crustaceans (Taylor and Lehrer 1996).

Table 1: Classification of shellfish arthropod species

Phyla	Sub-Phyla	Class	Order	Common name
Arthropoda	Crustacea	Malacostraca	Decapoda	Shrimps (Caridea) Prawns (Penaeidae) Crabs (Brachyura) Lobsters (Nephropidae) Crayfish (Cambarus)
				Spiders Mites
		Arachnida	Araneae	Dust Mites
Arthropoda	Chelicerates	Xiphosura		Horseshoe Crab
Arthropoda	Myriapoda	Chilopoda		Centipedes Millipedes

Table 2: List of identified and characterized shellfish allergens (Radauer et al. 2008)

Allergen	Molecular Weight	Heat Stability	IgE Binding	Route of Exposure	Physiological Function
Tropomyosin	34–38 kDa	Highly heat stable	Reactive	Ingestion Inhalation	Actin binding Regulation of myosin and troponin
Arginine Kinase	40–45 kDa	Labile	Reactive	Ingestion Inhalation	Catalyst Reversible transfer ATP phosphoryl group to arginine
Myosin Light Chain	17–20 kDa	Stable	Reactive	Ingestion	Regulation of smooth muscle contraction in the presence of MLC kinase
Sarcoplasmic calcium Binding Protein	20–25 kDa	Stable	Reactive	Ingestion	Calcium buffer Cytosolic calcium (Ca^{2+}) binding Regulation of calcium based signaling
Troponin C	20–21 kDa	Unknown	Reactive	Ingestion	Muscle contraction Calcium binding Regulation of actin and myosin
Triosephosphate Isomerase	28 kDa	Labile	Reactive	Ingestion Inhalation	Key glycolysis enzyme Catalyst Conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate

Ingested Arthropod Allergens

Seafood is an important part of human diet. Its consumption has increased in recent years due to growing international trade and distribution of marine products across many countries. However, seafood allergy is one of the most prevalent causes of food induced anaphylaxis in the world. It is more than twice as prevalent as peanut allergy (Sicherer et al. 2004). Shellfish are a common cause of allergic anaphylaxis, affecting children and adults of all ages however, it is 5 times more prevalent amongst adults than children (Lopata et al. 2016; Sicherer and Sampson 2010). Studies have shown that allergic reaction in sensitized individuals can not only be elicited by shellfish ingestion but through air-borne shellfish allergens as well. This is especially evident from occupational risk studies (Gautrin et al. 2010; Bonlokke et al. 2012; Kamath et al. 2014).

Shellfish and Crustacean Allergens

Shellfish are classified into one chordate and two invertebrate groups, Mollusca and Arthropoda (crustaceans). Phylogenetically, arthropod shellfish or crustaceans are related to arachnids and insects (Afzaal et al. 2016). Classification of arthropod shellfish species is shown in Table 1. More than 50,000 crustacean species have been described (Chan 1998). Many such varieties are used for human consumption, either cooked or raw. Shellfish allergy is the most prevalent of the eight common food allergens inculpated in 90% type-I hypersensitivity reactions to food (Rona et al. 2007). Majority (62%) of arthropod shellfish allergies are attributed to prawns, followed by crabs and lobsters (Sicherer et al. 2004). Six major shellfish allergens have been identified from crustaceans (Table 2) and are registered in the International Union of Immunological Societies (IUIS) Allergen Database (www.allergen.org) (Radauer et al. 2008). These allergenic proteins are highly water soluble light molecules with the ability to maintain stability at high heat and have an acidic isoelectric point (Sun and Lopata 2010). The list of characterized crustacean allergens is shown in Table 3.

Prevalence and Epidemiology

Approximately 2% of global population is affected by shellfish allergy. It has a particular impact in the Asia-Pacific region, where seafood consumption is the highest in the world (Lee et al. 2012). After Japan and China, America is the third largest seafood consumer in the world (Sicherer et al. 2004). Overall prevalence of shellfish allergy in the western world (Canada, USA and Europe) is estimated to be 0.6% (Rona et al. 2007). Iceland has the highest seafood consumption rates in Europe, followed by Portugal, Norway, Spain, France, United Kingdom and Germany (Lopata et al. 2010).

While majority of allergen studies in Pakistan are focused on aeroallergens such as pollen and HDMs, shellfish allergies are mostly ignored. A recent local study involving a small sample (n=149) has reported shellfish allergy as the most prevalent of the tested food allergens, implicating crabs (70%; n=39) and prawns (46%; n=26) (Hussain et al. 2020). However, clinical record has revealed that approximately 90% allergic patients suffered from aeroallergen sensitization, whereas only 10% had food allergies (Hussain et al. 2019). This may be due to lower seafood consumption rates in Pakistan. According to the Food and Agriculture Organization (FAO) food balance sheet (FBS) for South East Asia, Pakistan ranked the lowest in seafood consumption rates (0.9 kg per capita per year) in 2015. This figure was even less in 2011 (0.6 kg per capita). Even though seafood in Pakistan is consumed in coastal provinces of Sindh (1.6 kg) and Balochistan (2.4 kg), its consumption is recorded to be very less in Punjab (0.2 kg) and even less in the Northern regions of Khyber Pakhtunkhwa (0.05 kg) (FAO-FBS 2015 online resource). Some common shellfish species found in Pakistan are shown in Figure 4.

Clinical Manifestations

Allergic reaction to ingested arthropod allergens is symptomatically similar to any of the eight major allergic reactions. The severity of reaction may vary from tolerable to fatal. Atopic individuals are at a higher risk of developing anaphylactic reaction to food allergens. Itching and angioedema of the lips, mouth and pharynx is usually immediate i.e., within 2 hours of consumption, however late-phase reaction may take up to 8 hours (Villacis et al. 2006). Respiratory distress, particularly oral allergy syndrome, is experienced due to ingested allergens like crustacean (Dohi et al. 1991). Typical type-I hypersensitivity reaction due to ingested allergens are given in Table 4.

Table 3: Characterized crustacean allergens (IUIS Allergen Nomenclature)

		Shellfish Species	Common names	Tropo-myosin	Arginine Kinase	Myosin Light Chain 1 and 2	Sarcoplasmic Calcium Binding Protein	Troponin C, Troponin I	Triose-phosphate somerase
Crusta-ceans	Shrimp	*Penaeus monodon*	Asian Tiger Shrimp	Pen m 1	Pen m 2	Pen m 3	Pen m 4	Pen m 6	Cra c 8
		Penaeus aztecus	Brown Shrimp	Pen a 1	-	-	-	-	-
		Crangon crangon	Common Shrimp	Cra c 1	Cra c 2	Cra c 5	Cra c 4	Cra c 6	-
		Litopenaeus vannamei	Vannamei Shrimp	Lit v 1	Lit v 2	Lit v 3	Lit v 4	-	-
		Pandalus borealis	Pink Shrimp	Pan b 1	-	-	-	-	-
		Metapenaeus ensis	Sand Shrimp	Met e 1	-	-	-	-	-
		Archaeopotamobius sibiriensis	ND	-	-	-	-	-	Arc s 8
		Machrobrachium rosenbergii	Giant Fresh Water Shrimp	Mac ro 1	-	-	-	-	-
	Prawn	*Melicertus latisulcatus*	King Prawns	Mel l 1	-	-	-	-	-
		Penaeus indicus	Indian White Prawn	Pen i 1	-	-	-	-	-
	Crab	*Charybdis feriatus*	Crucifix Crab	Cha f 1	-	-	-	-	-
		Portunus pelagicus	Blue Swimmer Crab	Por p 1	-	-	-	-	-
	Lobster	*Homarus americanus*	American Lobster	Hom a 1	-	Hom a 3	-	Hom a 6	-
		Panulirus stimpsonii	Spiny Lobster	Pan s 1	-	-	-	-	-
	Crayfish	*Pontastacus leptodactylus*	Narrow-Clawed Crayfish	Pon i 1	-	-	-	-	-

*Allergens nomenclature is registered with International Union of Immunological Societies (www.allergen.org).

Table 4: Type-I hypersensitivity reaction due to ingested allergens

Time	Severity	Symptoms	
Immediate	Mild	–	Facial, mouth or tongue tingling
		–	Urticaria, itching or eczema
		–	Angioedema of the face, lips, tongue or throat
Within 1 to 2 hours	Tolerable	–	Wheeze or nasal congestion
		–	Breathing difficulty
		–	Nausea or vomiting
		–	Abdominal pain or diarrhea
Within 8 hours	Anaphylaxis	–	Airway constriction manifesting as a throat lump
		–	Severe drop in blood pressure and vitals
		–	Lightheadedness, dizziness or loss of consciousness
Chronic sensitization	Complication	–	Asthma exacerbations
		–	Extreme sensitivity to trace amounts of allergen
		–	History of food-induced anaphylaxis

Prevention and Management

As with all types of allergies, prevention of ingested allergens such as shellfish allergy is to avoid seafood and shellfish products. Even trace amounts may result in severe immunological reaction. Shellfish is rarely a hidden food ingredient, which makes it easier to avoid when dinning out. However, seafood flavoring, shellfish stock, oils and cross-contamination through pans and utensils pose a high risk to allergic individuals. Further, cross-contamination may occur in markets, food stores, factory packaged materials where shellfish are prepared, processed or stored. Some individuals may suffer an allergic reaction due to air-borne or even through contact with shellfish allergens. A medical alert bracelet worn at all times, especially when eating out, is also a good management practice.

Arthropod Aeroallergens (Indoor Allergens)

Arthropod derived aeroallergens are potent inducers of respiratory inflammation. Arthropod secretory or excretory materials induce IgE responses. Inhalation of environmental arthropod allergens (house dust mites, cockroaches, moths and butterflies) may induce persistent airway diseases such as asthma (Kang and Chang 1985). Population fluctuations in indoor arthropods are usually very slight, which consequently results in perennial allergy instead of seasonal allergy unlike pollen and other aeroallergens (Yong and Jeong 2009; Calderón et al. 2015). Although true allergens such as Anisakis simplex antigen (Ani s 1) have been identified (*Anisakis* spp. larvae), most arthropod-derived materials implicated in increased IgE levels are considered allergens (Ibarrola et al. 2008).

House Dust Mites (HDMs) Allergens

Dust mites are taxonomically classified in the class Arachnida, which makes them phylogenetic relatives of scorpions, spiders and centipedes (Colloff 1998; Esch et al. 2001). More than 40 HDM species have been described. The most common of these are shown in Figure 5. HDMs are medically important due to their role in allergy and atopy (Dhaliwal et al. 2021). Allergic asthma and rhinitis are associated with HDMs prevalence (Terreehorst et al. 2002; Li et al. 2013). HDM larvae have 3 pairs of legs, whereas adults have an additional pair of legs, totaling in 4 pairs. They are mostly found in human dwellings and indoor environments, such as settled dust, pillows, blankets, mattresses and bedding. Food sources include human skin scales, cotton fibers, wood, paper and synthetic materials. Water is absorbed from air. Most favorable growth and sustainability conditions include 23-30ºC temperature and 80-90% relative humidity, which makes HDM infestations, particularly destructive in tropical and sub-tropical coastal cities (Guerrant et al. 2006).

Mite-derived allergens enter the respiratory track through inhalation. They vary in size, ranging from very small (1.1-4.7 µm) to larger (>4.7 µm) particles. Large particles, even in smaller quantities, can elicit more substantial early-phase immune response (Casset et al. 2006), however smaller particles can penetrate far deeper into the lungs (Custovic et al. 1999). HDM allergens manifest as rhinitis, bronchial asthma, and rarely-conjunctivitis. To date more than 24 groups of HDM allergenic proteins have been identified and characterized (Table 5). HDM allergens are potent antigens, which orchestrate combined effects of both the innate and the adaptive immune responses. They distort the immune response by mimicking virulent pathogen-associated molecular patterns and activating multiple routes (Wang 2013). They may initiate IgE independent activation of mast cells, causing direct damage to the epithelial cells of the respiratory tract (Takai and Ikeda 2011). Whereas, HDM immunogenic components such as epitopes and proteases along with structural polysaccharides derived from HDM ligands and exoskeleton cause IgE-dependent allergic responses through $CD4^+$ and T_H^2 cell activation (Jacquet 2013; Wang 2013). This combined effect of the immune responses is the therapeutic barrier marring clinical efficacy.

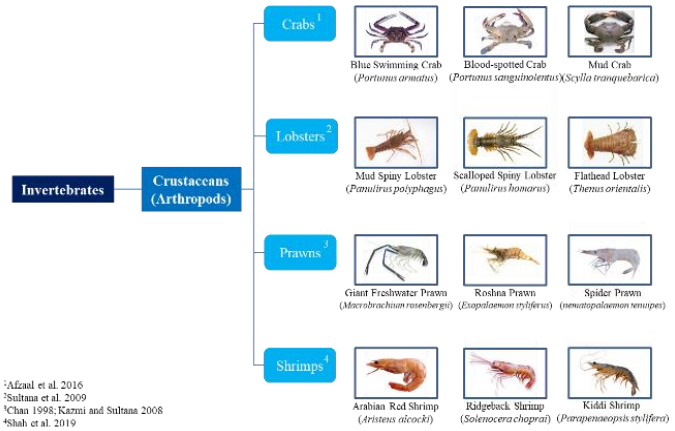

Fig. 4: Common commercial shellfish species from Pakistan.

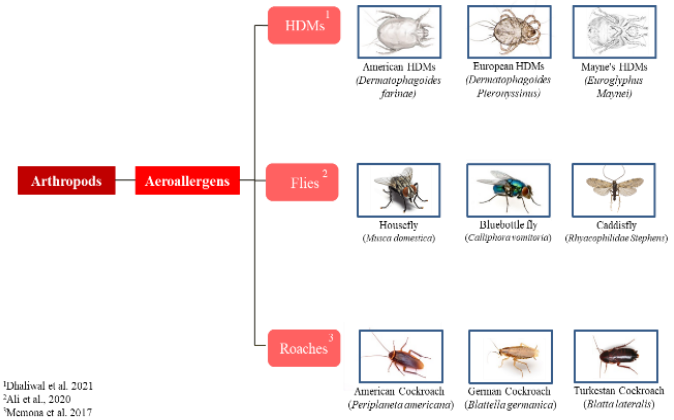

Fig. 5: Common aeroallergen producing arthropod species in Pakistan.

Prevalence and Epidemiology

It is estimated that 65 - 130 million persons or 1-2% of the global population is affected by HDMs (Colloff 1998). Three HDM species, *Dermatophagoides pteronyssinus*,

Dermatophagoides farinae and *Euroglyphus maynei,* are the most common dust mites by density and species prevalence (Arlian et al. 1992). Regional climate influences HDMs population density, HDM allergen being highest in summer and lower during winter. Indoor microclimate plays a dramatically crucial role. Air-conditioning has been reported to significantly reduce HDM allergens (Lintner and Brame 1993). However, geographical variations in HDM species dominance are strong indicators of specialist adaptation (Thomas 2010). Prevalence of HDM allergy is dependent on the density of exposure. Settled dust provides a detrital habitat for HDMs and serves as a reservoir of organic macromolecules, such as cellulose (textile fibers), keratin (human skin scales) and chitin (fungal hyphae and mite cuticles), along with other HDM dietary sources pollen, bacteria and microscopic spores (Calderón et al. 2015). It has been noted that HDM densities were decreased in houses furnished with new carpets, curtains and mattresses (Simpson et al. 2002).

Clinical Manifestations

HDM allergen is perennial, since HDM inhabit mattresses, bedcovers, blankets and pillows (Yong and Jeong 2009; Calderón et al. 2015). This causes year-round allergy symptoms, which mostly occur during late night or early morning. The most common HDM allergens are Der p 1, Der p 2, Der p 23, Der f 1 and Der f 2. These allergens target CD23 and CD25 triggering release of immunoglobulin E (Chapman et al. 2007). Catalytic inactivation of alpha antitrypsin by the most potent HDM allergen Der p 1, makes the lower respiratory tract vulnerable to proteinases mediated damage causing airway inflammation, which is extremely devastating in asthmatics (Wang et al. 2021).

Table 5: HDM allergen groups and their immunological roles (IUIS Allergen Nomenclature)

Allergen Group	Identified Allergens	Molecular Category	Molecular Weight	Quantitative Allergenicity	Species	Role in Immunity
1.	Blo t 1 Der f 1 Der m 1 Der p 1 Der s 1 Eur m 1 Pso o 1 Sar s 1	Cysteine Protease	25 kDa	80% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farina* *Dermatophagoides microceras* *Dermatophagoides pteronyssinus* *Dermatophagoides siboney* *Euroglyphus maynei* *Psoroptes ovis* *Sardinops sagax*	– Production of chemokines, cytokines and growth factors – Production and promotion of pro-T_H^2 polarization – Inflammatory cell recruitment – Airway remodeling – Disruption of epithelial junctions to increase permeability – Degranulation of mast cells and eosinophils – Fibroblast maturation – Proliferation of smooth muscles
2.	Aca s 2 Ale o 2 Blo t 2 Der f 2 Der p 2 Der s 2 Eur m 2 Gly d 2 Lep d 2 Pso o 2 Sui m 2 Tyr p 2	MD-2–like lipid-binding protein Niemann-Pick C2 homologue	14 kDa	80% IgE binding frequency	*Acarus siro* *Anacardium occidentale* *Blomia tropicalis* *Dermatophagoides farina* *Dermatophagoides pteronyssinus* *Dermatophagoides siboney* *Euroglyphus maynei* *Glycyphagus domesticus* *Lepidoglyphus destructor* *Psoroptes ovis* *Suidasia medanensis* *Tyrophagus putrescentiae*	– Production of chemokines and cytokines – Promotion of T_H^2 polarization – Inflammatory cell recruitment – Molecular mimicry of MD-2 or NPC2-like proteins – Activation of TLR2 and TLR4 on airway epithelium – C-Type Lectin Receptor binding on dendritic cells by glycosylation – Release of TNF-α
3.	Blo t 3 Der f 3 Der p 3 Der s 3 Eur m 3 Gly d 3 Lep d 3 Sar s 3 Tyr p 3	Trypsin-like serine protease	25 kDa	16% to 100% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Dermatophagoides siboney* *Euroglyphus maynei* *Glycyphagus domesticus* *Lepidoglyphus destructor* *Sarcoptes scabiei* *Tyrophagus putrescentiae*	– Production of chemokines, cytokines and growth factors – Production and promotion of pro-T_H^2 polarization – Inflammatory cell recruitment – Airway remodeling – Disruption of epithelial junctions to increase permeability – Degranulation of mast cells and eosinophils

						– Fibroblast maturation – Proliferation of smooth muscles
4.	Blo t 4 Der f 4 Der p 4 Eur m 4	α-Amylase	56 kDa	40% to 46% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Euroglyphus maynei*	Unknown
5.	Blo t 5 Der f 5 Der p 5 Gly d 5 Lep d 5	Lipid-binding protein	15 kDa	50% to 70% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Glycyphagus domesticus* *Lepidoglyphus destructor*	– Production of chemokines, cytokines and growth factors – Production and promotion of pro-T_H^2 polarization – Inflammatory cell recruitment – Stimulation of innate immunity by hydrophobic ligand binding – Activation of Toll-Like Receptor (TLR) Signaling Pathways
6.	Blo t 6 Der f 6 Der p 6	Chymotrypsin-like serine protease	25 kDa	40% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus*	– Production of chemokines, cytokines and growth factors – Production and promotion of pro-T_H^2 polarization – Inflammatory cell recruitment – Airway remodeling – Disruption of epithelial junctions to increase permeability – Degranulation of mast cells and eosinophils – Fibroblast maturation – Proliferation of smooth muscles
7.	Blo t 7 Der f 7 Der p 7 Gly d 7 Lep d 7	Lipid-binding protein	24 kDa	50% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Glycyphagus domesticus* *Lepidoglyphus destructor*	– Production and promotion of pro-T_H^2 polarization – Activation of Toll-Like Receptor (TLR) Signaling Pathways – Stimulation of innate immunity – Structural similarity to lipopolysaccharides (LPS)-binding proteins – Acts as ligand for other bacterial ligands
8.	Blo t 8 Der p 8 Gly d 8 Lep d 8 Pso o 8 Sar s 8	Glutathione-S-transferase	26 kDa	20% to 40% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides pteronyssinus* *Glycyphagus domesticus* *Lepidoglyphus destructor* *Psoroptes ovis* *Sarcoptes scabiei*	Unknown
9.	Blo t 9 Der f 9 Der p 9	Collagenolytic-like serine protease	29 kDa	90% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus*	– Production of chemokines, cytokines and growth factors – Disruption of epithelial junctions to increase permeability – Production and promotion of pro-T_H^2 polarization – Inflammatory cell recruitment – Airway remodeling – Degranulation of mast cells and eosinophils

						– Production of chemokines, cytokines and growth factors
10.	Blo t 10 Der f 10 Der g 10 Der p 10 Gly d 10 Lep d 10 Pso o 10 Tyr p 10	Tropomyosin	35 kDa	50% to 95% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermanyssus gallinae* *Dermatophagoides pteronyssinus* *Glycyphagus domesticus* *Lepidoglyphus destructor* *Psoroptes ovis* *Tyrophagus putrescentiae*	Unknown
11.	Blo t 11 Der f 11 Der p 11 Pso o 11 Sar s 11	Paramyosin	100 kDa	80% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Psoroptes ovis* *Sarcoptes scabiei*	Unknown
12.	Blo t 12 Der p 12 Lep d 12	Chitinase	14 kDa	50% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides pteronyssinus* *Lepidoglyphus destructor*	Unknown
13.	Aca s 13 Blo t 13 Der f 13 Gly d 13 Lep d 13 Try p 13	Lipocalin Fatty acid binding protein	15 kDa	10% to 20% IgE binding frequency	*Acarus siro* *Blomia tropicalis* *Dermatophagoides farinae* *Glycyphagus domesticus* *Lepidoglyphus destructor* *Tyrophagus putrescentiae*	– Activation of Toll-Like Receptor (TLR) Signaling Pathways – TLR and T_H^2 cells polarization
14.	Blo t 14 Der f 14 Der p 14 Eur m 14 Pso o 14 Sar s 14	Vitellogenin/ apolipophorin-like	177 kDa	90% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus* *Euroglyphus maynei* *Psoroptes ovis* *Sarcoptes scabiei*	– Activation of Toll-Like Receptor (TLR) Signaling Pathways – TLR and T_H^2 cells polarization – IL-4 and IL-13 production
15.	Der f 15 Der p 15	Chitinase	63 kDa	70% IgE binding frequency	*Dermatophagoides farinae* *Dermatophagoides pteronyssinus*	– Mostly unknown – T_H^2 cells polarization
16.	Der f 16	Gelsolin	55 kDa	50% IgE binding frequency	*Dermatophagoides farinae*	Unknown
17.	Der f 17	Calcium binding EF protein	30 kDa	35% IgE binding frequency	*Dermatophagoides farinae*	Unknown
18.	Blo t 19 Der f 18 Der p 18	Chitinase-like protein	60 kDa	55% IgE binding frequency	*Blomia tropicalis* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus*	– Mostly unknown – T_H^2 cells polarization
19.	Blo t 19	Antimicrobial peptide	7 kDa	10% IgE binding frequency	*Blomia tropicalis*	Unknown
20.	Der p 20	Arginine kinase	20 kDa	-	*Dermatophagoides pteronyssinus*	Unknown
21.	Blo t 21 Der p 21	Lipid-binding protein	-	-	*Dermatophagoides pteronyssinus*	– Mostly unknown – T_H^2 cells polarization
22.	Der f 22	Lipid-binding protein	-	-	*Dermatophagoides farinae*	Unknown
23.	Der p 23	Chitin-binding protein	14 kDa	-	*Dermatophagoides pteronyssinus*	Unknown
24.	Der f 24	Troponin C	18 kDa	-	*Dermatophagoides farinae*	Unknown
25.	-	α-tubulin	51 kDa	-	*Lepidoglyphus destructor* *Tyrophagus putrescentiae*	Unknown
26.	-	Heat shock protein 70	70 kDa	-	*Blomia tropicalis* *Dermatophagoides farinae*	Unknown

*Allergens nomenclature is registered with International Union of Immunological Societies (www.allergen.org)

Typical symptoms due to HDM allergy include coughing, sneezing, nasal or oral itchiness, shortness of breath, allergic conjunctivitis, rhinorrhea and lethargy. HDM mediated asthma may result in tightness or discomfort in chest, wheezing and dyspnea (Huss et al. 2001; Bourdin et al. 2009; Shafique et al. 2018). Cross reactivity due to HDM allergen sensitivity may also result in development of allergic sensitization to mollusks and shellfish.

Prevention and Management

Prevention is the most effective intervention against HDM allergy. Household bedding, mattresses, pillow etc. may be encased in plastic to prevent penetration. Infested items, heavy fabric drapes and carpeting should either be removed, periodically replaced or washed weekly in hot water (55-60°C). Vacuum cleaning should be the preferred method of cleaning over dry dusting.

Non-Biting Flies

Flies are a common environmental occurrence. They are not only responsible for communicating parasites and infectious diseases as vectors for various viral, bacterial, protozoan, and fungal pathogens (Khamesipour et al. 2018) but are also implicated in aeroallergen induced allergy (Sgambato et al. 1987). Urticaria, rhinitis, erythema, conjunctivitis and respiratory difficulties have been associated with exposure to flies and their maggots (Kino et al. 1987; Martinez et al. 1997). Occupational allergy due to flies is affected by several factors, such as availability of shelter, water and nutritional sources (Tee et al. 1985; Kraut et al. 1994). Some common biting and non-biting flies implicated in allergic reactions are shown in Figure 6.

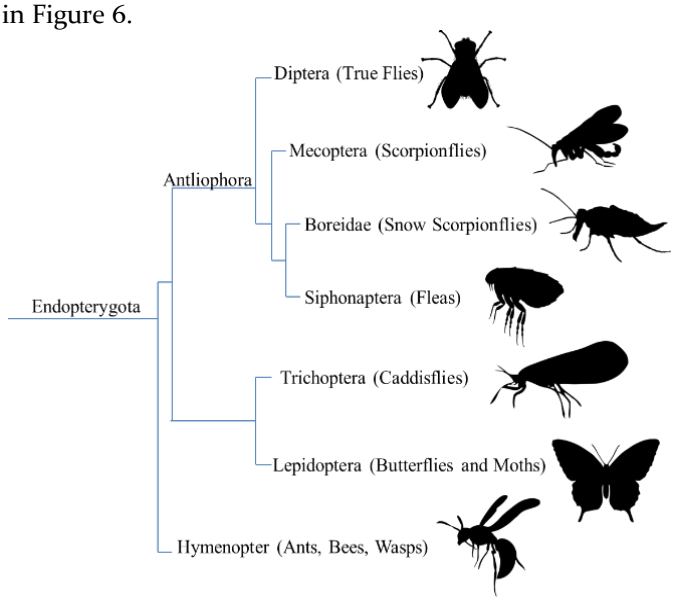

Fig. 6: Cladogram of biting and non-biting flies.

Common Housefly (*Musca domestica*)

Houseflies belong to the order Diptera, and are known as true flies. Although the common housefly (*Musca domestica*) is recognized as a cause of nasal allergy for many years (Jamieson 1938; Tee et al. 1985), relatively fewer incidences are reported as case studies. Housefly emanated particles (wings and body sheddings, follicles, excreta) and body secretions (saliva) are potent IgE-mediated aeroallergens responsible for asthma exacerbations, especially in children (Lierl et al. 1994). Previous studies have confirmed high-affinity IgE cross reactions between the sera of sensitized individuals against protein molecules from housefly extracts (Baldo and Panzani 1988; Martinez et al. 1997). Occupational allergy to houseflies has been reported in infested barn workers (Wahl and Fraedrich 1997), farmers (Focke et al. 2003) and pharmaceutical industry workers engaged in fly breeding (Tee et al. 1985; Tas et al. 2007).

Bluebottle Fly (*Calliphora vomitoria*)

The bluebottle fly (*Calliphora vomitoria*) is twice the size of a common housefly (Whitworth 2006). It is named so because of characteristic black and bright metallic blue abdominal markings. Wings of this fly are transparent, body and legs are covered with dark hair-like bristles, while antennae are short and club-shaped (Chinery and Legrand 2012). *Calliphora* maggots are used in live bait fishing. Bluebottle fly allergy is mostly reported by fishing bait breeders (Pazzaglia et al. 2003), occupational and recreational fishermen (Félix-Toledo et al. 2005). Symptoms typically appear within an hour of exposure, resulting in itchiness of the hands which extends to face and neck, accompanied by urticaria and rhinoconjunctivitis. Respiratory difficulties, such as congestion in chest and wheeze, appear after 6-8 hours and progressively worsen with time (Stockley et al. 1982; Siracusa et al. 2003). IgE-immunoblotting has revealed protein bands of 14, 28, 40, 46, 73 and kDa weights (Tideman and Elberink 2009; Porcel Carreño et al. 2013). *Calliphora* allergy prevalence has not been extensively investigated.

Caddis Fly (*Rhyacophilidae Stephens*)

Rhyacophilidae are free and parasitic insects. Adult flies appear like moths, since they are closely related to the Lepidoptera. They have bristly and hairy membranous wings. Larvae use silk to encase themselves as pupae (Wiggins 2004; Ali et al. 2020). Investigation of caddis fly extracts has revealed a spectrum of allergenically active low molecular weight proteins (Shulman et al. 1962; Rapp et al. 1962). Post exposure symptoms include epiphora, rhinorrhea, cough, wheeze, and shortness of breath. Incidences of hypersensitivity increase during summer season when new flies are hatched. Power plant managing workforce has been extensively studied in relation to occupational allergy to caddis flies. Caddis flies are attracted to station lights, water, nutritional sources and shelter due to human inhabitation. Adult caddisflies are attracted to both UV and visible spectrums (Kimura and Kuranishi 2020). These flies are sucked into and pulverised by the power turbines and dispersed as aeroallergens (Kraut et al. 1994; Miedinger et al. 2010; McNulty and Divekar 2017). A recent study has reported that professional cleaning workers exhibit airway inflammation and hypersensativity to caddis fly allergens (Lima et al. 2017). Skin prick tests (SPTs) invoked 60% positive results against laboratory prepared caddis fly antigen (LCFA) collected from power plant sites and 39% positive results from commercial caddis fly antigen (CCFA) (Kraut et al. 1994).

Roaches

Cockroaches are primitive but common Neopteran insects, having global distribution. Cockroaches don't have any specialized adaptations, apart from chewing mouthparts called mandibles, allowing them to feed on a great variety of nutritional sources alongside human foods such as starches (paper, leather, glue), fiber (clothing), organic debris (shedding, hair follicles, skin flakes) and other dead insects (Bell 1982). Of the total 4,600 cockroach species described so far, only 50 are considered pestilence

Table 6: Cockroach allergens and their biological roles (IUIS Allergen Nomenclature)

	Identified Allergens	Molecular Category	Molecular Weight	Species	IgE Prevalence	Biological Role
1.	Per a 1	Nitrile-Specifier	45 kDa	*Periplaneta americana*	9-100%	Microvilli-Like Protein with unknown function
	Per a 2	Aspartic Protease-Like	42 kDa		81%	Inactive
	Per a 3	Arylphorins	72 kDa		26-95%	Arthropod Hemocyanins
	Per a 5	Glutathione S-Transferase	23 kDa		25%	Protective enzyme against oxidative damage and insecticides
	Per a 6	Troponin C	36 kDa		14%	Calcium (Ca^{2+}) binding
	Per a 7	Tropomyosin	33 kDa		13-54%	Reduction of IL-12 and TLR9 expression in mastocytoma cells
	Per a 9	Arginine Kinase	43 kDa		80-100%	Unknown
	Per a 10	Serine Protease	28 kDa		82%	Unknown
	Per a 11	Alpha-Amylase	55 kDa		83%	Unknown
	Per a 12	Chitinase	45 kDa		64%	Unknown
	Per a 13	Glyceraldehyde-3-Phosphate Dehydrogenase	17 kDa		*N.R.	Unknown
2.	Bla g 1	Nitrile Specifier	46 kDa	*Blattella germanica*	20-40%	Microvilli-like protein with unknown function
	Bla g 2	Aspartic Protease	36 kDa		40-70%	Binding protein
	Bla g 3	Hemocyanin, Arylphorins	78.9 kDa		*N.R.	Arthropod Hemocyanins
	Bla g 4	Calycin, Lipocalin	21 kDa		17-40%	Unknown function
	Bla g 5	Glutathione S-Transferase	23 kDa		35-68%	Protective enzyme against oxidative damage and insecticides
	Bla g 6	Troponin C	21 kDa		14%	Calcium (Ca^{2+}) binding
	Bla g 7	Tropomyosin	33 kDa		18%	Unknown
	Bla g 8	Light Chain Myosin	21 kDa		*N.R.	Unknown
	Bla g 9	Arginine Kinase	40 kDa		*N.R.	Unknown
	Bla g 11	Alpha-Amylase	57 kDa		*N.R.	Unknown
	Bla g 12	Chitinase	58 kDa		*N.R.	Unknown

*Not reported.

associated with human dwellings (Roth and Willis 1952; Cornwell 1968). Five of the most frequently occurring cockroach pests are the American cockroach (*Periplaneta americana*), German cockroach (*Blattella germanica*), Asian cockroach (*Blattella asahinai*), Oriental cockroach (*Blatta orientalis*) and Turkestan cockroach (*Blatta lateralis*) (Helm et al. 1990; Kang et al. 1996; Memona et al. 2017). Cockroaches are not only vectors of infectious diseases and parasites (Koehler et al. 1990), but allergens derived from cockroach secretions, saliva, excreta, exoskeletons, egg casings and dead bodies are source of powerful aeroallergens implicated in allergic reactions (Lehrer et al. 1991; Arruda and Chapman 2001). Allergic rhinitis, eczema and asthma are amongst the most prevalent chronic disorders in the world, especially in children. An alarming increase in disease incidence and economic burden has been observed in the past few decades (Beasley et al. 2000; Fineman 2002). Genetic variability of a population is not as rapid as environmental variations, which makes aeroallergens largely responsible for allergic diseases (Sears et al. 1989; Sporik et al. 1990).

Particles bearing cockroach allergens are heavy and settle rapidly, becoming air-borne only when disturbed (de Blay et al. 1997). Various investigative studies have detected clinically significant cockroach allergen concentrations in settled dust in kitchen surfaces, lounges, libraries, gymnasiums, cafeterias, hallways, offices, mattresses and floors (Sarpong et al. 1997). Some closely related cockroach species cause cross reactivity due to their similarities. Per a 1 (*Periplaneta americana*) and Bla g 1 (*Blattella germanica*) allergens show cross-reactivity with female *Anopheles gambiae* mosquitoes, with 30% homology (Melen et al. 1999). Other proteins from these two species show moderate homology with other allergenic arthropods, including glutathioneS-transferase of dustmites and other insects, and tropomyosins of shellfish and dust mites (Reese et al. 1999). List of 3 most prevalent cockroach species is given in Table 6.

Prevalence and Epidemiology

Cockroach allergy has been attributed to both genetic and environmental factors (Sohn and Kim 2012). Socioeconomic factors and population density are also important factors (Alp et al. 2001; Leaderer et al. 2002; Mendy et al. 2020). Earliest clinical investigations have implicated cockroach allergen sensitization in 40% asthmatics (Bernton and Brown 1964; Kang et al. 1979). A study in Korea found cockroach infestations in 62% of investigated homes in Seoul. Four species were discovered, *Blattella germanica* (36.2%), *Periplaneta americana* (33.3%), Periplaneta japonica (1.1%) and *Periplaneta Fulginosa* (1.7%) (Lee 1998). Immunological investigations against cockroach extracts in Korean population have revealed 18.7% SPT positivity, elevated IgE levels and 37.5% bronchial constriction (Lee et al. 1993).

Mounting evidence has suggested that cockroach allergen sensitized patients are exposed to cockroach allergens in their own homes (Call et al. 1992; Gelber et al. 1993; Chapman et al. 1996). Cockroach infestations are a major problem in inner city areas and schools, where allergen

levels are clinically significant (Call et al. 1992; Sarpong et al. 1997; Wang et al. 2009). In United States, 36.8% children living in inner city areas were allergic to cockroaches (Rosenstreich et al. 1997). Development of wheeze in one year old children in metropolitan Boston was significantly associated with exposure to cockroach allergens during the first 3 months of their life (Gold et al. 1999; Donohue et al. 2008). Although suburban houses have relatively lower allergens, however 30% suburban and middle-class homes showed detectable levels of cockroach allergens (Gaffin and Phipatanakul 2009). Improved garbage disposal systems and frequent use of pesticides may have contributed to relatively decreased infestation rates (Lee et al. 1993). Cockroach extermination, along with frequent and through cleaning in homes, schools and restaurants, can reduce allergens to clinically insignificant or even undetectable levels (Liccardi et al. 2000; Eggleston and Arruda 2001).

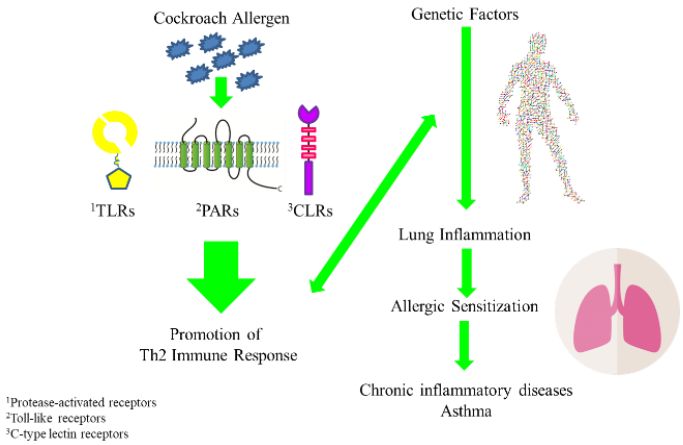

Fig. 7: Mechanism illustrating cockroach allergen-induced allergic sensitization (Gao 2012).

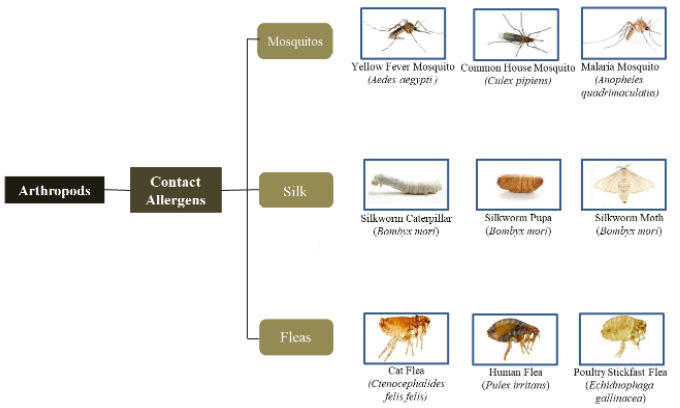

Fig. 8: Common arthropods species implicated in contact allergy.

Cockroach allergy has been reported as one of the most frequent environmental allergens in Pakistan (Abbas et al. 2015). Memona et al. (2017) conducted a collection-based survey and reported that *B. germanica* had the highest diversity indices and is the most dominant indoor cockroach species in Lahore, Pakistan. Furthermore, a survey-based study carried out in Southern Punjab, Pakistan revealed that about 80% people, who participated in the survey, were unaware of diseases transmitted by cockroaches (Naeem et al. 2014). A population-based study in Karachi, Pakistan concluded that out of 27 allergens tested on 88 individuals including children and adults, 33% exhibited moderate rates of reactivity to cockroach allergens, with 4.5% showed high reactivity rates (Abbas et al. 2015).

Clinical Manifestations

Cockroach allergens increase cellular penetration by disturbing the airway epithelial integrity, which not only leads to increased sensitization to cockroach allergen but also activates the innate immunity's cellular components such as dendritic cells, promoting Th2 response ultimately culminating in lung inflammation (Figure 7). Cockroach allergen sensitization is overwhelmingly associated with allergic respiratory distress, as well as development of asthma and asthma-like symptoms. Exposure to higher levels of cockroach allergens has greater asthma related hospitalizations and morbidities in cockroach antigen sensitized patients, especially in children. Studies show that a quarter of asthmatic children are sensitized to cockroach allergens (Rosenstreich et al. 1997; Stelmach et al. 2002.). Cockroach allergens responsible for asthma exacerbations are usually found in detectable levels in the house.

Cockroach allergen is a strong risk factor associated with severity and frequency of childhood allergies and asthma, especially in inner city residents, where higher cockroach infestations are recorded. Rates of emergency room visits, hospitalizations and days of school or work days missed are also higher in inner city residents, mostly due to cockroach allergen induced IgE levels (Gao 2012; Fukutomi and Kawakami 2021). Certain clinical markers, such as wheeze, inflammation and IgE monitoring, are potent indicators of sensitization to cockroach allergen and surrogate measure of the amount of exposure. The probability of cockroach allergen sensitization increases with increased exposure to the allergen. Sanitization often acts as a gateway to development of asthma and inflammatory respiratory diseases.

Prevention and Management

Exposure to cockroach allergen is of public health concern. Sufficient data suggests its association in the development of chronic respiratory diseases, such as asthma (Bourdin et al. 2009; Wang et al. 2021). Experts recommend eradication of cockroaches to reduce environmental allergens. A combination of pest management through pesticides, traps and general cleanliness, combined with patient and family education, are the effective approaches. Reservoir cleansing may be achieved through cleaning by using vacuum cleaners, washing of carpets, rugs and drapes with hot water and detergents. Professional cleaning and installation of HEPA filters can also reduce cockroach infestations.

Immunotherapy coupled with preventive measures can result in beneficial treatment for the sensitized patients. Several FDA standardized extracts are commercially available for immunotherapy; however their efficacy, besides in limited clinical trials, is debatable (Portnoy et al. 2013). There is no effective dosage and no standard symptom-based medications have so far been developed. However, placebo studies in India have shown significant clinical improvement after one year of immunotherapy (Srivastava et al. 2011).

Contact Allergens

Contact mediated irritation or allergic reaction to certain arthropods has been recorded as early as ancient Rome (Burgess 1993). Incidences of silk worm caterpillar allergy have existed as long as sericulture itself. Acute or chronic exposure to larvae, caterpillar or moth hairs cause irritation, usually followed by inflammation. The irritants are characterized either as histamines or soluble proteins which trigger histamine. Direct contact with larvae and caterpillar hair can cause permanent damage, such as when shed hair are blown due to wind and lodged in eyes. Arthropod contact allergens have seasonal density proportional to moth populations.

Occupational allergies associated with arthropods are well documented (Fukutomi and Kawakami 2021). Workers in arthropod breeding facilities, such as in laboratories, fly fishing farms and sericulture industry, routinely report contact allergies. Typical symptoms exhibited are rhiniconjunctival reactions, urticaria and asthma. Clinical symptoms develop rapidly in atopic individuals in comparison to non-atopic individuals. Specific IgE levels correspond to the levels of exposure to allergens. In most cases, patients are unaware of their allergies (Burgess 1993; Naeem et al. 2014).

Mosquitoes

Dermal inflammatory reactions to mosquito bites are common, especially in tropical and sub-tropical regions. However, anaphylactic reactions to mosquito bites are very rare (Larry 2002). A total of 19 proteins found in mosquito salivary gland extracts induce IgE mediated allergenic responses (Boorman 1987). Mosquito shedding, including wings, hair and feces, may become aerosolized and cause allergic reactions if inhaled. This is especially evident in occupational hazard studies, where workers are exposed to mosquitos such as when rearing mosquitos or when working in rice fields (Fukutomi and Kawakami 2021). Allergic reactions to inhaled mosquito allergens are typical hypersensitivity-I reactions, culminating in sneezing, dizziness, shortness of breath or even in some severe cases anaphylaxis (McCormack et al. 1995).

Positive skin prick tests using *Aedes* sp., *Culex* sp. and *Anopheles* sp. along with elevated specific IgE levels suggest sensitivity to these commonly found mosquito species. Immunotherapy has proved effective, however, it is not widely used and its effectiveness has not yet been clinically determined. Preventive measures include mosquito eradication or use of repellents. Evidence of natural desensitization due to repeated bites has also been recorded (McKiel and West 1961).

Silk Proteins

Allergic reaction to silkworm caterpillar (*Bombyx mori*) has been reported and many of its metabolites are now recognized as allergens capable of inducing severe hypersensitivity reactions (Suzuki et al. 1995). A recent study analyzed silkworm feces larva, pupa, moth and silk for potential allergens and identified 45 allergens. Furthermore, homology comparison analysis suggested cross-reactivity with several other arthropod allergens, including *Aedes aegypti*, *Dermatophagoides farinae*, *Malassezia furfur*, *Triticum aestivum* and *Tyrophagus putrescentiae* (He et al. 2021). Mounting evidence suggests that components of the silkworms cocoon and even silk pose allergic threat to sanitized populations.

Fleas

Fleas are quite common in occurrence, and infest humans and domestic animals. They are disease vectors and are responsible for transmission of pathogens and allergens (Souza 1997). Flea bites may induce hypersensitivity responses characterized as Flea Allergic Dermatitis (FAD), due to allergens present in the salivary glands (Halliwell 1984; Esch et al. 2001). Adult fleas are permanent ecto-parasites. This ensures continued longevity, a constant source of nutrition, reproductive opportunities and large egg production. Common fleas are shown in Figure 8.

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BIOLOGY AND ECOLOGY OF TICKS OF MEDICAL AND VETERINARY IMPORTANCE

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INTRODUCTION

The economy of Pakistan is agriculture based and the livestock sector is one of the major subsectors of agriculture. According to the Pakistan Bureau of Statistics, the growth rate of the livestock sector in Pakistan was 2.58% during the last fiscal year 2020, which contributed 60.6% to the agriculture sector and 11.7% to the national GDP of the country. The gross value addition of livestock showed an increase of 2.5%, as it increased from 1430 billion rupees in 2018-19 to 1466 billion rupees in 2019-20. The estimated population of different livestock species in Pakistan is as buffalo 41.2 million, cattle 49.6 million, sheep 31.2 million, goats 78.2 million, camels 1.1 million, horses 0.4 million, asses 5.5 million and mules 0.2 million (Zia *et al.* 2011). The survival of most of the livestock farmers in rural areas of the whole world depends on animal raising for their livelihood. They earn money by selling milk, meat, hides, and live animals. As the population of the developing world is increasing, the demand for animals and their products, especially food, is also increasing. Productivity of these animals, which ultimately ensures food security, is at risk by the infestation of various ectoparasites.

Ticks, flies, fleas, mites, and midges are important ectoparasites, out of which ticks are the most significant, because they cause a decrease in production by sucking blood (Elhaig *et al.* 2016; Wen *et al.* 2016). Ticks also indirectly affect animal production by transmitting various diseases (bacterial, viral, and protozoal) and toxins, and by causing tick paralysis (Rajput et al. 2005; Durrani et al. 2008; Zulfiqar et al. 2012). In this scenario, ticks and tick-borne diseases (TTBDs) are a huge threat to animal productivity, which ultimately threatens food security (Sibhatu et al. 2015). The ticks are included in the order Acarina and sub-order Ixodides, and are characterized by the presence of chelicerae and pedipalps on their mouthparts, while mandible and antennae are absent (Cheng 1964). Ticks are obligate blood sucking parasites that infest a wide range of hosts, including humans, with a worldwide distribution (Domingos et al. 2013; Hassan et al. 2013). They are the most important ectoparasites, affecting almost 800 million cattle and sheep worldwide (Sutherst and Wilson 1986). Almost 900 species of ticks have been described and some of them are very common in transmitting various disease causing agents. Ticks are divided into three families: Argasidae (soft ticks) with 191 species, Ixodidae (hard ticks) with 701 species, and Nutalliellidae which consists of only one species (Pfäffle et al. 2013; Guglielmone et al. 2014).

Understanding the biology and ecology of the most prevalent ticks of medical and veterinary importance will help to design effective tick control and eradication programs. This chapter describes the important biological and ecological factors, which play a vital role in the prevalence of TTBDs.

Prevalence of TTBDs in Pakistan

In the livestock industry, almost 80% of the cattle population is infested with TTBDs worldwide (Ghosh et al. 2006), including Pakistan. Direct losses caused by ticks are the damage of skin by wound opening that leads to paralysis and secondary bacterial infections. Indirect effects of TTBDs are even greater than the direct losses because of their ability to act as vectors of various diseases, such as Babesiosis and Theileriosis (Jongejan and Uilenberg 2004; Schroder and Reilly 2013). Ticks have a unique ability that they can survive for three years without food, however, they are not in a position of oviposition during this time because they need to be engorged for this phenomenon. Ticks affect their hosts in many ways, such as reduction in growth rate and milk production, damage to hides and transmission of disease causing organisms that can cause paralysis and injuries. The underlying tissues and the host's integument are severely damaged by tick bites.

To date, many studies have shown that more than 80% of buffaloes and cattle in Pakistan are exposed to two major species of ticks (Sajid et al. 2008; Iqbal et al. 2013; Farooqi et al. 2017; Rehman et al. 2017) i.e. *Rhipicephalus* (*R.*) and *Hyalomma* (Hy.), which transmit *Theileria, Babesia* and *Anaplasma* species to buffaloes and cattle (Jabbar et al. 2015; Karim et al. 2017). Many studies have found that *Hy. anatolicum* is the most abundant tick species in Pakistan (Sajid et al. 2009; Karim et al. 2017; Rehman et al. 2017), followed by *R. microplus* (Ghafar et al. 2020). Karim et al. (2017) reported 17 hard tick species from Pakistan, which include *Hy. anatolicum*, *Hy. bispinosa*, *Hy. isaaci*, *Hy. hussaini*, *Hy. dromedarii*, *Hy. turanicum*, *Hy. kumara*, *Hy. scupense*, *Haemophysalis* (*Ha.*) *cornupunctata*, *Ha. kashmerensis*, *Ha. sulcata*, *Ha. montgomeryi*, *R. microplus*,

R. sanguineus, *R. annulatus, R. haemaphysaloides* and *R. turanicus*. The same study reported the highest diversity of ticks in Azad Kashmir and Baluchistan, most probably due to nomadic lifestyle of the people in these regions (Karim et al. 2017). Ticks are equally important in the case of equines, because they cause blood loss and transmit various diseases, such as Equine Granulocytic Anaplasmosis, Piroplasmosis and Equine Lyme Borreliosis (Sigg et al. 2010). Hemolytic conditions can be observed in horses, donkeys, mules, and zebras due to equine tick-borne hemoparasitic diseases (Traub-Dargatz et al. 2010).

It has been reported in many studies that ticks are the most prevalent during the summer months of June, July and August (Kosar 1965; Khan 1967; Durrani 1992; Sajid et al. 2009) and comparatively absent during the winter months of December, January, and February (Sajid et al. 2009). The summer temperature of Pakistan, specifically Punjab Province, remains between 38-48°C, which is ideal for the reproduction, development and growth of ticks (Sajid et al. 2009). This monthly variation is also justified by the questing activity of ticks. Lower temperatures and higher altitudes delay the activity of ticks and vice versa (Jouda et al. 2004). The males have a higher prevalence of ticks than females (Sajid et al. 2009) and the possible reason behind this could be that the males receive less attention from the farmers because of their interest in the dairy animals. European breeds (Jersey, Friesen and their crosses) are relatively more affected ticks. Their denser and longer hairs are considered to be the factors for greater tick infestation (Verissimo et al. 2002; Sajid et al. 2009). Predilection sites for the larvae of *Boophilus microplus* include dewlap, axillae, flank and escutcheon (Reik 1962), while for *Hyalomma* these sites are lips of vulva, inside of thighs, ears and neck (Mattioli et al. 1997). Cattle are found to be more prone to the tick infestation than buffaloes due to swamp habitat and thicker skin of buffaloes as compared to dry habitat and thinner skin of cattle (Sajid et al. 2009).

Morphological Features

The ticks have a segmented body but these are not clearly visible. They possess two body parts namely; the capitulum or gnathostoma and the idiosoma. The capitulum is comprised of basis capituli, which is heavily sclerotized, bearing mouthparts (Gregson 1960). The mouthparts are located on gnathostoma, and comprise three different types of structures. First is a toothed and elongated hypostome, which is located on the ventral side of the mouth. The hypostome projects anteriorly from its free end. The second part is a pair of chelicerea, present on each dorsolateral surface of the hypostome. The basis capitulum is connected to a sheath that is encasing each chelicera. Each chelicera contains teeth on its outer surface. The third part is a pair of pedipalps or palpi, which arise from the basis capitulum at the anteroventral margins. The ticks of family Ixodidae have rigid palps and they are associated with the hypostome, however, the Argasidae have flexible pedipalps associated with the hypostome. Family Argasidae ticks possess a leathery integument that covers the whole dorsal surface. This cuticle contains ornamentations of granules, tubercles and even circular discs in some cases. Three pairs of legs are present in all the tick larvae; however, a fourth pair is found after the molting of the larval stage in the nymph and adult tick stages (Cheng 1964).

The adult females possess a scutum, which is a rigid area and a genital pore is ventrally situated between the third or fourth pair of legs. Nymphal stages can be differentiated from adult females because they possess a size equal to half of the adult females. Additionally, scutum is present but genital pore is lacking. If it is confirmed that the specimen is adult, the next part for examination is the position of a cuticular fold near the anus, called the anal groove. The anal groove may be present in front of or behind the anus. Palps are sensory in their function and are supported by the basis capituli. The shape, length and width of these palps is also used to differentiate between different genera of ticks. The term 'decorated' (ornate ticks) is used for ticks when their scutum appears to be metallic, patterned or brightly colored. These features are useful in the identification of different genera of ticks and then their further evaluation at the species level (McGarry 2011).

Life cycle and Behavior of Ticks

All the ticks are obligate parasites of vertebrate animals, and are characterized by a complex life cycle. The life cycle of ticks includes hatching of eggs and development into larvae, then after feeding, the larvae drop on the ground and molt into nymphs. The nymphs need to find a suitable host for further feeding and then again return to the ground for further molt, resulting in an adult. The adult will also feed and mate, while the females will lay a huge clutch of eggs, which are left in protected sites like decaying vegetation, where survival is ensured by the high relative humidity (Kahl et al. 2002). Ticks take a blood meal during every active stage and ingest a sufficient quantity of blood, which is necessary for oviposition and molting. The mating phenomenon takes place on the host in most of the tick species, however, some species mate in vegetation during questing for a host. Most of the ticks have a three-host life cycle, but some species possess two hosts, where feeding of larvae and nymphs occurs on the same host and molting does not occur at the ground. Some ticks of family Ixodidae have a single host life cycle, where each step of feeding, molting and mating occurs on the same host (Estrada-Pena and de la Fuente 2014).

The search for a host by the active stages of ticks is known as questing or host-seeking behavior (Estrada-Pena et al. 2013b), which is an important process in the life cycle of a tick that also affects the pathogen transmission ability of ticks. Before starting to quest, most of the ticks are found in the lower layers of vegetation in an inactive form, and this process is triggered by a unique combination of photoperiod and climate, influenced by the light and darkness hours in a day (Belozerov 1982). Photoperiod acts on the molting stages of ticks, causing activation or delay in the molting until the availability of favorable

conditions, while in the case of questing stages, the onset of activity can be initiated or delayed. Although the role of photoperiod has been ignored during the investigation of tick seasonal activities, this single factor has a strong impact on the epidemiology and transmission of viral diseases (Estrada-Pena et al. 2013a). Ticks have an interesting phenomenon that they detect their host by sensing carbon dioxide and if this phenomenon exists in various tick species, it is an indication that the capability of host detection was present in the ancestral tick lineage (Mans 2014).

During questing and development, ticks need a relative humidity of more than 80% or longer periods because they are exposed to desiccation during these stages (Gray 1998). Questing of ticks can be continued for several months but they have to refill their water reserves by frequent visits down to the base from surface vegetation. Water is obtained from sub-saturated air, where they secrete a hygroscopic fluid from salivary glands on the external mouthparts and then the water-enriched fluid is re-ingested (Kahl and Knülle 1988). The time taken by the tick during the questing phase depends upon some factors including micro-climatic conditions, host-seeking success and the energy reserves available for replenishment of water. The length of the questing period for individual ticks can vary from several weeks to months in a conducive environment and is stage dependent because adults quest for the longest periods, while larvae quest for the shortest. Different factors like relative humidity, temperature, sunshine and rain are important for questing behavior and vary in different habitats, affecting energy depletion and water balance, and ultimately affect the survival of ticks (Gray et al. 2016).

Seasonal climate change and regional events of weather are the consequences that restrict questing in a defined period in temperate regions. The contact rates between ticks and their hosts are controlled by specific principles: the frequency of contacts is directly related to the abundance of hosts and ticks, ultimately questing of ticks will occur in a lesser time and; the higher host density will also help to reduce tick mortality because of the availability of abundant suitable hosts.

Physiology of Ticks

Off-host osmoregulation

This is a matter of debate that how do the hard ticks survive for several months when they are off-host? Family Ixodidae share a common feature of integument with insects that is impermeable to water. Another common property between ticks and insects is that they can absorb water vapors from the environment when the following two conditions are fulfilled: (i) there should be a certain degree of dehydration and (ii) the level of relative humidity should be specific in the atmosphere. The value of critical equilibrium humidity (CEH) should be in a range of 80-95% and it is species specific. However, in some insects, the level of CEH can be lowered up to 50% (Edney 1977). This value ranges from 75-95% in ticks, which is dependent on the developmental stage and tick species (Needham and Teel 1986; Gaede and Knulle 1997; Yoder et al. 2006). Ticks have to utilize the metabolic energy for taking up the water vapors from the atmosphere if the level of relative humidity is less than 100% (Edney 1977). The uptake of water vapors is best observed in unfed ticks; however, this phenomenon can also be studied in the larvae and nymphs of fully engorged ticks when they are absorbing water vapors from the unsaturated atmosphere.

For water uptake, a major role is played by the salivary glands and this process occurs through the oral cavity (Rudolph and Knulle 1974). If the atmosphere has an increased level of CEH, a tiny droplet of saliva is secreted by the tick on mouthparts. As the saliva is hygroscopic, atmospheric water vapor is condensed on it. This enlarged droplet is now swallowed by the tick, a fresh aliquot is secreted and this process is repeated until the tick attains its normal hydration level. This is called rehydration saliva and is secreted by the type-I acini of salivary glands. These acini help in the processes of active transport system because they have the ultra-structural properties of tissue functioning (Fawcett et al. 1986). Some organic solutes which are still unknown, are assumed to be responsible for the hygroscopic nature of saliva (Knulle and Rudolph 1982; Needham and Teel 1986).

On-host Osmoregulation

Blood sucking insects maintain the osmotic equilibrium by using Malpighian tubules for excreting excess fluid from the blood meal (Maddrell 1981). Like insects, the ticks of families Ixodidae and Argasidae also use Malpighian tubules for excretion of nitrogen, these two major tick families have developed a unique system to deal with the excess fluid of blood meal. For this purpose, salivary glands are used by the hard ticks and coxal organs by the soft ticks. In the case of hard ticks, salivary glands are only used by the female ticks. Males take only a small amount of blood meal, thus the osmotic stress is not significant in them and up till now, no one has explored osmoregulation in feeding males. Female salivary glands develop ultra-structural properties during feeding, however, this feature is absent in males (Kaufman 2010).

Blood Feeding

Hematophagy (blood feeding) is a vital physiological process for ticks to maintain their nutrient requirements for reproduction and development. Ticks have a unique blood feeding process compared to other hematophagous arthropods. Hard ticks feed blood once in their lifetime and females lay down a large number of eggs and die after several days of egg laying (Coons and Alberti 1999). Feeding of ticks has been divided into three stages: attachment, slow feeding and feeding (Kemp et al. 1982; Anderson and Magnarelli 2008). The attachment phase further includes multiple steps including identifying a vertebrate host, penetration deep into the dermis through barbed mouthparts, a secure attachment through the

hypostome encasement, and provision of a suitable environment during a blood meal to transmit the pathogens (Alekseev et al. 1995; Anderson and Magnarelli 2008).

Feeding Patterns of Families Ixodidae and Argasidae

Ticks ingest the blood and tissue fluid by anchoring through a mouthpart structure, called hypostome, which creates a lesion during feeding. Argasidae have a rapid speed of blood feeding and they feed only on tissue fluid. On the other hand, Ixodidae complete the feeding process in several days and they feed on blood, lysed tissues and lymph around the mouthparts. The best time for the transfer of pathogens is during feeding because cutaneous damage is caused to the skin during this time. Stages of blood feeding in the life cycle of ticks include small larvae, nymphs and adult females (McGarry 2011). Slow feeding of Ixodidae female ticks takes 6-9 days in the first stage, which is followed by the detachment stage that occurs 12-24 hours before detachment from the host. Only females that take part in mating can go for a rapid engorgement period, indicating an uncharacterized mechanism of physiological control (Sojka et al. 2016). The feeding duration of Ixodidae varies greatly between and within species, and ranges from 3-5 days for larvae, 4-8 days for nymphs and 5-20 days for adult females. Interestingly, males also attach to the host's skin but only feed a very small amount of blood and enlarge insignificantly. For initial 24 hours, females remain flat and then start to swell gradually with blood, leading to full engorgement after 48 hours as the rate of blood intake increases rapidly. *Hyalomma* and *Amblyomma* tick species ingest larger quantities of blood from animals up to 2ml, increasing in weight from 0.04 g to 4.0 g and size from 10 mm to 25 mm (Wall and Shearer 2001). In animals, Ixodidae have specific sites for blood feeding, however, in humans, they can be found on any part of the body (Felz and Durden 1999) and can feed un-noticed (Hatwick et al. 1978; Krinsky 1983).

Ticks have the ability to feed on the host un-noticed for extended periods due to highly adaptive mechanisms of feeding (Binnington 1978; Binnington and Stone 1981). Numerous proteins are produced by the tick salivary glands to create the stable environment for feeding through alteration of the host's immune response, haemostasis and inflammation (Francischetti et al. 2009; Mans 2011; Fontaine et al. 2011). Depending on the species, most of the adult ticks remain attached to the host for 7-21 days, where a cement cone is required for a firm attachment. Mouthpart is protected from the immune system of the host by the cement cone (Alekseev et al. 1995) and helps the ticks to enter the host's dermis. Different tick species secrete a proteinaceous matrix from cement cone, both by long mouthparts (longirostra) and short mouthparts (brevirostra) (Kemp et al. 1982). In response to the feeding of ticks, the host reacts by vasoconstriction, activation of the coagulation cascade, formation of a hemostatic plug, inflammatory responses and tissue remodeling, leading to the wound healing. This leads to the rejection of tick feeding with detrimental effects on tick reproduction and viability. However, ticks complete their successful blood meal because various biologically active molecules are present in salivary glands, displaying antiplatelet, anticoagulation, immune-modulatory, vasodilatory and anti-inflammatory activities.

Digestion of Blood Meal

The process of blood digestion is different in ticks than that of hematophagous insects where digestion of proteins takes place rapidly in the gut lumen. Digestion is an intracellular process in ticks that proceeds slowly for which the optimum pH of proteases is 3 and for gut contents, optimum pH is 6.5. Three different phases are involved in tick feeding, namely the preparatory phase, growth phase and expansion or rapid engorgement phase. These phases are involved in the digestion process, with the exception of the preparatory phase which lacks digestion. Continuous digestion starts in the growth phase, followed by the expansion phase that involves a slow digestion process. After detachment, the process of slow digestion starts again and it continues till the end of oviposition (Friedhoff 1990). The protease activity is decreased (only 10%) at the end of the rapid engorgement phase. Thus, the hostile environment is no more available in the tick gut because proteases are intracellular and cannot work at the pH of vertebrate blood that entered the tick gut. In Argasidae ticks, concentrated blood meal is stored as a food reserve, however, the process of blood digestion is similar to that of Ixodid ticks. Since the feeding process is rapid in Argasidae, the following steps are involved in the blood digestion; no digestion phase, rapid and slow digestion phase (Akov 1982; Coons et al. 1986). The process of blood protein digestion is rapid in the lumen of the gut and is assisted by alkaline serine proteases in most hematophagous insects (Briegel and Lea 1975). On the contrary, ticks have a slower process of blood digestion in gut cells inside the acidic vesicles (Grandjean and Aeschlimann 1973).

Harmful Effects of Ticks

Following are some direct and indirect methods by which ticks may harm the animal and lead to production and economical losses to the farmers.

Tick Paralysis

Although ticks cause many problems through direct effects on the host, they are also harmful indirectly by transmitting many disease causing organisms. They transfer toxic substances through their saliva while feeding steadily on the host; these result in severe clinical cases, ultimately leading to toxicosis and paralysis (Jongejan and Uilenberg 2004; Estrada-Pena and Mans 2014). Salivary secretions of ticks play an important role in the transmission of diseases because of the injection of toxic compounds, causing flaccid paralysis and affect the general metabolism (Cupp 1991). Salivary neurotoxins, which are transferred to the host during the feeding of

ticks, are considered to be responsible for paralysis. It has been suggested that toxin generation is associated with the prevention of blood coagulation, reduction in host mobility and excretion of local anesthesia during feeding (Stone et al. 1989). Ticks remain attached to their host for longer period because inflammatory and immune responses are suppressed by the components of tick saliva. These toxins in the salivary secretions are responsible for the destruction of host tissues (Bowman et al. 1997). Many cases of heavy mortalities have been recorded due to tick toxins and the best example is ascending motor paralysis due to ticks in vertebrate hosts (Estrada-Pena and Mans 2014). Some species of ticks secrete toxins that affect the nervous system of the host. Only small quantities of these toxins are secreted by larval and nymphal stages; thus paralysis is caused by adult female ticks, which secrete a sufficient amount of toxins to the host. The terminal part of the motor nerve fiber is altered by these toxins, which further leads to the failure of animal mobilization.

Paralysis usually continues during the presence of ticks because this is the phenomenon of chemical induction of toxins by the ticks. Thus, the symptoms of paralysis are diminished quickly after the removal of ticks from the host. However, in certain cases, there are chances of development of profound paralysis, which can also become fatal before the awareness about the presence of ticks. As the tick attach to their host, the symptoms of weakness may take five days to set in. Effects of toxins can be metabolized rapidly or diminished within 12-24 hours after the removal of ticks (Mondal et al. 2013). Paralysis through tick bites is characterized by the loss of sensation and muscular functions; and even in some cases, the respiration is affected that may lead to death. Thus, further investigation is required for molecular characterization and identification of toxins causing paralysis, which will help to sort out the mysterious structure of these potent toxins. Many low molecular weight effectors and proteins are present in the tick saliva to target the host defense mechanism. Tick saliva with abundant proteins offers a great opportunity to discover various pathways of effectors targeting specific physiological pathways of mammals.

Transmission of pathogens

The importance of ticks for wildlife and livestock can be realized by the fact that they pierce the skins of hosts, resulting in the transmission of many disease causing agents as vectors (Allen 1994). In this scenario, a few examples of tick-borne diseases can be quoted, which include Bovine Anaplasmosis caused by *Anaplasma marginale* (Kocan et al. 2010), *Babesia bigemina* causing the disease African red water or Bovine Babesiosis (Bock et al. 2004; Suarez and Noh 2011; Farooqi et al. 2017), *Ehrlichia rumanntium* causing heart-water (Allsopp 2010) and strains of *Theileria parva* causing East Cost Fever, Corridor Disease and January Disease (Bishop et al. 2004). The carriers of these diseases are mostly the wildlife species, with an additional importance to cause the zoonotic diseases in humans (Smith and Parker 2010), indicating their great medical importance. Salivary secretions transmit most of the pathogens, although the Argasidae can transmit vectored pathogens through coxal fluid, which is used for irrigation of feeding lesions (Burgdorfer 1951). The efficacy of transmission of a diseased pathogen by ticks to the host can be determined by the amount of secreted saliva because salivary glands are the most important route of pathogen transmission (Kaufman and Phillips 1973; Koch and Sauer 1984). After feeding on a blood meal, the pathogens reside in the gut contents with ingested blood, and showing different mechanisms to pass through the membranes of the gut. Laboratory trials have indicated that certain pathogens may find their way to the gut cells through the expression of molecules that allow recognizing the specific receptors in the cell membrane (de la Fuente et al. 2001).

Some well-known tick species transmit pathogens to humans and they have been very well studied due to their public health significance. In this case, an example of *Hyalomma* genus can be given, because it transmits the most important disease, Crimean Congo Hemorrhagic Fever (CCHF) to humans, thus researchers have focused their attention for its investigation (Hoogstraal 1979). In Pakistan, the first case of CCHF was reported in 1976 and has become endemic (Alam et al. 2013; Ansari et al. 2018). This virus is particularly important in people, who are closely related to livestock activities (Lugaj et al. 2014) and Pakistan being an agricultural country, is at risk of this vector-borne disease. As *Hyalomma* tick species are very common in livestock in Pakistan, this is a major threat for animals and humans (Rehman et al. 2017). However, some tick species transmit pathogens in cryptic cycles without their direct involvement, and ultimately pathogen transmission becomes perpetuated in the ecosystem. The active foci of pathogen transmission can be sustained in the ecosystem by these cryptic cycles. Thus, the clinical cases will not be noticed in the human population because of the absence of vector linkage between reservoir hosts and humans.

Trans-ovarial and Trans-stadial Transmission

Epidemiologically, ticks have two important events to maintain the transmitted pathogens. Ticks feed only once at each stage, the pathogen needs to persist at every stage after molting, so that it may be passed to the next vertebrate cohort by an infective bite. This course of the stage to stage transmission is called trans-stadial passage. In this phenomenon, tick feeds on a reservoir host and acquires a pathogen, thus the pathogen has to further persist in the newly molted stage of the tick. The vertical or transovarial passage is another important feature involved in the transmission of a pathogen by ticks. The tick acquires a persisted pathogen, while engorged female ticks transfer it to the cohort of eggs. This phenomenon is considered to be responsible for the transfer of many pathogens to unaffected hosts and the new generation of ticks. The rate of transfer of pathogens to the next generation of ticks is a species specific mechanism and

both phenomena of pathogen transfer are dependent upon this mechanism (Hartemink et al. 2008).

The mechanism of transmission and maintenance of pathogens by the ticks and their hosts cannot simply be determined by the field studies, because such studies only report a small sequence of nucleic acids from a big population of ticks. Complementary laboratory studies should also be adopted as an essential part to testify the hypothesis made in the field trials. The ticks should possess the following qualities to be a successful vector of pathogens, (a) feeding on infectious vertebrates, (b) pathogen acquiring during feeding on blood meal, (c) maintenance of pathogen during single or more trans-stadial molts, and (d) transmission of the pathogen to previously unaffected hosts (Kahl et al. 2002). The status of the carrier or non-carrier state of the host is determined by the feeding of ticks and transmission of the pathogen to the host. According to this definition, pathogen carrying hosts are called the carrier hosts, however, it is not necessary that the carrier hosts are infected by the ticks, thus infectivity status must be defined by the terms reservoir and non-reservoir. Non-reservoir hosts are those which are unable to transmit the pathogens to uninfected ticks (Estrada-Pena and de la Fuente 2014).

It is suggested that if the tick species responsible for pathogen transmission to wild vertebrates are present in the ecosystem, the presence of pathogens will go un-noticed until the availability of ticks that will link it to humans. Furthermore, seasonality and abundance of hosts, regional weather and variations in the abundance of ticks can change the epidemiological landscape by their interaction. Therefore, a specific mechanism of pathogen transmission in a community of ticks, hosts and pathogens cannot be implemented in another community because these are realized as regional or local processes.

Host Immune Response against Ticks

The host immunity against ticks was first described in 1918, but little was known during 1918-1973 about the bovine immunity towards ticks. However, *Bos indicus* cattle were considered to be resistant against ticks when compared with *Bos taurus* cattle. It has been described that immediately after the feeding of ticks, cellular density is increased up to 80% because of the arrival of the vasoactive amines at the feeding site. Immunity of the host against ticks has been described in many studies due to the active role of basophil derived mediators such as (i) resistant animals had twice the level of tissue histamine as compared to susceptible animals (Willadsen et al. 1979; Wikel 1982), (ii) histamine injection caused the tick withdrawal or mortality at the attachment sites along with the interruption in the feeding when histamine was added to the artificial media, and (iii) histamine receptor antagonist caused the reduced skin reactivity and resistance (Tatchell and Bennett 1969; Bagnall 1975; Brossard 1982; Wikel 1982).

Histopathologically, it has been observed that tick feeding sites possess neutrophil infiltration, which is accompanied by eosinophil response as the cutaneous cellular mechanism in naive animals. The restricted pathological lesion to the site of feeding is due to the formation of a feeding cavity that leads to hemorrhages as the feeding process advances. Perivascular cuffing is caused by neutrophils in the vessels of the lower dermis. Additionally, a little degranulation is also observed because basophils are accumulated at the border of the epidermis and dermis (Brown 1988). Microscopically, feeding sites have been observed to possess intra-epidermal vesicles. Here, leukocytes showed intense cellular response adjacent to the tick's hypostome that mainly contains basophils. Interestingly, circulating leukocytes consist of only 1% basophils because they are not the normal residents of the majority of vertebrate tissues (Schermer 1967; Bloom and Fawcett 1975), however, their number can increase up to 500% in the hosts affected by ticks (Gordon and Allen 1979; Brown and Askenase 1982). It has been suggested that a specific immunological response involving T-cells and antibodies is the reason for the accumulation of these cells in tissues (Askenase 1977).

The hemostasis phenomenon is triggered by vascular injury, accompanied by a triad of platelet aggregation, blood coagulation and vasoconstriction. The mechanism of hemostasis is started within few seconds of tissue injury. Scar formation is induced by the mechanism of tissue repair, which starts with the injury and continues for several days. Cellular and humoral immune responses are triggered by the immune system that causes modification at the site of tick feeding. Such types of immune responses are immediate, as seen in antigen/antibody/complement reactions, or may take longer periods in their occurrence similar to cellular reactions where leukocyte infiltrates are formed like basophilic infiltrates (Allen 1973). As Argasidae and Ixodidae take time from few hours to several days during feeding, various feeding strategies are involved to influence the host immune response. However, the activation of the host immune system depends upon many factors, such as health status, the genetic background of the host and species of the ticks and hosts involved. Many authors are of the view that the role of histamine in the immunity of the host against ticks is secondary because basophil derived mediators are considered to play a major role in this immune response. Furthermore, ticks have the ability to induce immunomodulation in the host immune response because of the presence of many immunomodulators in their salivary glands. Thus, there is a need of deep understanding and investigation of the tick salivary glands having biologically active molecules which they use to inactivate the host resistance against ticks.

Factors Affecting Tick Abundance and Distribution

Climate Change

In Pakistan, ticks are most prevalent during the summer months and comparatively absent during the winter months (Sajid et al. 2009). The primary factor in the prevention of tick distribution is considered to be climate

change, given that the tick distribution is not necessarily affected by its hosts. However, broad scale factors about tick distribution have yet not been established (Cumming 2002). It has been suggested that an increase in the temperature will lead to an increased tick distribution, particularly in endemic areas. Additionally, this change can cause several implications related to tick infestation and incidence, and control of tick-borne diseases. In this regard, the example of South Africa can be quoted, where increased temperature caused the displacement of *R. decoloratus* with the Asian intruder *R. microplus* (Tønnesen et al. 2004; Nyangiwe et al. 2013). This species of Ixodidae is now present in all of Africa with warm and humid climates, except for extremely cold areas. Similarly, another tick species, *Amblyomma herbraeum,* had its natural habitat of warm and moist coastal areas (Coetzer et al. 1994), but now its distribution has also been reported in the semiarid areas (Nyangiwe et al. 2011). This changed distribution of bont tick has been hypothesized due to intense drought periods in highlands areas, as happened in Zimbabwe (Estrada-Pena et al. 2008).

Tick populations are also affected by short term alterations in local weather conditions. For instance, variations are expected in the development rates of tick populations during milder temperatures in winter and autumn. Similarly, the normal temperature in the winter season, which is high enough to stimulate questing, ticks may quest for the host. These factors must be considered while predicting the tick distribution because ticks have an increasing trend towards those areas which were previously uninhabited.

Nidicolous and non-nidicolous Ticks

On the basis of mobility and site of living, ticks are divided into two types; including nidicolous (nest-dwelling) and non-nidicolous (field-dwelling). Thus, ticks with nidicolous behavior would have a stronger population structure (Anderson and Magnarelli 2008). The movement of nidicolous ticks is usually low because they occupy places having the availability of favorable habitats and food (Lane et al. 1999). On the contrary, non-nidicolous ticks have a more widespread range with different time alterations, including the time spent free in the environment and the time spent on the host during feeding. Field dwellers have the opportunity to disperse in wide ranges because they are not limited to seek sites near nests. These are the reasons which suggest that nidicolous ticks will have a stronger population structure compared to non-nidicolous. It is further suggested that the ticks in one nest will have more association than those in another nest (Araya-Anchetta et al. 2015).

Other Vital Factors

The abundance and distribution of ticks are also affected by some other vital factors, including availability of alternative hosts, grazing management, natural resistance and acaricide use (Cumming 2002). Alternative hosts gain importance in the absence or sparse availability of natural hosts, influencing the tick distribution. Alternative hosts also act as a source of the movement of ticks to different habitats and thus, increasing the tick distribution (Ruiz-Fonsa and Gilbert 2010). Tick distribution can also be affected by the prolonged use of acaricide treatments, along with the absence of alternative hosts (Norval et al. 1994). Microclimate in the lowest vegetation layers is another important factor to regulate the tick abundance (Belozerov 1982). In temperate regions, longer periods of summer coupled with an increased temperature and high desiccating power of the air undoubtedly cause an increased mortality rate in the questing or molting stages of ticks. On the other hand, extremely low temperatures during the winter season can also induce significant mortalities in ticks. However, it is a well-known fact that when snow covers the ground, it provides a protective effect for ticks overwintering in the ground during low temperatures (Childs and Paddock 2003).

In Pakistan, tick density can be reduced by the modification of habitat and pasture management with possible methods like drainage, herbal treatment, controlled burning, removal of leaf litter and clearing of bushes mechanically. Habitat alteration can also result in the change of availability of the host for ticks. However, these methods of reducing tick density can only be applicable for shorter period, and additionally, the majority of these methods are labor-intensive and require periodical repetition. Furthermore, altered habitat may lead the farm animals at risk of tick-borne diseases posed by the ticks that came from other habitats, thus, this habitat modification is not always favorable.

Ultimately, tick control strategies in Pakistan need to be revolved around alternate methods, minimizing chemical residues, preventing production losses, reducing chemical treatment and reducing the number of ticks to acceptable levels. Although various studies have been conducted on TTBDs and sufficient data has been generated since the last 45 years, still there is need to focus on systematics, functional genomics, proteomics and transcriptomics in Pakistan.

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PATHOBIOLOGY OF THE TICK-BORNE PIROPLASMOSIS

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INTRODUCTION

Tick-borne haemoparasitic protozoa, *Piroplasms* are classified into genera *Theileria* (*T.*) and *Babesia* (*B.*). Piroplasmosis is also known as the cause of Texas fever, which is an endemic disease of domestic animals in tropical, temperate, and subtropical areas of the world (Niu et al. 2012; Aydin et al. 2015; Siddique et al. 2020; Niaz et al. 2021). These parasites belong to phylum Apicomplexa, family Theileriidae and Babesiidae. They are termed as *Piroplasms* because of their pear-shaped morphology (Soulsby 1982; Smith and Wall 2013).

These parasites are different from *Plasmodium* sp. due to absence of schizogony, characteristic shape, and no association with the blood pigment (Cheng 1986; Cox 1987). *Piroplasms* are transmitted through tick bite; so, their prevalence is directly associated with the prevalence of ticks and indirectly with the environmental changes that favor the growth and propagation of ticks (Niu et al. 2012). Piroplasmids have been classified exclusively on biological and morphological grounds into 111 *Babesia* and 39 *Theileria* species (Schnittger et al. 2012).

These organisms were identified first time by Babes (1988) in cattle erythrocytes, and later in sheep RBCs, and were given the name of *Babesia* by Starcovici (1983). Later on, due to their peculiar shape, they were named *Piroplasms,* which is still in use (Uilenberg 2006). Babesiosis, an important tick-borne disease (TBD), affects the tropical and sub-tropical countries of the world. Babesiosis is caused by organisms of the genus *Babesia,* which are highly successful intracellular parasites, having specialized survival mechanism with coevolution with their hosts. So, these organisms possess a complex life cycle involving hard ticks (definitive hosts) and vertebrates (intermediate hosts) (Mehlhorn and Shein 1984). More than 100 *Babesia* species are identified, affecting a wide range of wild and domestic animals and occasionally humans. Among these species, the spectrum of pathogenicity is quite variable; some are more pathogenic, while others are less pathogenic. Identified species include *B. crassa*, *B. ovis* and *B. microti,* causing ovine babesiosis, *B. caballi* infecting horses, *B. canis* and *B. gibsoni* infecting dogs, *B. bovis, B. bigemina* and *B. divergens* infecting cattle and *B. divergens, B. venatorum*, *B. microti* and *B. duncani* associated with human babesiosis (Soulsby 1982; Radzijevskaja et al. 2008; Hasle et al. 2010; Atif et al. 2012; Najm et al. 2014; Moumouni et al. 2015; Bhat et al. 2017; Abdela et al. 2018; Vieria et al. 2019; Siddique et al. 2020; Neelawala et al. 2021). Figure 1 presents the frequency distribution of various species of *Babesia* in various parts of the globe.

Babesia bovis and *B. bigemina* are among the major causative agents of babesiosis in bovines of the tropical and sub-tropical regions and by far the most studied agents. In Europe, *B. divergens* is also associated with bovine babesiosis and has been reported as a zoonotic pathogen, infecting immunocompromised humans (Beugnet and Moreau 2015). Bovine babesiosis is causing numerous socio-economic impacts on the beef industry in the world (Suarez and Noh 2011). Live vaccines are available for babesiosis, but still this disease is poorly controlled in some continents and its control is so far, unsuccessful, due to climate change that is favoring the growth of vector i.e. hard ticks (Acari: Ixodidae), thereby increasing its survival and vectorial capacity (Florin-Christensen et al. 2014; Dantas-Torres et al. 2017; Sonenshine 2018).

Bovine theileriosis or East Coast fever (ECF), caused by the genus *Theileria*, is considered a significant tick-borne disease of cattle in tropical and subtropical regions, causing significant morbidity and mortality (Ota et al. 2009; Adjou et al. 2015; Kho et al. 2017; Hassan et al. 2018; Mohamed et al. 2018). *Theileria* parasites are transmitted by different genera of the hard ticks, including *Rhipicephalus*, *Hyalomma,* *Haemaphysalis*, and *Amblyomma* (Bishop et al. 2004; Kho et al. 2017). Genus *Theileria* possesses certain unique characteristics which differentiate its species from other apicomplexan parasites e.g., they do not reside in the parasitophorous vacuoles in comparison to *Toxoplasma* and *Plasmodium,* are non-motile and lack apical complex (Bishop et al. 2004; von Schubert et al. 2010). *Theileria* species, causing infection in livestock population include: *T. annulate, T. parva*, *T. ovis*, *T. lestoquardi, T. separate, T. mutans, T. velifera,* and *T. recondite* (Zaeemi et al. 2011; Kumsa et al. 2013; Li et al. 2014; Aydin et al. 2015; Guo et al. 2018; Remesar et al. 2019; Niaz et al. 2021; Roy et al. 2021). *Theileria* species are different from *Babesia* sp. due to transstadial transmission and schizogony inside lymphocytes (Mehlhorn et al. 1994; Schnittger et al. 2012).

The geographical distribution, economic significance, pathophysiology, clinical picture and diagnostic approaches of babesiosis and theileriosis have been discussed below:

Babesia

Economic Importance

Bovine babesiosis is considered as an important disease from the economical point of view, causing huge production losses. Meat and milk production losses due to death of animals and convalescence, along with those of treatment and immunization costs, add up the economic burden. Early diagnosis and effective treatment can reduce the mortality rate up to 5% (Uilenberg 2006).

Risk Factors Associated with Babesiosis

Host-related factors

Among cattle breeds, *Bos indicus* is comparatively more resistant to the infection with babesiosis than *Bos taurus*. This observation is considered as a result of an evolutionary relationship among *Bos indicus*, *Rhipicephalus* sp. and *Babesia* sp. Zebu cattle show higher resistance for *B. bovis* infection as compared to European breeds; however, cross-bred cattle occupy an intermediate position. Zebu cattle are almost free from the disease due to their innate resistance against tick infestation. In Australia, the infection caused by *B. bigemina* is of lower pathogenicity as compared to that of *B. bovis*. *Babesia bigemina* inoculation studies have revealed that *Bos indicus* and *B. indicus* cross-bred cattle are more resistant to babesiosis compared to *Bos taurus* (Radostits et al. 2007).

In cattle, the infectivity of parasites and severity of the disease vary with increase in age of the host. Calves from the naïve dams are more susceptible to the infection. Clinical illness begins from birth and is sustained for up to 2 months of age during which they develop resistance that can persist up to 6 months. Calves from the immune dams receive antibodies through colostrum and this immunity persists for 3-4 months after birth. Higher infection rate is reported in animals of 6-12 months of age. Infection is rarely seen in animals older than 5 years. Animals that are less than 1 year of age are mostly infected with *B. bigemina,* while animals of above 2 years of age are infected with *B. bovis*. In endemic areas, the average age for the calves to become infected is 11 weeks and at this stage, mild clinical signs and pathogenesis occur. After 6 month of age, infected number increases in enzootic areas. Serum antibodies are at their lowest level in housed cattle in the beginning of spring, when they came out of the barn; however, their level gradually increases with exposure to the infected ticks. Cattle that are reared for breeding and slaughter purposes are susceptible to infection in enzootic areas; while in endemic areas, indigenous cattle are rarely affected due to the presence of natural resistance in youngstock and passive immunization through colostrum that is gradually replaced with the active immunity. In endemic areas, severe cases are due to exposure to the stress during parturition, intercurrent disease, and starvation (Gohil et al. 2013).

Environmental factors

Seasonal variations are reported in the prevalence of babesiosis, which might be attributable to the seasonal fluctuations in the vector population; hence, affect its prevalence. Higher surge in incidence of babesiosis occurred during the period of peak tick population. Among the environmental factors, temperature is the most important one because it is directly proportional to the tick activity. Other climatic factors, like rainfall and humidity, have a limited effect on the disease prevalence. In marginal areas, heavy losses have been recorded, as in these areas tick population is highly variable. During winter, the infection is less, presumably due to decreased fecundity of ticks or their death; but, as soon as the conducive environment prevails, ticks multiply, resulting in increased infection rate in the susceptible hosts (Constable et al. 2016).

Pathogen-related factors

Like some other intra-erythrocytic haemoparasites, *Babesia* also evade the host immune response through antigenic shift and/or drift mechanisms (Allred and Al-Khedery 2006). The relationship between *Babesia* sequestration and cytoadherence has been examined and different strains/antigenic variants of *B. bovis* and *B. bigemina* have been reported. In cattle, *Babesia* infection is related to superinfection with some other antigenically distinct parasites. Antigenic variations provide a temporary respite from immune system of the host and prolong the duration of infection. Antigenic variation and strain differences usually have no effect on the vaccine, as cross-immunity between/among same *Babesia* species/strains provides reasonable protection against each another (Hunfeld et al. 2008).

Clinical signs of babesiosis

Clinical picture of babesiosis is presented in Fig. 2. The disease course is 3-7 days, with fever of 104°F that can persist for several days, followed by depression, weakness, inappetence, and loss of movement. Haemoglobinuria, also known as red water, is often present and characterized by dark red to brown colored urine with froth. In advance cases, anemia, jaundice, diarrhea, muscle wasting, recumbency, coma, metabolic acidosis and tremors are also developed. Severally infected animals usually die during the first 24 hours of the disease period. In case of severe disease, pregnant cows may undergo abortion, and the breeding bulls may suffer from sterility for 6-8 weeks. In case of involvement of nervous system incoordination, convulsions, and mortality occur. Clinical signs of babesiosis include fever, profound anemia, and haemoglobinuria lasting up to 3 weeks, and abortion in pregnant animals (El-Ashker et al. 2015), while in severe cases, death may occur within 24 hours of the infection (Radostits et al. 2007). In case of *B. divergens* infection, anal sphincter spasm also occurs, causing the passage of faeces with great force, the situation is referred as pipe-

Fig. 1: Physical map of the world, showing the prevalence of *Babesia* species in various regions (Source: Altay et al. 2008; Adjou et al. 2015; Farooqi et al. 2017; Abdela et al. 2018; Adelabu et al. 2020; Zimmermann et al. 2021).

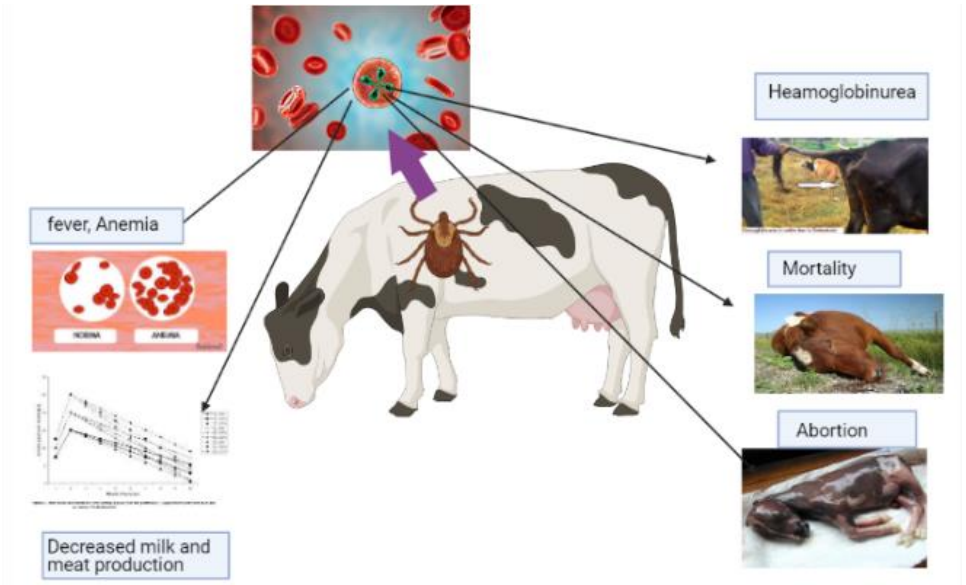

Fig. 2: Clinical picture of Babesiosis (Source: Modified from Reichel et al. 2013).

stem faeces. However, in case of *B. bigemina* infection, haemoglobinuria is seen earlier, while fever is not a common feature. In acute cases, disease severity is less as compared to severe cases, with no cerebral involvement and higher recovery rate. Recovered animals remain infective for ticks for 4-7 weeks. Direct losses caused by bovine babesiosis are meat and milk reduction, ill thrift, cost of control measures, abortion, and mortality (Cunha 2000; Benavides and Sacco 2007).

Pathogenesis and pathophysiology of babesiosis

The complete life cycle of *Babesia* sp. consists of two phases, one occursring in ticks and the other in vertebrate hosts. Gametogenesis mainly occurs in the tick gut after emergence of the oocyte and fertilization of micro and macrogametes. Emerged oocytes invade the epithelial cells of midgut and are transformed into kinetes in the tick haemolymph. Kinetes can migrate towards other tick tissues and ovaries, where they can penetrate into the eggs and result in the transovarian transmission. Further, these kinetes penetrate the salivary glands, where they are transformed into sporozoites. These sporozoites are the infective stages for babesiosis and can be injected into the blood and sub-cut tissues in the bovine hosts through blood-feeding of larval or nymphal stages of the hard ticks (Suarez and Noh 2011).

Injected sporozoites enter into the blood stream and penetrate into the red blood cells (RBCs). Terminally-differentiated RBCs are lacking their genetic information, are capable of carbohydrates, proteins, and lipid synthesis and provide a safe environment for pathogen due to low metabolic activity. Red blood cells are also considered ideal sites for most pathogens due to easy access to every organ through circulation. While in the case of *Babesia*-infection, RBCs are under control of genetic material of the pathogen, the latter can control the activity RBCs by introducing genetic information, hence, the parasites are

free to control and change the RBCs according to their requirements. *B. bovis* like *Plasmodium* sp. can modify the appearance and composition of *Babesia*-infected RBCs i.e., change in protein and carbohydrate composition and permeability, absence of lysosomes, and inability to present antigen to the immune system to facilitate parasites in masking the immune system (Florin-Christensen et al. 2000; Allred and Al-Khedery 2006). Infected RBCs are also not capable of phagocytosis and endocytosis of nutrients. Sexual reproduction takes place in vertebrate hosts and merozoites are produced in the infected RBCs, which invade neighboring RBCs. In comparison to *Plasmodium* sp., sporozoites of *Babesia* sp. only invade RBCs, considering them as the only target for infection in the bovine hosts (Mehlhorn and Shein 1984; Cheng 1986; Singh 2008; Chauvin et al. 2009).

In infected RBCs, rapid division of parasites causes destruction of erythrocytes along with acute haemoglobinemia, fever and haemoglobinuria, decreased packed cell volume (PCV) below 20%, and death in few days. Less pathogenic species and resistant hosts are contributing factors of the milder form of babesiosis characterized by anorexia, fever, and slight jaundice for several days. Nervous signs like hyperexcitability and incoordination due to clumping of erythrocytes in the brain are associated with *B. bovis* and *B. canis* infections (Suarez et al. 2019). Pathogenesis of Babesia species is illustrated in Fig. 3.

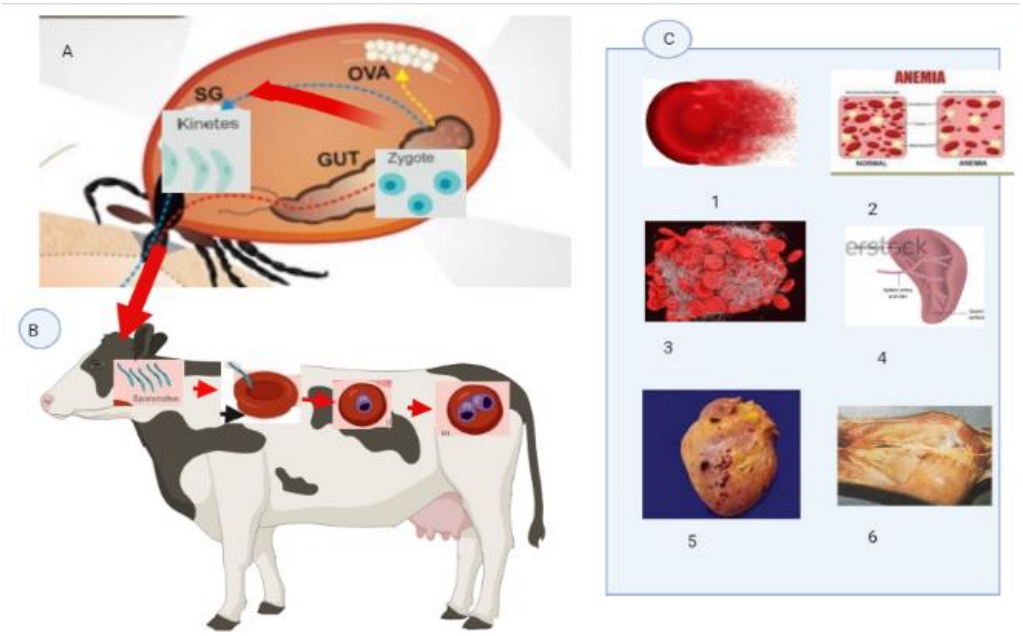

Fig. 3: Pathogenesis of *Babesia* sp. A= lifecycle events occurring in ticks i.e. sexual reproduction of *Babesia;* B= lifecycle events occurring in host i.e. asexual reproduction; C= pathological changes occurring in host due to Babesia: 1= destruction of RBCs, 2= anemia, 3= clumping of RBCs in the vital organs, 4= splenomegaly, 5= myocardial haemorrhages 6= jaundice carcass (Source: Modified from Herenda et al. 2000).

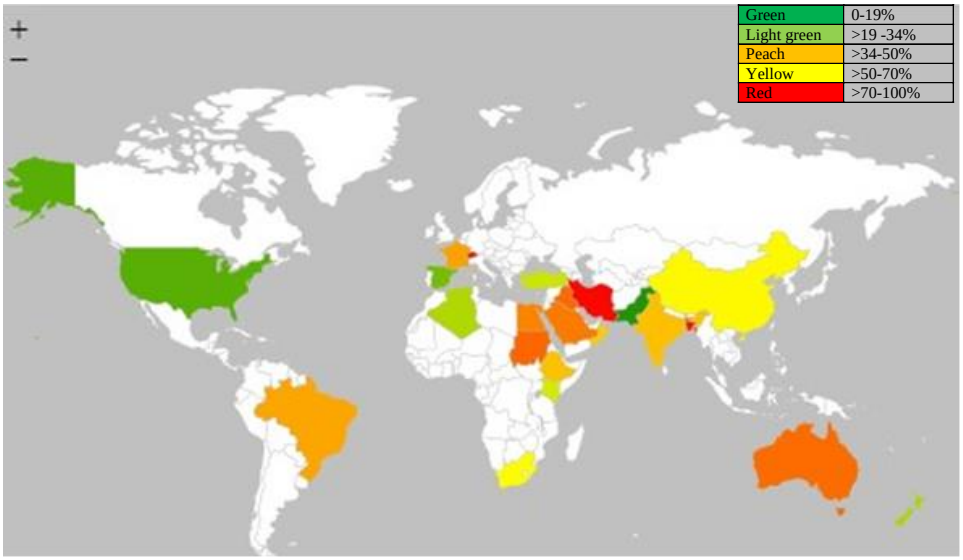

Fig. 4: The prevalence of *Theileria* species in different regions of the world (Source: Jeong et al. 2005; Hussain et al. 2014; Moumouni et al. 2015; Hayati et al. 2020; Jia et al. 2020; Zimmermann et al. 2021).

Virulence of Babesia species is due to parasite-mediated host exploitation and infection-related immunopathology. In case of acute infection, the immunocompromised adult hosts show exploited and uncontrolled reproduction of the parasite, resulting in host RBCs destruction and decreased hematocrit values (Brown and Palmer 1999). The infected animals show decreased tissue oxygen level in vital organs, increased body temperature, haemoglobin degradation in the liver, followed by red-coloured urine, jaundice due to high level of RBCs degradation, kidney damage, and splenomegaly because of overactivity and respiratory distress (Wright et al. 1988). Increased parasitemia in *B. bigemina* infection contributes towards the trapping of parasites in the spleen and results in increased haemoglobin degradation, kidney damage, and haematuria, while in *B. bovis* infection neurological signs are present due to clumping of infected RBCs in the microvasculature of the brain (Clark and Jacobson 1998).

The incubation period of *B. bovis* is 8-15 days; in acute cases, animals remain ill and death occurs in 4-8 days. The infected animals show multiplication of parasites in peripheral and visceral vessels, resulting in clinically detectable haemolysis after 7-20 days of incubation. The first symptom is the sudden rise in temperature i.e., 106°F to 108°F that can persist for a week. Animals become dull and lethargic, with loss of appetite. This haemolysis results in anemia, haemoglobinuria, and jaundice. Moreover, in fatal cases, anoxia also accompanies anemia. Destruction of erythrocytes occurs up to 75%. In survived animals, ischaemic changes are noted in heart and skeletal muscles (Uilenberg 2006). In the case of *B. bovis* infection, vasoactive substances are produced, resulting in vasodilation, hypotension, increased vascular permeability, shock, disseminated intravascular coagulation (DIC), circulatory stasis, and pulmonary thrombosis. Susceptibility to *Babesia* infection decreases with age of the hosts; however, the severity of infection increases with increase in age e.g. in 5-6 months calves, limited clinical signs are observed with *B. bovis* infection; cattle aged 1-2 years show moderate form of the disease, while in above 2 years aged cows, severe disease often results in fatality. In case of *B. bovis*, intrauterine infection is also reported. Survived animals remain carrier with a subclinical infection, which is maintained due to an immunogenic balance between protozoa and antibodies which can be disturbed by stress during transportation, pregnancy and food shortage. Carrier animals remain resistant to *B. bovis* infection for 2 years (Cunha 2000; Mohiuddin 2007; Kumar et al. 2009).

Immune responses to *Babesia* infection

Immunity against *Babesia* parasites in youngstock, as well as in adults, needs strong triggering of innate and adaptive immune responses. Persistently infected and/or immunized animals can control parasites due to the presence of an antigen-specific CD4+ T cells, which produce cytokines (interferon gamma; IFN- γ), the latter can activate macrophages required for the clearance of parasites, and increase neutralizing IgG2 antibody production (Brown and Palmer 1999; Homer et al. 2000; Brown 2001; Estes and Brown 2002); neutralizing antibody IgG2 along with IgG1 provides protection against parasites (Mahoney 1986). Still unexplored are the mechanisms through which the T-cells are activated and the roles of distinct T-cell population in the adaptive immunity. So, a quick innate response is required for survival in case of acute infection, followed by the production of antibodies for the control of infection in persistently-infected and vaccinated animals.

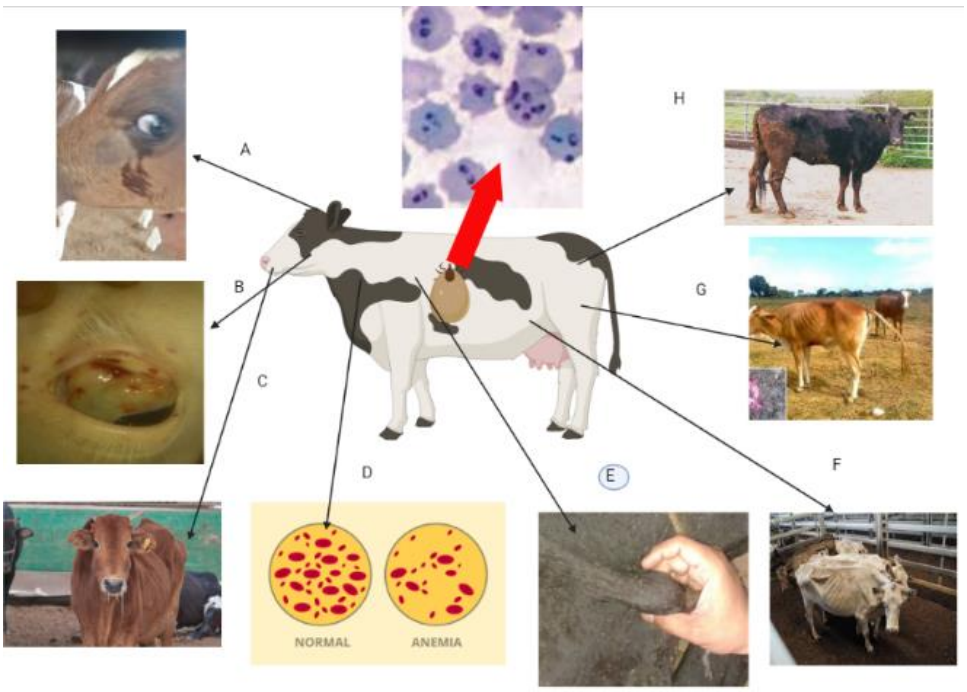

Fig. 5: Clinical picture of theileriosis, A= running eyes, B= petechial haemorrhages on conjunctival mucosa, C= runny nose, D= anaemia, E= swelling of lymph nodes, F= emaciation, G= haematuria, H= diarrhea (Sources: Joshi et al. 2016; Reis et al. 2016; Alsaad, 2018).

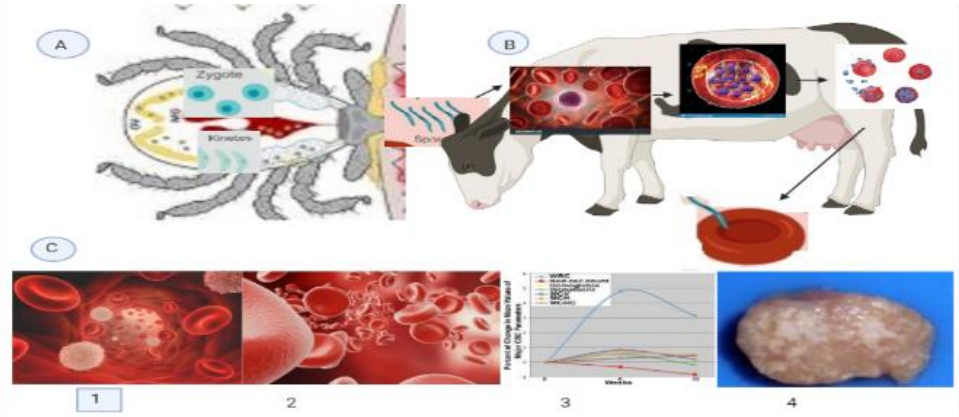

Fig. 6: Pathogenesis of theileriosis and associated changes, A= sexual reproduction of *Theileria* in tick gut and haemolymph, B= asexual development of *Theileria* in vertebrate hosts including, schizogony, merogony and merozoite's penetration into the RBCs, C= pathological changes: 1= leukopenia, 2= decrease Hb concentration, 3= reduction on PCV, MCHC values, 4= lymphadenitis (Source: Herenda et al. 2000).

In acute *B. bovis* infection, strong innate immune response along with pronounced infiltration of large leukocytes i.e. macrophages, immature dendritic cells and the natural killer (NK) cells, IFN-γ-based activation of macrophages, release of metabolites of toxic macrophages e.g. nitric oxide (NO) due to priming of the immune cells and parasite-derived products, protect the young animals (Goff et al. 1998; Brown and Palmer 1999; Brown 2001; Goff et al. 2001, 2003, 2010). According to Schneider et al. (2011), immune response of young animals showed crosstalk between NK and immature DC in the spleen, presumably mediated by IL-15. Similarly, Goff et al. (2001) studied the immunological arguments for age-related resistance in *B. bovis* infection for young and adult animals and reported an increased expression of IFN-γ and IL-12 in the spleen of youngstock after 3-6 days and inducible nitric oxidase synthase after 7-8 days post-infection as compared to adult animals.

Various *in vitro* studies have shown that release of NO and IFN-γ is necessary for parasite clearance and upregulated expression of IFN- γ and NO are associated with the resistance in youngstock. However, conflicting results were reported in *in vivo* studies about NO release by macrophages as a slight growth-inhibitor and not associated with the evading of parasites (Gale et al. 1998; Shoda et al. 2000; Chauhan 2010). Immunopathological effects in immunocompromised hosts are overwhelmed, which might be attributable to the lack of parasite priming by the immune system and non-specific clearance. The severity of pathogenesis is related to the over-production of mediators like IL-18, IL-12, TNF-α, IFN-γ, and late induction of IL-10, causing increased inflammatory responses and tissue damage (Goff et al. 2001, 2002).

Clinical pathology and necropsy findings of babesiosis

In clinical cases, severe anaemia, reduction in haemoglobin level and reduced RBCs count (3g/dL and 2 million/ µL, respectively) have been reported. Anemia remains at peak level for 9-16 days of the infection. Moreover, reduction in platelets and fibrinogen contents also occur in clinical cases (Mohiuddin 2007; Singh 2008; Stockham and Scott 2008; Chauhan 2010; Constable et al. 2016).

At necropsy, jaundice, pale tissues, thin and watery blood, dark brown discoloration, hepatomegaly, splenomegaly, distended gall bladder with viscous, granular consistency of the bile, enlarged and darkened kidneys, and urinary bladder containing red-colored urine are observed in acute cases. Endocardium and epicardium show ecchymotic haemorrhages, with an increased amount of the blood-tinged fluid in the pericardial sac. Intravascular clotting is also visible on necropsy analysis. In chronic cases, absence of haemoglobin and emaciated carcass are observed along with other acute findings. For confirmatory diagnosis, blood from heart, kidneys, and brain in *B. bovis* infection is used for smear examination. Blood should be taken within 8 hours of mortality from tissues and 28 hours from the brain for Giemsa staining. For direct fluorescent antibody staining, older tissues are preferred. Organ smears stored at 72°F are usable till 5 days after collection, as post-mortem morphometric changes rapidly occur in *B. bigemina*. For serum antibodies, serological testing and dead animal's blood are also usable (Stockham and Scott 2008; Constable et al. 2016).

Diagnosis and identification of *Babesia* sp.

Diagnosis of babesia is an important component of the control program. Different methods are used as given below:

Blood smear examination

Diagnosis of babesiosis in infected animals depends upon the detection of piroplasms in the stained smears of capillary blood, as the venous blood can give false-negative results. No direct correlation is present between the protozoa containing RBCs and disease severity. In case

of *B. bigemina*, numerous piroplasms are observed in the peripheral blood, while in *B. bovis*, less number of piroplasms is seen in the peripheral blood and this difficulty can be overcome through using thick smears of the blood sample. A thick blood smear examination gives 10 times more sensitive results as compared to thin blood smears and can detect a low level of *B. bovis*. General circulation samples showed 20 times less *B. bovis* in blood as compared to capillary blood samples. Microscopy is less expensive and rapid tool of detection but has low sensitivity and specificity (Mohiuddin 2007).

Transmission test

Inoculation of the infected bovine blood in susceptible and splenectomized calves is the most sensitive technique for *Babesia* detection. For this purpose, 50 to 100 mL of blood is taken from the infected animal and injected into the splenectomized calves through intravenous or subcutaneous route. Recipient calves are examined on daily basis for the number of protozoa in the blood (parasitemia) at the febrile reaction. Carrier cattle are difficult to diagnose due to low *Piroplasma* level in the peripheral blood (Chauhan 2010).

Culturing of Babesia

Some *Babesia* species are identifiable by using *in vitro* culture analyses. Babesia stages can be isolated after 9 months of acute infection and can be cryopreserved. Culture techniques are useful tools for the detailed investigations of the parasite (Holman et al 1988; Malandrin et al. 2004; Rojas-Martínez et al. 2017).

Molecular methods

Detection of *Babesia* species through the use of nucleic acids (DNA) and their amplification techniques is reliable and sensitive. The polymerase chain reaction is used for rapid identification of pathogenic species and is a comparatively more reliable and sensitive method for parasitic identification. DNA probe that can detect low-level infections, can also detect protozoa in necropsy samples and tissues. This method is used in low parasitemia and can identify three merozoites per reaction, using primers for *Babesia* small subunit rRNA gene. For persistent infections, even if last up to 27 months in untreated animals and 13 months in treated animals, PCR is the most widely used test for identification of *Babesia* species (Krause et al. 1998; Rozej-Bielicka et al. 2017; Ganzinelli et al. 2020).

Serological methods

Seroassays are commonly used for the detection of sub-clinical infections in cattle and during surveillance studies in herds (Mosqueda et al., 2012). Serological tests are well established, but they have some limitations regarding specificity and sensitivity. It is also not possible to distinguish between current and the past infection on an individual basis in sero-assays. Complement fixation test (CFT) is commonly used in bovine babesiosis, along with other tests under field conditions. The other tests include microplate enzyme immunoassay (EIA), passive agglutination, latex agglutination, indirect fluorescent antibody test (IFAT), capillary agglutination, card agglutination, indirect haemagglutination, and slide agglutination; these tests show satisfactory results.

Immunofluorescence antibody test (IFAT)

Immunofluorescent antibody test (IFAT) is commonly used for differentiation among *Babesia* species. and detection of antibodies in animals. This serological test can differentiate *B. divergens* from other *Babesia* species but it is unable to differentiate *B. divergens* from *B. caprolei* in the red deer (Vercammen et al. 1995; Geurden et al. 2017).

Enzyme linked immuno sorbent assay (ELISA)

In this test, crude detection of IgM antibodies is achieved with higher specificity and sensitivity (94% and 100%, respectively) from *B. bovis* antigenic preparations (Radostits et al. 2007). Production of the specific IgM antibodies against *B. bovis* infection begins on the 11th day of inoculation with an infected tick and on the 19th day of the infected blood inoculation. Competitive ELISA (cELISA) is also used for the detection of serum antibodies in haemoparasites. Gene encoding for *B. bovis* rhoptry-associated protein 1 (RAP-1) was used to develop this assay and this can be used to differentiate the *B. bovis* specific antibodies-containing animals from uninfected animals with high sensitivity and specificity (Radostits et al. 2007; Alvarez et al. 2019).

Latex agglutination test (LAT)

It is a simpler, rapid, economical, and relatively sensitive test for detecting antibodies against *B. equi* by using recombinant merozoite antigen 1 (RMA-1). The LAT assay is a good alternative for IFAT and ELISA with equal sensitivity and specificity ratio (Brown et al. 2006).

Differential diagnosis of babesiosis

Babesiosis should be differentially diagnosed from some common infections i.e. theileriosis which is clinically similar to babesiosis, and can be differentiated throughthe laboratory examination. Post parturient haemoglobinuria is also clinically similar to babesiosis, but the former is not a vector-borne condition and is reported in recently calved cows, full lactating animals, and those having low phosphorus concentrations in their diets. Blood smear examination for parasites is the gold standard i.e. absence of protozoa in the blood smear examination is a characteristic differential point between these two diseases. Babesiosis can also be differentially diagnosed from the bacterial haemoglobinuria (characteristic necrotic infarction in the diaphragmatic surface of the

liver), S-methyl-L-cysteine-sulfoxide (SMCO) poisoning (animals grazed on *Brassica* sp. or other rape crops), and leptospirosis (calves kept in unsanitary conditions with wet underfoot) (Roberts and Janovy 2009; Constable et al. 2016).

Babesiosis in humans

Different *Babesia* species cause diseases in humans. These include: *B. microti, B. crassa, B. dancani, B. venatorum*, and *B. divergens*, KO-1, XXB/Hang Zhou, *B. sp.* CA1, *B. sp.* CA3, and *B. sp.* CA4. Human babesiosis is almost reported in all parts of the world. Over the past 10 years, survey of the diseases is reported as endemic and increase in geographic spread of this disease is reported in northeastern and mid-western United States (Joseph et al. 2011; Smith et al. 2014; Stein et al. 2015; Walter et al. 2016; Mareedu et al. 2017; Goethert et al. 2018; Scott et al. 2021). Increase in the disease prevalence is supposed to be due to the increase in white tailed deer, which act as amplifying host for *Ixodes* ticks, the latter are the main vectors of the disease. Further, increase in the construction of houses in woody areas and availability of better diagnostic facilities are also the reasons of reporting more *Ixodes*-borne diseases (Spielman et al. 1985).

Babesia microti spread started from Southern New England to north, south and west but this spread was slow as compared to that of *Borrelia burgdorferi* (Dunn et al. 2014; Walter et al. 2016). Human babesiosis is also widespread in Europe and most important species are *B. venatorum, B. divergens, and B. microti*. Most important vector which transmits the disease is cattle tick *Ixodes ricinus*. Around 50 cases were reported from different parts of the Europe, including Ireland, Sweden, Portugal, Croatia, Poland, Turkey, Spain, Norway, Finland and Georgia and these cases were due to *B. divergens* (Zintl et al. 2003; Hunfeld et al. 2008; Gray et al. 2010). In case of *B. microti,* only three cases were reported from Europe and among these two were asymptomatic. These *B. microti* cases were reported in Poland and Germany (Hildebrandt et al. 2013; Welc-Faleciak et al. 2015). The cases of *B. venatorum* were also reported from different parts of Europe and first described as EU-1. Germany, Sweden, Austria, and Italy are considered as the most-infected areas with *B. venatorum (*Herwaldt et al. 2003; Hunfeld et al. 2008; Gray et al. 2010).

In Asia, cases of human babesiosis are reported from China, Taiwan, Japan and Korea. Species reported from Asia include *B. venatorum*, *B. crassa* and *B. microti*. *B. crassa* infection has been reported from Heilongjiang province of China (Jia et al. 2018); while infection due to *B. venatorum* is reported from northwestern and northeastern China (Sun et al. 2014; Jiang et al. 2015). Case of human babesiosis caused by KO-1 was reported in Korea (Kim et al. 2007). In India, a case of human babesiosis was reported but the precise species was not identified (Vannier and Krause 2012). Isolated cases of human babesiosis have also been reported from different parts of world, including Australia, Mexico, Cuba, Canada, and Egypt (Senanayake et al. 2012; Vannier and Krause 2012; Bullard et al. 2014; Peniche-Lara et al. 2018). Recently, Scott et al. (2021) have reported for the first time two of the 19 PCR-confirmed (18S rRNA gene) cases of human babesiosis (*Babesia odocoilei*) from Ontario, Canada. The symptoms shown by the same subjects included fever, chills, fatigue and night sweats. This also provided substantial evidence that *B. duncani* cross-reacts with *B. odocoilei* and that the latter is pathogenic to humans.

Theileriosis

Economic importance of theileriosis

Theileriosis, also called as the East coast fever, is considered as an important disease causing a great impact on the animal production in different regions of the world. Severe cases of the disease in exotic and indigenous breeds are responsible for huge economic losses in terms of reduced milk and meat production, mortality, morbidity, treatment cost, and management expenses (Uilenberg 2006). Almost 1/6 of the world cattle population is at risk of theileriosis, with an estimated economic loss of US $130,000-598,000 per annum in endemic areas of Turkey (Cicek et al. 2009). In Tunisia, most of the losses are in the form of milk production in carrier animals (Gharbi et al. 2011). The prevalence of Theileria species in different regions of the world is shown in Fig. 4.

Contributing factors of theileriosis

The most important risk factor in theileriosis is the prevalence of ticks in a given area and tick burden per animal, with an understanding that only a single infected tick can establish a fatal infection. On an average, infestation of five ticks per animal will allow sustaining endemicity at low infestation rates, 1-4 ticks per animal can invite the epidemic, while less than one can cause sporadic outbreaks. In endemic areas, infection rate in ticks is 1-2% and recovered animals are no longer considered sterile. Indigenous cattle breeds and young animals are less affected compared to exotic and adult animals. Buffaloes are considered as the carriers for the East coast fever. Based on the environmental factors, too hot and too cold areas are not favorable for the tick development and reduce the transmission potential of theileriosis by nymphs or adults (De Deken et al. 2007; Constable et al. 2016).

Clinical sign of theileriosis

During the first phase, subclinical infection occurs with the entry of protozoa and the clinical signs are not detectable. In the second phase, regional lymph nodes are involved; while in the third phase, lymph nodes destruction, leukocytes depletion, and death occur (Urquhart et al. 1996). The incubation period for theileriosis is 1-3 weeks and depends upon the strain virulency, and number of infecting parasites. First clinical sign is the lymph node enlargement after 8-16 days of tick

attachment. After one or two days, there is the onset of depression, anorexia, and fever. In later stages, dyspnea, nasal and ocular discharge, splenomegaly and lymph nodes enlargement occur. Emaciation, recumbence, weakness, and death due to asphyxia occur in 7-10 days, often with a frothy nasal discharge. Brain involvement is occasional and characterized by circling and termed as the cerebral theileriosis or turning sickness with tremors, convulsions, head pressing, and profuse salivation. The clinical symptoms of theileriosis include depression, nasal discharge, dyspnea, runny eyes, high fever, weakness, anaemia, emaciation, lymph nodes swelling, haematuria, petechial haemorrhages on conjunctival mucosa, diarrhea and blood in faeces (Shiono et al. 2004; Jeong et al. 2005; Islam et al. 2011).

Pathogenesis of theileriosis

Ticks acquire infection through the infected RBCs containing piroplasms from the infected cattle, followed by the fusion of micro and macrogametes in the tick gut and development of kinetes. Kinetes are released into the tick haemocoel and sporogony takes place in type III acini of the tick salivary glands (Fawcett et al. 1982; Norval et al. 1992). After 4-8 days of attachment of ticks, 30,000-50,000 *Theileria* sporozoites are released from the tick salivary glands and enter into the lymphocytes through the zipper process (Shaw and Young 1995). Compared to other apicomplexan parasites, *Theileria* sporozoites are non-motile and oval in shape, having 0.75-1.5 µm diameter, and lack a well-developed apical complex (Fawcett et al. 1982; Shaw 2003). Within a vertebrate host, sporozoites develop into the multinucleated stage (schizonts) and acquire metastatic phenotypes, which are mainly responsible for pathophysiology of the parasite (Dobbelaere and Heussler 1999; Shaw 2003; Chauhan 2010). Sporozoites also infect the dendritic cells and macrophages and are converted into early schizont stage (Shaw et al. 1993). Schizonts produce merozoites which are released by the rupture of the host cells. Merozoites enter into the neighboring RBCs and are converted into the piroplasms, which are infective stages for ticks (Shaw and Tilney 1996). A limited number of cell divisions take place in piroplasms and anemia occurs due to RBCs destruction, but it is not a prominent feature of the East coast fever (Cheng 1986; Norval et al. 1992).

In *Theileria* infection, change in the complete blood cell count (CBC), decreased PCV, MCHC, Hb concentration, and increased MCV are the main haematological changes that occur in the host (Çöl and Uslu 2006; Singh 2008; Somu et al. 2017; Lawrence et al. 2018). Leukopenia is also reported in theileriosis and depends upon the infecting *Theileria* species (Omer et al. 2002). At necropsy, atrophy of the cellular content, presence of ecchymoses, petechial haemorrhages on the gastrointestinal mucosa, lymphadenitis, oedema, splenomegaly, and emphysema are reported. Rarely, neurological signs are reported and associated with the presence of schizonts in the cerebral capillaries (Urquhart et al. 1996).

Immune responses in *Theileria* infections

In the cases of bovine tropical theileriosis, there is an active involvement of innate, as well as adaptive, immunity in protecting the cattle. The front line of defense is provided by innate immunity by producing rapid and non-specific defense with the entrance of pathogen in the host (Seitzer and Ahmed 2008). Upon entrance of *Theileria* parasites, cytokines are secreted by the macrophages, NK cells, and T lymphocytes which modulate interaction among cells of the immune system. This interaction enhances the synergy of the NK cells and macrophages, and increases IFN-γ level, resulting in the activation of macrophages and IL-12 (Fearson and Locksley 1996). Adaptive immune response activates phagocytic activity of the macrophages and in turn, promotes immunity. Similar cytokines are secreted by schizont-infected and uninfected macrophages, hence affecting the innate and adaptive immunity. So, NK cells and CD8+ T cells produce IFN-γ in macrophages. Macrophages, in response to this, produce NO, which helps in the clearance of infected cells. Natural killer cells cause lysis of schizont-infected cells and macrophages clear these cells and undergo apoptosis (Preston et al. 1999). Monocytes and macrophages secrete IL-12, tissue necrosis factor alpha (TNF-α), IL-β and other pro-inflammatory cytokines, which in turn, mediate the acute phase protein involvement. These acute phase proteins allow the T helper cells (CD4+ T cells) to differentiate and form a linkage between innate and adaptive immunity (Razavi et al. 2010). Activated T-helper lymphocytes secrete IFN-γ and IL-2, which are required for clonal expansion of the cytotoxic T cells (Ahmed and Mehlhor 1999). Host macrophages are stimulated by IFN- γ and secrete TNF-α, which inhibits the schizont infected cells and provides defense against *Theileria* pathogen (Preston et al.1999).

Theileria parva antigens block the apoptotic pathways and secure parasite survival in the host cells (Heussler et al. 2001). This can cause changes in the T and B lymphocytes through their transformation and harmonize their own direction in the host cells. During the mitotic phase of the host cells, schizonts attach at the spindle fiber and enhance transfer of both daughter cells containing schizonts (von Schubert et al. 2010). So, in the case of East coast fever, lymphoproliferative mediated parasite multiplication takes place (Hayashida et al. 2013). Ability of the infected cells to proliferate and avoid apoptosis is dependent on the suppression of p53 activity, which is primarily used to mediate host cell apoptosis (Haller et al. 2010). This protein (p53) is a tumor suppressor and in the normal conditions, it is present at low levels due to its degradation by proteosome enzymes (Vogelstein et al. 2000; Vogelstein and Kinzler 2004). In stress conditions, p53 accumulates and controls cell cycle progress (Hayashida et al. 2013). This protein is mainly present within the nucleus of the infected cells and is associated with the schizonts' membrane (Haller et al. 2010; Hayashida et al. 2013).

Immunohistochemical analysis showed an increased number of CD163+ macrophages, which in turn cause increased secretion of IL-17 in infected cattle (Fry et al. 2016). A high level of infiltrating CD163+ macrophages is associated with poor prognosis (Yang et al. 2015). Schizonts-infected lymphocytes produce a higher level of IL-17 and ultimately upregulate the expression of CD163+ cells (McKeever et al. 1997). Parasite-induced activated macrophages challenge the energy trigger and T cell apoptosis as an evasion method from immune system of the host (Tomioka et al. 2012). Lysis of the infected lymphocytes, and erythrocytes results in the release of free-iron, pro-inflammatory cytokines, activation of CD163+ macrophage response, and necrosis (Fry et al. 2016). Cytokine IL-17 is associated with the severe pathological tissue damages and can be suppressed by administrating anti-IL-17 antibodies (Del Cacho et al. 2014). Major histopathological finding in *T. parva* infection is the histiocytic vasculitis of the small to medium-sized lymphatic and blood vessels, which is due to a increased level of IL-17 secreting macrophages (CD163+ macrophages). Hence, a higher number of macrophages in enlarged spleen, liver, lymph nodes, and lungs are reported (Fry et al. 2016). Excess cytokines production is associated with the pathogenesis of theileriosis (Preston et al. 1993; Visser et al. 1995; Forsyth et al. 1997). Therefore, schizonts of *T. annulata*-infected schizonts have the ability of the immune subversion and change the immune cells into metastatic lymphocyte population. Along with cytokines, some other factors like NO and matrix metalloproteinases also play important role in suppressing the growth and infection outcome of parasitized cells (Preston et al. 1999). Cytokine profile of the schizonts-infected macrophages consists of IFN-α, IL-1α, IL-1β, IL-6, IL-10, and TNF-α (Brown et al. 1995).

Clinical pathology and necropsy findings of theileriosis

Schizonts are present in circulating lymphocytes and in the smear prepared from biopsy of the lymph nodes. Piroplasms are easily visible in erythrocytes from day 16 after infection and their number increases with the stage of infection. Levels of intra-erythrocytic piroplasms are not related to disease severity. Blood counts showleukopenia, minor anaemia, and thrombocytopenia. The parasite can grow on lymphoblastoid cells (Singh 2008; Stockham and Scott 2008; Constable et al. 2016).

On necropsy, most important lesions are pulmonary edema, hydropericardium, hydrothorax, hyperemia, emphysema, blocked airways with copious froth and emaciated carcass with several haemorrhages in different organs and tissues. Enlargement of the liver, spleen, kidneys, and lymph nodes are also observed along with ulceration of intestines and abomasa. Nodules are usually present in kidneys, gastrointestinal tract, and liver. Necrosis in lungs, kidneys, lymph nodes, gastrointestinal tract, liver and other tissues resembles to that seen in lymphoid tumors. Giemsa-stained lymphoblasts show presence of schizonts. In case of nervous system involvement, lymphoblasts are found clumped in the cerebral blood vessels and cause infarction. For pathological identification, various organs are examined for the presence of gross lesions (Stockham and Scott 2008; Constable et al. 2016).

Diagnosis and identification of *Theileria* sp.

Different serological tests are used for the diagnosis of theileriasis like indirect hemagglutination (IHA) test, indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). The most commonly used serological test for sero-epidemiological studies is ELISA with high sensitivity and specificity compared to IFAT (OIE 2008; Chauhan 2010). Polymerase chain reaction (PCR) is more sensitive and specific and can detect infection in the carrier animals as well. Multiplex PCR is used for simultaneous detection of *B. bigemina*, *T. annulate*, and *A. marginale*. This is a simple, sensitive, and specific test that can be used for epidemiological studies (Khattak et al. 2012; Bilgiç et al. 2013). Theileriosis can be differentially diagnosed from babesiosis, anaplasmosis, trypanosomiasis, malignant catarrhal fever, bovine viral diarrhea, and rinderpest. Liver biopsy and blood examination can be used to confirm a clinical diagnosis (Constable et al. 2016).

Theileriosis in humans

No evidence of theileriosis in humans has been reported worldwide (OIE, 2020).

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CRYPTOSPORIDIOSIS

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INTRODUCTION

Cryptosporidiosis is an important parasitic disease. It leads to profuse watery diarrhea of clinical significance around the world, affecting gastric mucosa of humans and animals who have ingested infectious oocysts of Cryptosporidium species. Cryptosporidium oocysts are widespread in nature and many species of Cryptosporidium can induce this illness. Cryptosporidium was first identified in 1907 by Ernest Edward Tyzzer, a well-known medical parasitologist at the Harvard University at Boston, USA. The name Cryptosporidium has been given due to the lack of sporocyst within the oocysts, which is unique to other coccidians. More than thirty species of genus Cryptosporidium have been described worldwide. Some of them are host-specific, while others have been reported to affect a wide variety of hosts. *Cryptosporidium hominus* (*C. hominus*) and *Cryptosporidium parvum* (*C. parvum*) are most widely identified for human infections. However; *C. parvum* is also associated with ruminants infections (Yang et al. 2021).

Human Cryptosporidiosis is a protozoan disease that causes gastrointestinal problems to individuals all over the world. It is the disease of major public health concern in children between the ages of 1-5 years, particularly in developing nations (Kotloff et al. 2019). Cryptosporidium-related diarrhoea was reported to be the leading cause of death worldwide in children under the age of five years in 2016, with 48,000 deaths attributable to severe illnesses (Khalil et al. 2018). Cryptosporidium spreads from animals to humans, animals to animals or humans to humans through contaminated food or water, and therefore, is an important example of zoonosis and reverse zoonosis. Infectious oocysts may live in the atmosphere for a long time and are not harmed by traditional disinfection methods (including chlorination and iodine), so that they may spread easily via water (Guy et al. 2021).

Outbreaks of human Cryptosporidiosis were reported during 1987, 1993, 2005, 2007, 2008, 2009, 2014 and 2017 in the United States of America (Gharpure et al. 2019; Alleyne et al. 2020), in 1995, 2005, 2008 and 2010 in UK (Chalmers et al. 2011), in 1996 and 2001 in Canada (Iqbal et al. 2015; Guy et al. 2021), in 2002, 2007, 2011 and 2013 in Ireland (Thomson et al. 2007; Mahon and Doyle 2017), in 2003, 2007, 2010 and 2011 in Australia (Ng-Hublin et al. 2015; Ng-Hublin et al. 2018), in 2010, 2011 and 2017 in Sweden (Insulander et al. 2013; Bjelkmar et al. 2017), in 2020 in New Zealand (Garcia et al. 2020), in 2006, 2009, 2017 and 2019 in France (Costa et al. 2020), in 2001 and 2005 in Kuwait (Majeed et al. 2018), in 2010, 2014 and 2016 in Pakistan (Raja et al. 2014; Khushdil et al. 2016), in 2010 and 2014 in India (Sarkar et al. 2014) and in 2012 in Malaysia (Rossle et al. 2012). Sporadic cases of human Cryptosporidiosis have been reported in Iraq, Jordan, Saudi Arabia, China, Iran and Lebanon (Feng et al. 2012; Osman et al. 2015). Cryptosporidium is a parasitic protozoan that can live in both humans and animals. Cryptosporidiosis is considered to be a zoonotic disease due to its broad host range. Humans and animals become infected when they come into touch with animal waste or consume contaminated food and water. This is because both human pathogen oocysts and non-human pathogen oocysts are of the same anatomical structure (Zakir et al. 2021). Since the 1980s, cattle have been the primary source of zoonotic Cryptosporidiosis. Many cases of Cryptosporidiosis occurred due to contacts with infected calves, veterinary students, researchers, practitioners and children, who attended farming camps and animal markets or shows. Many waterborne and foodborne incidents of Cryptosporidiosis have been associated with water and food contamination by animal waste (Caffarena et al. 2020). Interactions between birds and humans have been documented to spread zoonotic Cryptosporidiosis, leading to severe illnesses and deaths, especially in immuno-compromised individuals. It has been found that the incidence of Cryptosporidiosis in Asian countries varies greatly, the range being 8-35%. From 2009 to 2017, the number of human Cryptosporidiosis outbreaks in the United States increased at an annual rate of 12.8%. In under-developed countries, the situation could be even worse (Zaheer et al. 2021). Foodborne diseases cost the global economy by $15.5 billions per year due to infections caused by enteric microorganisms. Cryptosporidium is a protozoan parasite that can spread through food, causing 800,000 foodborne illnesses each year. Foodborne disease impacts have still not been thoroughly studied but are estimated at US$10-83 billion, US$86 million, US$1,289 billion and US$171 million per year in the United States, New Zealand, Australia and Sweden, respectively (Toljander et al. 2012). Cryptosporidiosis is a protozoan infection caused by Cryptosporidium, which resulted in

8.6 million foodborne diseases, with 3759 deaths worldwide in 2010 (Ryan et al. 2018). According to a 2015 survey by the Global Enteric Multicenter Study (GEMS), Cryptosporidium is the second most common cause of diarrhea-related mortality in children under 5 years of age, accounting for 0.6 million deaths (Sow et al. 2016). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recently rated food-borne parasites based on public health, animal health, microbial ecology, agribusiness and social and economic consequences. As a foodborne pathogen, Cryptosporidium ranks fifth among the 24 possible foodborne parasites, next only to *T. solium, E. granulosus, E. multilocularis and T. gondii* (Ryan et al. 2018). Cryptosporidium is one of 10 foodborne pathogens tracked by the United States Food Net surveillance system (Crim et al. 2014). Most of the parasitic diseases associated with diarrhea have not been reported, particularly in the developing countries, due to poor identification and monitoring systems.

Micro and Macro Characteristics of the Pathogen

Cryptosporidium is a protozoan pathogen genus, sometimes informally referred to as Crypto, belonging to the Apicomplexa phylum that is responsible for causing gastrointestinal diseases in both animals and humans (Betancourth et al. 2021). Two types of diseases, intestinal Cryptosporidiosis and respiratory Cryptosporidiosis, have been reported in both the immune-deficient and immuno-compromised individuals (Madbouly et al. 2021). The phylum Apicomplexa covers pathogens of public health concern, including the Plasmodium species and the Toxoplasma species. Cryptosporidium species have a variety of peculiar characteristics, including an endogenic growth period of epithelial microvilli, pathogenic and nonpathogenic oocysts have same morphology, oocysts containing smallest number of sporozoites, and organelles are multi-membranous. DNA studies have shown a correlation with gregarious instead of coccidia. The taxonomic classification of Cryptosporidium has not yet been established (Zahedi et al. 2020).

Cryptosporidium species have a wide variety of host species. Polymorphism, anatomy, genetics and molecular data within the species recommend that Cryptosporidium be classified into a specific species. To date, twenty species of Cryptosporidium have been isolated and identified on the basis of the global results, and have been presented in Table 1 (Swaffer et al. 2018). Among these, *C. parvum* and *C. hominis* are more predominant in humans (Krumkamp et al. 2020). Many Cryptosporidium species have infected a range of domestic, wild, and companion animals, as listed in Table 2.

Molecular genotyping tools, such as polymerase chain reaction (PCR; 830 bp), restriction fragment length polymorphism (RFLP; SspI and VspI restrictions fragment), quantitative polymerase chain reaction (qPCR) and nested PCR, have been used to identify different Cryptosporidium species e.g. *C. parvum, C. hominis, C. meleagridis* at genetic level (Falohun et al. 2021; Fan et al. 2021; Mohammad et al. 2021). The glycoprotein gene (gp60/gp40/15) with a molecular weight of 60 kDa is widely used to subtype Cryptosporidium species such as *C. hominis, C. parvum, C. meleagridis* and *C. ubiquitum* (Li et al. 2021).

Table 1: List of Cryptosporidium species that cause disease in humans

Sr. No.	Name of Species	Reference
1.	*C. hominis*	Krumkamp et al. 2020
2.	*C. parvum,*	Krumkamp et al. 2020
3.	*C. canis*	Lee et al. 2019
4.	*C. muris*	Silva and Sabogal-Paz 2020
5.	*C. felis*	Ayres et al. 2020
6.	*C. meleagridis*	Yildirim et al. 2020
7.	*C. ubiquitum*	Braima et al. 2021
8.	*C. andersoni*	Haghi et al. 2020
9.	*C. cuniculus*	Chalmers et al. 2011
10.	*C. viatorum*	De-Lucio et al. 2016
11.	*C. garnhami*	Yang et al. 2021
12.	*C. enteriditis*	Díaz et al. 2018
13.	*C. suis*	Liu et al. 2020
14.	*C. tyzzeri*	Díaz et al. 2018
15.	*C. scrofarum*	Danišová et al. 2017
16.	*C. erinacei*	Yang et al. 2021
17.	*C. cervine*	Insulander et al. 2013
18.	*C. horse*	Shahiduzzaman and Daugschies 2012
19.	*C. xiaoi*	Dessì et al. 2020

Genetic heterogeneity, such as mutation in tri-nucleotide repetition (TCA, TCG or TCT), has been seen at the 5′ end of the gp60 gene coding sequence, although major phenotypic mutation is also observed in the remaining portion of the gene. It should be noted that the widely used gp60/gp40/15 PCR primers do not amplify the DNA of *C. felis, C. ubiquitum, C. canis* and species not related to C. hominis and *C. parvum* (Lee et al. 2019). The Cryptosporidium subtype nomenclature is based on the gene gp60. According to this nomenclature, the subtype family represents letters e.g. *C. hominis* (Ia, Ib, Id, If, etc.); *C. parvum* (IIa, IIb, IIc, etc.); *C. meleagridis* (IIIa, IIIb, etc.), followed by the number of trinucleotide repetition sequences such as A is represented by TCA, G is described by TCG and T is defined by TCT. For example, IeA11G3T3 subtype nomenclature indicates that le is a subtype family of C. hominis, 11, 3 and 3 copies belong to the trinucleotide repeat regions of TCA, TCG and TCT, respectively (Rojas-Lopez et al. 2020). The possible organization among subtype families and phenotypes of *C. parvum* and *C. hominis* is one advantage of using gp60 for subtyping. The potential for correlation among subtype families and phenotypes of *C. hominis* and *C. parvum* is beneficial for the use of gp60 for subtypes. Glycoprotein gp60 has also been discovered on the parasite's surface epithelium. It has the ability to suppress human antibody response to parasitic invasion (Wang et al. 2020; Zahedi et al. 2021). Micro and mini satellite genomic sequences of various Cryptosporidium species have been analyzed using whole genome sequencing methods, such as locus fragment typing (MLFT) or multilocus sequence typing (MLST) (Innes et al. 2020; Liu et al. 2020).

Table 2: List of Cryptosporidium species which cause disease in different animals

Sr. No.	Host	Name of Species	Reference
1.	Cattle	*C. parvum, C. bovis, C. ryanae and C. andersoni, C. felis, C. hominis, C. scrofarum, C. serpentis*	Haghi et al. 2020; Mohteshamuddin et al. 2020
2.	Sheep/Goat	*C. parvum, C. ubiquitum, C. xiaoi*	Dessì et al. 2020; Kabir et al. 2020
3.	Pigs	*C. suis, C. scrofarum*	Wang et al. 2020
4.	Dear	*C. ubiquitum, C. parvum*	Yang et al. 2020
5.	Camels	*C. andersoni*	Silva and Sabogal-Paz 2020
6.	Alpacas	*C. parvum*	Shrivastava et al. 2017
7.	Rabbits	*C. cuniculus*	Yang et al. 2021
8.	Birds	*C. meleagridis*	Novaes et al. 2019
9.	Guinea pigs	*C. wrairi*	Ježková et al. 2021
10.	Snakes	*C. serpentis*	Laroucau et al. 2020
11.	Fish	*C. molnari*	Golomazou and Karanis 2020
12.	Kangaroos	*C. fayeri*	Braima et al. 2021
13.	Tortoise	*C. ducismarci*	Rostad et al. 2019

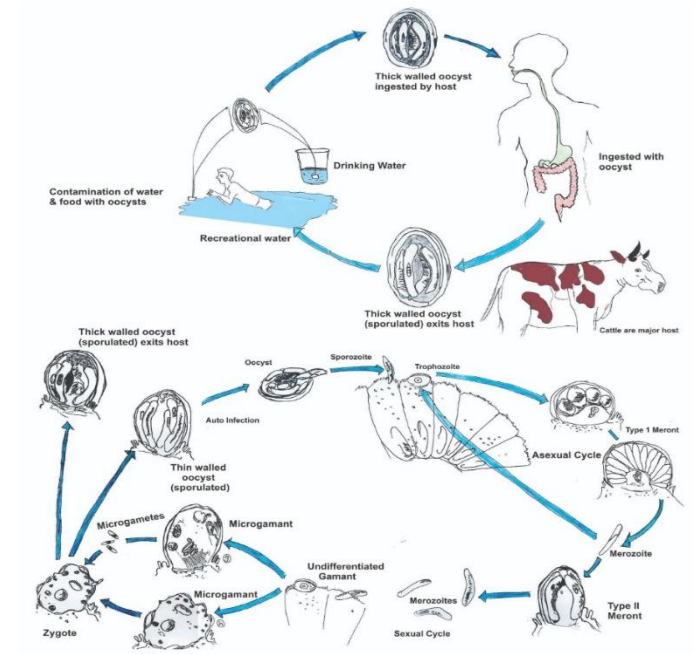

Fig. 1: Life cycle of *Cryptosporidium parvum.*

Life Cycle of Cryptosporidium Species

The peculiarity of *C. parvum* or *C. hominis* life cycle began with the intake of thick walled sporulated oocysts presented in Figure 1 (Pinto and Vinayak 2021). Cryptosporidium oocyst wall proteins and surface receptors have the ability to withstand various disinfectants, such as the bleach and environmental exposures, e.g. sunlight. Bile salts, optimum temperature of 37°C and taurocholic acid play a significant role in the oocyst excystation of *C. muris, C. andersoni*, *C. parvum* and *C. hominis* (Silva and Sabogal-Paz 2020). Sporozoites are released and penetrated by excystation into the outer small intestine enterocytes. Sporozoite apical complex has a gliding potential to adhere to intestinal epithelium cells to establish parasitophorous vacuole (Guérin and Striepen 2020). CP47 is a membrane-associated sporozoite protein that binds specific cell receptors to host enterocytes. After attachment, the micronem and the dense granules move towards binding sites. The complex is engulfed by the host cell and the surrounding microvilli enlarge. The parasitophorous vacuole has revamped the cytoskeleton of the infected cells and has been responsible for taking nutrients from the host cytoplasm. Sporozoite converts into trophozoite after enclosure of the sporozoite within the parasitophorous vacuole, which is the establishment of the asexual cycle. Mitosis division in sporozoites results in the production of type I meront. Each type I meront converts into 6-8 type I merozoites, which resemble with the sporozoites. Type I merozoites escape from enterocytes and convert into 4 type II merozoites. It infects the nearby enterocytes, producing male and female microgametes. These male microgametes (microgamont) and female microgamete (macrogamont) can fertilize to form a diploid zygote, which differentiates into an oocyst. Meiosis division in oocyst results in 4 sporozoites formation. This is the sexual life cycle of Cryptosporidium species. At the end, fully developed oocyst is excreted into the environment (Tandel et al. 2019).

Epidemiology of Cryptosporidiosis

Cryptosporidium pathogens may be zoonotic or Anthroponotic *(*Nader et al. 2019*)*. Cryptosporidium oocysts are transmitted from one host to another through the faecal oral route via contaminated faeces, droppings, food, water and utensils *(*Zahedi et al. 2020*)*. Person-to-person transmission of Cryptosporidium oocysts may be observed *(*Chalmers et al. 2019*)*. Cryptosporidium infection is thought to be responsible for 30 to 50% of all youth deaths worldwide, and it is the second most common cause of diarrhoea and death in children after infection with some viral disease. Polluted outdoor recreation or drinkable water, raw vegetables, contamination of fruit with animal feces, direct contact with Cryptosporidium, travelling to affected regions, changing diapers, caring for an infected person, or an infected animal such as handling a cow and calf have been implicated in the spread of Cryptosporidiosis (Bouzid et al. 2018; Khan et al. 2019; Makawi et al. 2021). Although Cryptosporidium species do not reproduce in milk, infected oocysts may withstand. Cryptosporidium species have been found in raw milk from cattle, goats and sheep in Norway, Iraq, Australia and Thailand. In areas where

Cryptosporidiosis is widespread and linked to a specific burden of gastric illness and death, particularly among children, the hazards of Cryptosporidiosis from raw milk should not be underestimated (Ursini et al. 2020). Drinking water contamination with Cryptosporidium species may be very dangerous, as many people can get the infection in a short period of time. Cryptosporidium oocysts are quite small, making it difficult to remove them from the public water supply, as the filters used are wider than that of the oocyst itself. Oocyst cannot be chemically inactivated by chlorination or other techniques commonly used for the treatment of drinking water. In certain regions, the use of ozone and ultra violet light has recently proven to be very efficient in inactivating Cryptosporidium oocysts present in drinking water (Silva and Sabogal-Paz 2020).

To date, the largest epidemic caused by Cryptosporidium was found in Milwaukee, Wisconsin, USA in 1993, where about 0.4 million out of 1.6 million people became infected and 54 died (Choy and Huston 2020). Initially, the epidemic was thought to be caused by a malfunction in the clean drinking water system, following the contaminated feces of the animals entering the system. However, Milwaukee's wastewater gene typing showed that *C. hominis* was the most common species, stating that the epidemic was Anthroponotic, not zoonotic. In 1993, the Milwaukee epidemic was estimated to cost US$ 96 million, including maintenance and loss of production costs, highlighting the economic value of ignoring such contamination in the purification of waste water (Chyzheuskaya et al. 2017). Cryptosporidiosis distribution has also been associated with adulterated entertainment water, such as that found in parks and swimming pools (Hassan et al. 2021). *C. hominis* is the primary causal species in most human Cryptosporidiosis studies. Individuals in rural communities, particularly children, may be much more likely to be infected with *C. parvum* than children and youth, where *C. hominis* predominates. Because of the abundance of farm animals and other domestic pets in remote regions, there is a wider diversity of Cryptosporidium species in such regions (Yang et al. 2021). Outbreaks of *C. parvum* have been reported in urban areas by veterinary students, who worked with calves and livestock farm workers and zoo workers (Mravcová et al. 2019; Thomas-Lopez et al. 2020). The foodborne transmission of Cryptosporidium occurs when food, such as vegetables and fruits, are exposed to contaminated water during washing and processing. Foodborne Cryptosporidiosis transmission is slightly lower than that of water-borne transmission, probably due to the low risk of detection or lack of reporting (Karanis 2017). Anthroponotic transmission of *C. hominis* has been observed in people working in hospitals and nurseries, and can be in close contact with contaminated human faeces. The spread of disease by drinking water and recreational water has been linked to human-to-human transmission (King et al. 2019). The infective dose of Cryptosporidium oocysts has been quantified in various host species. When the same Cryptosporidium and host species were studied, the results differed from study to study. The infective doses of *C. parvum* oocysts as 10-30 oocysts, 16.6 oocysts and 87.0 oocysts in different hosts have been determined (Craighead et al. 2021). The main Cryptosporidium oocyst peeling was recorded in calves such as 10^4-10^8 oocysts/gm of faeces (Mammeri et al. 2019).

Immunopathology of Cryptosporidium

Cryptosporidium species are obligate intracellular protozoan parasites that are responsible for gastroenteritis in humans and animals worldwide. *C. hominis* and *C. parvum* are the main causative agents of zoonotic Cryptosporidiosis. Profuse watery diarrhoea is a pathognomic sign of Cryptosporidiosis. In immuno-competent individuals, the disease is self-limiting, whereas severe and life-threatening diseases have been observed in immuno-compromised individuals, particularly AIDS patients (Weerasooriya et al. 2020). *C. parvum* mainly infects the small intestine enterocytes. Sexual, as well as asexual, life cycle starts in host epithelial cells, particularly in the ileum.

Comprehending the intricate evolutionary biology of *C. parvum*, as well as the regular underlying biosynthetic processes that cause disease throughout host parasite relationships, has received less attention. Infection with *C. parvum* begins with oocysts ingested by humans from infected animals, food and water. Sporozoites are released from the excystation. Thus, sporozoite adhesion to intestinal epithelium of the host and subsequent invasion are critical first steps in the pathophysiology of Cryptosporidiosis. Electron micrograph showed that sporozoites bind to host cell membranes of intestinal epithelium via the anterior pole. After engulfing, parasitophorus vacuole is developed. The ability of sporozoites to attach to the host epithelial cells depends on several factors, such as the infective dose of sporozoites, incubation period, pH, cations and the immune status of individual hosts. Various low molecular weight glycoprotein receptors, such as thrombospondin-related anonymous proteins (TRAPs, 22), GP900, gp15/40, CSL glycoprotein, lectin and galactose-N-acetylgalacto-samine (Gal/GalNAc), have recently been recognized on the exterior of sporozoites (Guérin and Striepen 2020).

GP900 is an antigen found in *C. parvum* trophozoites and merozoites and is recognized by immunoglobulin found in colostrum milk. GP900 is abundant on sporozoite and merozoite surfaces. The immune system of the host identified this antigen. GP900 consists of a large N-glycosylated proximal membrane core region, cysteine and polythreonine domains. It is found in micronemes and also at the surface of invading phases and contributes in the cascade of host epithelial cell penetration (Pinto and Vinayak 2021). *C. parvum* sporozoite surface contains Gal/GalNAc-specific lectin ligands, which help to bind sporozoite to the epithelial and biliary cells of the host. The 49 kDa precursor protein is distinguished between gp15 and gp40 glycoproteins after proteolytic cleavage. Both encode the same gene but differ in position and antigenicity. Gp15 is located on the entire surface, whereas gp40 is present in the apical complex of sporozoites.

Research has shown strongly that both of them are of key importance for host cell binding to establish pathogenicity (Fereig et al. 2018). Thrombospondin-related anonymous proteins (TRAPs) have been detected at the apical end of sporozoites and have been identified to be essential for the attachment, movement and penetration of host cells. Recently, a total of 12 genes, with TSP1-like domains, have been identified in the *C. parvum* genomic sequence. CqTSP3 and TRAPC1 are developed at both early and later stages of infection with *C. parvum*, while CpTSP5 and CpTSP2 are found during later stages of infection. CP47 and circumsporozoite-like (CSL) glycoprotein are other receptors present at the anterior surface of sporozoites and merozoites that have the ability to bind to host cells. Early research has also shown that the binding ability of CP47 is dependent on the concentration of manganese (Lendner and Daugschies 2014).

The attachment of Cryptosporidium to intestinal mucosa is crucial for both parasitic proliferation and immune system activation (Hemphill et al. 2019). Intestinal epithelial cells (IECs) have a protective barrier to infectious microbes, due to the presence of numerous physiological and biochemical factors and stimulate innate immune response. They contain pattern-recognition receptors, such as intracellular Nod-like receptors and Toll-like receptors, which help to detect parasites and stimulate innate immune response by caspase-1 inflammasome and eNF-kB pathways. The major class I and class II histocompatibility complex (MHC) molecules are also present on the surface of the IECs. IECs also contain antigen presenting cells, particularly dendritic cells that recognize *C. parvum* antigen and stimulate the production of chemokines. The concentration of IL-8 and IL-18, b-defensin-2 and cathelicidin LL-37 was found to be increased after *C. parvum* infection in mice (Tessema et al. 2009). The findings of research on *C. parvum* infection have revealed a low concentration of epithelial b-defensin-1 on the intestinal mucosal surface.

IFNγ plays an important role in activating innate immunity and cell mediated immunity against *C. parvum,* as demonstrated in laboratory animals. The concentration of IFNγ has been increased in the human and calves enterocytes after *C. parvum* challenge infection. However, the exact mechanism for stimulation of IFNγ is not clear. Tumor necrosis alpha has been reported to increase the production of IFNγ and to inhibit the replication of *C. parvum* by decreased oocyst shedding. Macrophages and neutrophils produce nitric oxide that eliminates invasive microorganisms. Nitric oxide production is increased significantly during Cryptosporidium infection, possibly due to the production of nitric oxide enzyme by the infected host gastric mucosa. The role of dendritic cells in Cryptosporidium defense has not been fully investigated. Dendritic cells are involved in the removal of *C. parvum* through stimulating the production of pro-inflammatory cytokines by enterocytes in response to pathogens. Complement is an integral part of humoral and innate immune system and is triggered by three paths such as lectin pathway, classical pathway and alternative pathway. Among these, lectin pathway plays a significant role in clearing *C. parvum* infection (Petry et al. 2010).

Humoral immunity has more significant effect against Cryptosporidiosis as compared to cell mediated immunity. First time T-cell role against Cryptosporidium infection was reported in congenitally athymic (SCID) mice in which T-cells were absent. It has also been shown that MHC-11 deficient mice are more prone to *C. parvum* infection. Human research has shown the need of CD^{4+} T-cells for protection, as HIV/AIDS patients with minimum CD^{4+} T-cell count are most seriously affected. T-cells are further divided into Th-1 and Th2 on the basis of protein expression studies. Th1 cells produce interferon alpha (*IFN-α*), interferon gamma (IFNγ) and IL-12, which are especially effective in the defense against intracellular pathogens such as *C. parvum*. IL-2 is a key regulator for the up-regulation of IFNγ through an IFNγ dependent mechanism that directly inhibits the growth of *C. parvum*. Th-2 cells produce different forms of interleukin, such as IL-13, IL-5, IL-10, IL-4, which stimulate and promote the development of B-cells (Stoyanova 2020).

Cryptosporidiosis Disease

All species of Cryptosporidium do not cause the same disease in all animals and humans. In most mammals, the illness can be self-limiting to chronic. The main clinical signs and symptoms of Cryptosporidiosis are loss of appetite, watery diarrhoea, and abdominal pain (Yang et al. 2020). Some species of Cryptosporidium, such as *C. hominis, C. meleagridis, C. serpentis, C. xiaoi* and *C. varanii,* produce similar type of disease in humans, birds, snakes, goats and lizards, respectively, whereas other *Cryptosporidium* species produce different types of clinical signs and symptoms. C. *baileyi* is the causative agent of respiratory Cryptosporidiosis and produce respiratory signs and symptoms in birds. Other Cryptosporidium species, such as *C. rayanae* and *C. bovis,* are non-pathogenic in some hosts and normally present in calves. *C. parvum* causes severe disease in immuno-compromised individuals, which may lead to death. It causes two type of diarrhea, malabsorptive diarrhea (MD) and secretory diarrhea (SD). In MD, villi of intestinal cells are destroyed, resulting in reduced intestinal surface area, which may lead to decreased water and nutrient absorption. In SD, microorganisms produce *Vibrio cholera* like toxins, which stimulate the secretion of epithelial cells of the host, resulting in profuse watery diarrhoea (Di Genova and Tonelli 2016).

Animal Cryptosporidiosis

Cryptosporidium species cause Cryptosporidiosis disease in large and small ruminants worldwide, particularly in neonatal calves and lambs (Thomson et al. 2017). Four species of Cryptosporidium including *C. parvum*, *C. andersoni, C. rayanae* and *C. bovis* have been found in large ruminants, particularly cattle and buffaloes (Haghi et al. 2020). *C. parvum* is responsible for severe infection in neonatal calves, causing profuse diarrhea and possibly death, but the disease is asymptomatic in animals older than six weeks. In all of these species, the organism

possibly infects the epithelial cells of the small intestine of weaned calves (Yang et al. 2020). According to age-related hypotheses of Cryptosporidium species found in many host species, it may be due to changes in normal intestinal microflora and may also be the result of changes in diet, both of which reduce the capacity of Cryptosporidium species to infect adult animal enterocytes. However, no experimental trials have been documented to confirm this in cattle. No age-related disease has been observed in *C. rayanae* and *C. bovis* infections (Díaz et al. 2018).

C. anderson affects the epithelial cells of adult cattle abomasum compared to young cattle and causes a decrease in milk production in adult cows. *C. parvum* infection in neonatal calves may lead to lethargy, profuse watery diarrhea, dehydration, inappetence and death in severe cases. Diarrheal signs are observed after 3-4 days of absorption of oocysts and may last of about 1-2 weeks. It has been seen that the oocyst shedding is often not linked with gastroenteritis. Infected neonatal calves, however, shed 1×10^{10} oocysts per day (Mohteshamuddin et al. 2020; Shaw et al. 2020; Wu et al. 2020).

Cryptosporidiosis in sheep was first seen in lambs less than 3 weeks of age suffering from severe diarrhoea on a small ruminant farm in Australia. Australia was also the first country to report goat Cryptosporidiosis. *C. ubiquitum, C. xiaoi and C. parvum* have been found to cause infection in small ruminants. *C. ubiquitum* infects animals of all age groups but *C. xiaoi* and *C. parvum* predominantly infect lambs and goat kids *(*Dessì et al. 2020*)*. Cryptosporidiosis in small ruminants causes more severe disease as compared to large ruminants in terms of morbidity (100%) and mortality (50-70%). The parasite infects the epithelial cells of the small and large intestines, particularly jejunum and ilium. Clinical signs and symptoms, such as anorexia, abdominal pain, depression and diarrhea, have been reported due to infection with Cryptosporidium (Mammeri et al. 2019; Santin 2020; Kabir et al. 2020).

Avian Cryptosporidiosis

Cryptosporidium species infect different avian species, like chicken, quail, turkey etc. Various types of Cryptosporidiosis such respiratory, ocular, renal and intestinal have been observed based on clinical signs and symptoms. Respiratory Cryptosporidiosis is more common in domestic poultry birds than intestinal Cryptosporidiosis except turkey (Zaheer et al. 2021). *C. meleagridis, C. baileyi* and *C. galli* are the important causative agents in avian Cryptosporidiosis that infect chickens, turkeys, quails, ostriches, Passeriformes and Psittaciformes (Novaes et al. 2018; Kabir et al. 2020). *C. meleagridis* is found in the bursa fabricius, cloaca and intestinal tract in chickens and turkeys but clinically disease is present in turkeys. The main clinical signs and symptoms of avian Cryptosporidiosis include diarrhoea, small intestinal gas and mucus edoema, reduced weight gain, and coughing (Holubová et al. 2017). *C. baileyi* causes respiratory Cryptosporidiosis in ducks, turkeys and chickens. It is the most common source of Cryptosporidiosis in chickens, where it usually appears as a respiratory disease with only small intestine symptoms. Infection with *C. baileyi* has also been linked to reduced body weight in broilers (Baines et al. 2020). *C. galli* does not cause infection in the epithelial cells of the intestines and respiratory tract, but it infects the proventriculus, causing diarrhoea and high mortality rates in birds (Holubová et al. 2017). Cryptosporidium species also infect different species of pigeons, particularly carrier pigeons. Recently, three species of Cryptosporidium including *C. meleagridis, C. hominis* and *C. baileyi* have been isolated in pigeons from Spain and China (Li et al. 2015). Enteritis, with other major clinical signs such as diarrhoea, has been seen in pigeons (Oliveira et al. 2017). Cryptosporidium species such as *C. baileyi, C. ornithophilus,* and *C. ubiquitum* have been also isolated from ostriches farmed in Vietnam and China (Holubová et al. 2019).

Human Cryptosporidiosis

Cryptosporidiosis is a disease that affects humans in both developed and developing countries, as presented in Table 3. Young persons are more resistant to disease than children and the elderly persons. Infections usually attack babies after or during weaning. Cryptosporidium infection in people with strong immunity usually causes self-limiting diarrhoea, but a recent report states that Cryptosporidium is the second leading cause of neonatal diarrhea and death in various African and Asian countries, indicating that Cryptosporidiosis is not always moderate and self-limiting disease (Guy et al. 2021). To date, twelve species of Cryptosporidium such as *C. parvum, C. canis, C. hominis, C. cuniculus, C. viatorum, C. bovis, C. muris, C. ubiquitum, C. suis, C. fayeri, C. meleagridis,* and *C. felis* have been found in human beings worldwide (Danišová et al. 2017; Ayres et al. 2020; Yildirim et al. 2020; Braima et al. 2021). Among these, *C. parvum* and *C. hominis* more frequently infect humans (Xiao and Cama 2018). The average incubation period ranges from 8 to 14 days. *C. hominis* produces more sever disease in humans than *C. parvum* (Shrivastava et al. 2018). The infection is usually found in the small intestine, but it can also spread to the large intestine and stomach. Human infections concentrated in the small intestine are more severe and cause more severe watery diarrhea. Distal ileum and intestinal infections are often asymptomatic. Since diarrhoea lasts longer than one week, there is a risk of dehydration and weight loss (Chalmers et al. 2019; Zhang et al. 2020). Cryptosporidiosis may be fatal in patients with AIDS, chemotherapy and genetic disorders. People living with HIV/AIDS with blood CD4+T cell counts of <150 per ml are particularly vulnerable to Cryptosporidiosis, which causes life threatening profuse watery diarrhoea in these people (Shrivastava et al. 2017; Zakir et al. 2021).

Diagnosis of Cryptosporidiosis

Definitive sample collection technique is a pre-requisite for diagnosis of Cryptosporidiosis. Faecal samples are collected from infected animals for diagnosis purposes.

Different techniques are used for diagnosis of Cryptosporidiosis in humans, animals and birds. Wet mounting and staining techniques have been used for microscopic examination of Cryptosporidium oocysts. After collection, stool or faecal samples are preserved in sodium acetate, formalin and polyvinyl alcohol. The image of oocysts under bright field microscopy incorporating differential interference contrast is spherical to ovoid, colorless, smooth, and the width can be 4-8 um. The better outcomes from wet mount are attained when the concentration of oocysts has been achieved. Various methods have been used for achieving oocysts concentration, such as saturated salt (sucrose) flotation method, Allen and Ridley's formol-ether method and centrifugation method. Among these, formol-ether method is more sensitive one (Khurana and Chaudhary 2018). Because Cryptosporidium oocyst is so small, it may be difficult to detect in a faecal sample. Different staining techniques have been reported in the literature to identify oocysts in stool samples, including Safranin staining, Nigrosin staining, Ziehl-Neelsen staining, Giemsa staining, Malachite green staining, trichrome stain and auramine-rhodamine staining (Shanmathi et al. 2020). Light pink to bright red color, bright reddish-orange color, pale green and colorless oocycsts have been seen in Ziehl-Neelsen staining, Safranin-methylene blue stains, Giemsa staining and Trichrome staining, respectively (Cengiz et al. 2017; Xiao and Griffiths 2020).

Electron microscopy has also been reported for the identification and detailed structural morphological characterization of Cryptosporidium oocysts in humans. However, the main drawbacks are challenging processing, high machinery and installation costs and the inability to recognize a large sample size (Cunha et al. 2019). Multiple sero-immuno-based techniques, such as enzyme linked immunosorbent assay (ELISA), ELISA Sandwich, monoclonal antibodies and indirect immunofluorescence, have also been used to identify Cryptosporidium species in clinical specimens based on either antigen or antibody detection. These methods are more specific and sensitive in the 90-100 percent range (Fereig et al. 2018; Aboelsoued et al. 2020).

Table 3: Worldwide prevalence of Cryptosporidiosis in human beings

Country Name	Host species	Prevalence	Reference
Pakistan	*C. parvum*	28% - 53%	Raja et al. 2014; Khan et al. 2019
Iran	*C. parvum, C. hominis, C. meleagridis*	1% - 11%	Ghafari et al. 2018; Najafi-Asl et al. 2020
Kenya	*C. hominis, C. parvum*	4% - 75%	Deichsel et al. 2020
Turkey	*C. parvum, C. cayetanensis*	0.5%	Hayriye et al. 2017
Germany	*Cryptosporidium species*	1.1%	Kern et al.1987
Iraq	*C. parvum, C. hominis*	8% - 46%	Salim et al. 2018; Nasir et al. 2020
Egypt	*C. hominis, C. parvum*	19% - 38%	Ibrahim et al. 2016; Gabr et al. 2019; Mohammad et al. 2021
United Arab Emirates	*Cryptosporidium sp.*	19.4%	El Bakri et al. 2018
Nicaragua	*C. parvum*	35.7%	Munoz et al. 2011
Nigeria	*C. parvum, C. hominis*	2.9% - 80%	Falohun et al. 2021
Nepal	*C. parvum, C. cayetanensis*	10.7% - 29.4%	Sherchand et al. 2016; Bhattachan et al. 2017
Canada	*C. parvum, C. hominis*	15.7% - 76.33%	Iqbal et al. 2015; Guy et al. *2021*
Malaysia	*C. feli, C. hominis, C. meleagridis*	3% - 25%	Lim et al. 2011; Lim et al. 2011
Bulgaria	*C. parvum*	2.18% - 14%	Harizanov et al. 2020
Kuwait	*C. parvum, C. hominis*	3.4% - 38.4%	Alyousefi et al. 2013
Yemen	*C. parvum, C. hominis*	1% - 34.7%	Alyousefi et al. 2013
Lebanon	*C. parvum, C. hominis*	10% - 11%	Osman et al. 2015; Osman et al. 2016
Jordan	*C. parvum*	5% - 37%	Hijjawi et al. 2017
Thailand	*C. meleagridis, C. canis, C. parvum, C. hominis*	5% - 67%	Sannella et al. 2019
South Korea	*C. parvum, C. hominis*	1.5% - 57.0%	*Lee* et al. *2019*
Ethiopia	*C. viatorum, C. parvum, C. hominis, C. felis, C. viatorum, C. parvum C. hominis, C. meleagridis*	4.6% - 26.9%	De-Lucio et al. 2016
Zimbabwe	*C. parvum*	9%	Gumbo et al. 1999
Cambodia	*C. parvum, C. hominis*	2.2% - 23.5%	Nuchjangreed 2018
Uganda	*C. parvum*	12% - 13%	Siobhan et al. 2010
Poland	*C.parvum*	11.4%	Wesolowska et al. 2016
Peru	*C. parvum, C. meleagridis*	13.3%	Ulloa-Stanojlović et al. 2016
Mexico	*C. parvum*	28.4%	Quihui-Cota et al. 2015
Slovakia	*Cryptosporidium spp*	44.4%	Mravcová et al. 2019
Guinea	*C. parvum*	7.4%	Mølbak et al. 1993
Israel	*Cryptosporidium spp*	3.25%	Grossman et al. 2019
Liberia	*Cryptosporidium spp*	8.4%	Højlyng et al.1986
Mozambique	*C. belli, C. hominis, C. parvum, C. felis C. viatorum*	8.3% - 30.8%	*Casmo* et al. *2018*
Oman	*C. parvum*	1.9%	Farsi et al. 2020
Portugal	*C. parvum, C. hominis*	2.7%	Almeida et al. 2006
Australia	*C. hominis, C. parvum C. meleagridis, C. viatorum*	1% - 86%	Braima et al. 2019; Braima et al. 2021

Characterization of genetic materials of Cryptosporidium species is a useful tool for the diagnosis, epidemiological analysis and taxonomic assessment of causative agents (Cunha et al. 2019). Genomic strategies, such as polymerase chain reaction, nested PCR, DNA hybridization methods, multiplex-PCR, qPCR and single-stranded polymorphism PCR (PCR-SSCP), have been effectively used to identify and characterize different species of Cryptosporidium (Claudel et al. 2021; Yin et al. 2021).

Treatment of Cryptosporidiosis

Previous studies have shown that the humans and animal species infected with Cryptosporidium usually recover spontaneously without any specific treatment. It could be due to their strong immune system. Some supportive treatments, such as electrolyte liquid treatment, pain killer administration, antiemetics and anti-nausea drugs, can help to overcome certain clinical signs such as dehydration, fever, headache, vomiting, nausea and abdominal pain. These drugs may help to treat symptoms of Cryptosporidiosis, but antiprotozoal treatment is also required in certain situations. The anti-Cryptosporidial Nitazoxanide drug has been approved for human use by US Food and Drug Administration (Bamaiyi and Redhuan 2017; Innes et al. 2020). However, its effectiveness in immune-suppressed patients would be questioned. Few updates on the use and effectiveness of nitazoxinide in animals have been documented globally, which show that nitazoxanide may mitigate the release of Cryptosporidium oocysts (Pumipuntu and Piratae 2018).

A variety of chemical agents, such as azithromycin, sinefungin, paromomycin, Letrazuril and roxithromycin, have been used to control clinical signs and symptoms caused by Cryptosporidiosis (Rossignol 2010). The combination of nitazoxanide and anti-retroviral therapy in HIV/AIDS patients has shown satisfactory results (Leitch and He 2011). Paromomycin is an anti-Cryptosporidial aminoglycoside used in the treatment of Cryptosporidiosis in cattle, calves, lambs and goats; this has resulted in reduced oocyst shedding. The appropriate dose of paromomycin is 100 mg/kg body weight up to 3 weeks after birth (Brainard et al. 2020). Halofuginone lactate and Decoquinate drugs are also used in animals for the treatment of Cryptosporidiosis. Halofuginone lactate has also been used for the treatment of avian Cryptosporidiosis caused by *C. baileyi* infections (Shahiduzzaman and Daugschies 2012). Paromomycin, azithromycin and tylosine have been used in the treatment of feline Cryptosporidiosis (Lappin et al. 1997).

Prevention of Cryptosporidiosis

Cryptosporidiosis is caused by various Cryptosporidium species through fecal-oral route. Infected oocysts are secreted into the environment. Cross-contamination with oocysts in drinking water and raw food from domestic and companion animals in community, slaughterhouses and certain other vectors such flies, rats and mice are the common sources of infection. Contaminated water supply and raw food can result in human illness (Ayres et al. 2020).Thus, the most effective strategy for preventing Cryptosporidium spp. spread in humans is to start practicing hygienic practices, which include hand washing before preparing food and eating, after using the lavatory, and after contact with diarrhoeal patients, children, and domestic and pet animals (Pumipuntu and Piratae 2018).

Water or raw food should be properly cleaned, rinsed, warmed, boiled, or cooked before intake. Patients with diarrhoea should also be conscious that they should not swim in a public swimming pool, beach resort, or pond to avoid spreading the disease to others. Moreover, people who swim in a pool, beach club, or sea should be aware of the consequences of disease if the water is contaminated (Ng-Hublin et al. 2015). The standard recommended dose of various commercially available disinfectants, such as Sorgene®5, FAM® 30, and Virkon® S, was unable to eliminate Cryptosporidium species oocysts, particularly in livestock farm areas. Little research on the field trials of disinfectants has been reported against Cryptosporidium oocysts in cattle farms (Delling et al. 2017).

Good hygienic farm management practices are the only methods to reduce the spread of Cryptosporidiosis. In livestock farms, the spread of Cryptosporidiosis through faeces was reduced by using clean feeding and watering cans. Colostrum must be given to newborn calves within 24 hours of birth, and malnutrition should be avoided. Dairy calves of the same age group should be kept in separate pens that are disinfected daily. In calf farms, where *C. parvum* has already been found, halofogenone can be used for 7 days to treat Cryptosporidiosis 24 hours after calving (Innes et al. 2020). The beddings should be kept dry at all times in poultry farms, with special attention to bedding near water sources or breeding wells. Always keep fonts above the drain pan or landfill to prevent water from entering the landfill. Feeding and watering equipment must be of a specific type and height to avoid contamination by feces. Batch cultivation, population reduction, and proper disinfection processes would help keep infection levels under control (Cunha et al. 2019).

Conclusion

Although Cryptosporidium has a history of more than a century, it is still one of the most challenging microbes to manage in livestock farms and the human animal related environment.

Cryptosporidiosis is a protozoa infection that causes gastrointestinal side effects in humans and animals all around the globe. It is a major health-related problem among children between the ages of 1–5 years, especially in the developing countries. It is transmitted to humans and animals through infected food and water. The disease begins after ingestion of thick-walled infectious oocysts that remain in the environment for a long time and are not harmed by traditional disinfection methods. Human and animal Cryptosporidiosis is caused by *C. parvum*. The disease inhibits itself in immunocompetent peoples, but

persistent and life-threatening disorders have been observed in immunosuppressed people, particularly in AIDS patients. The drug nitazoxanide has been sanctioned for human use by the US Food and Drug Administration. The only way to combat Cryptosporidiosis is through good farm and human hygiene practices.

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TOXOPLASMOSIS IN PUBLIC HEALTH

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INTRODUCTION

Toxoplasmosis is categorized as a parasitic disease, caused by *Toxoplasma gond*ii (*T. gondii*); it infects humans all over the world. Toxoplasmosis spreads through multiple intermediate hosts including, rodents, marine mammals, cattle, birds, goats, sheep, pigs and humans by ingestion of speculated oocytes. Due to the emergence of different ongoing, as well as previously reported, pandemics (human deficiency syndrome virus) which severely damaged the immune system, the risk of *T. gondii* increases in humans and it became a worldwide public health concern (Hooshyar et al. 2007; Gonzalez et al. 2007; Rafique 2017). *T. gondii* remains asymptomatic in healthy persons but can be very severe in immunocompromised population. *T. gondii* is most common zoonotic pathogen/ parasite that can be transferred from animals to humans and cause disease (Hill et al. 2005). *T. gondii* is excreted in cat feces, which had been transferred from other animals in the form of toxoplasma oocysts. *T. gondii* causes huge economic losses in livestock industry through neonatal losses, abortions and stillbirth (Buxton 2000). The global distribution of toxoplasmosis in humans depends on the geographical distribution. *T. gondii* causes the primary infection in pigs, sheep and goats during their pregnancy period and causes abortions, stillbirths or infertility according to the stage of pregnancy. In the case of abortion, a doe or ewe in mid gestation period gives birth to stillborn lamb before the predicted end of pregnancy period.

History

T. gondii was discovered first time in a rodent "Gundi", a small rodent lived in hilly area and mountains in north of African sub-continent. Similar discovery was reported in Sao Paolo, Brazil in rabbits (Splendore 1908). In New York, *T. gondii* was isolated from the tissue of congenitally infected infant's tissue in 1939. Moreover, Sabin and Feldman (1948) created serological test based on the patient's antibodies to alter the staining of Toxoplasma. This test proved more significant in sheep abortion storms during 1957. In 1970s, bone marrow transplant and immune suppressant treatment were on peak, AIDS pandemic in 1980s gave more importance to Toxoplasma (Ferguson 2009).

Toxoplasma Genotypes/Strains

Various strains of Toxoplasma have been reported in literature with passage of time (Keymer 1981; Levine 1985). In the current era with the aid of molecular techniques, Toxoplasma has been separated from protozoan on the basis of morphology and physical characteristics. Overall, it is accepted worldwide that Toxoplasma has only one species, *T. gondii*. Differences can be observed on the basis of pathogenicity in variety of hosts (Dubey 2010). Before the evolution of genetic markers, *T. gondii* was grouped on the basis of virulence in outbred mice. In 1980s-1990s, discoveries in genetics made easier to identify the genetic differences between *T. gondii* isolated from humans and animals (Pfefferkorn and Pfefferkorn 1980; Darde et al. 1988; Tibayrenc et al. 1991; Sibley et al. 1992; Howe and Sibley 1995; Darde 2008). Howe and Sibley (1995) classified the organism on the basis of DNA RFLP (restriction fragment length polymorphisms) into three types (I, II, III) and related them with the virulence in mice. The result showed that type I was 100% virulent and lethal, while type II and type III proved avirulent strains in mice (Howe et al. 1996). The type I and type III *T. gondii* are more frequently prevalent in clinical Toxoplasmosis than type II. The limited molecular and genetic characterization of clinical isolates of Toxoplasmosis shows the suggested results. As concerned to general human population, the genetic analysis and diversity of *T. gondii* is not much reported, with diminutive literature is available about humans. The recent revolutionary change adopted by *T. gondii* is direct oral transmission through recombination among other parasites and discrete clonal lines of parasites. The direct transmission of *T. gondii* aids in very quick and global distribution (Montoya and Liesenfeld 2004).

Epidemiology

Toxoplasmosis is a worldwide disease that is prevalent in both animals and humans. The disease is usually found in the asymptomatic to mild states, but its spread and prevalence depends on the age and lifestyle of the host. Globally, half a billion humans have the *T. gondii* antibodies. The frequency of disease may vary within a country in humans and animals. The variation factors of Toxoplasmosis in a country are still unknown. The severity and transmission of the disease may be depending on factors such as different animal species, physical and environmental factors and cultural habits. According to the CDC (2013), nearly 22.5% population aged ≥12 year in USA has been infected with Toxoplasma. The rate of Toxoplasmosis increased in Europe from 50 to 75%, while

in Asia, Africa and South America the disease rate is high (up to 90%), probably due to the awareness about the disease and improved diagnostic facilities. The rural areas and farms with poor hygiene conditions have Toxoplasmosis as an endemic disease. A seroprevalence based study in USA shows the 10.8 and 11.0% Toxoplasma prevalence in 6-49 years and 15-44 years old persons, respectively. In immunocompetent individuals, Toxoplasmosis infection causes latent chronic infection that is efficiently handled by immune system, while there is high risk of active disease in the babies and immunocompromised or immunosuppressive persons. The immunosuppressive category includes AIDS patients, organ or bone marrow transplant individuals and hematologic malignancy patients (Hodgkin's disease) (Frenkel et al. 1975). The prevalence of Toxoplasmosis manifestation is unusual, with >200 CD_4 cells/ul in patients. However, 30% of AIDS patients without getting any Toxoplasma prophylaxis or antiviral therapy (HAART) showed Toxoplasma encephalitis with <100 CD_4 cells/ul. By implying the prophylaxis measures against Toxoplasmosis, HAART against viral infections and through proper awareness, the occurrence of Toxoplasma and Toxoplasma related encephalitis mortality of Toxoplasmosis was significantly decreased in USA.

Toxoplasmosis can be transmitted in humans and animals through ingestion of oocysts from uncooked food, contaminated hands and infected animals (cats etc.). The oocysts in spores form may be present in soil and can be transmitted to humans while handling cats, gardening, contaminated vegetables, fruit and water (Bahia-Oliveira et al. 2003). Undeniably, in rural areas during pregnancy, impure or contaminated water has been reported as main source of infection (Andiappan et al. 2014). Most of the farm animals including goats, sheep, pigs, lambs, game animals and chickens have been reported as major source of Toxoplasma cysts in USA (Dubey et al. 2005; Dehkordi et al. 2013).

Causative Agent

The causative agent of Toxoplasma is *T. gondii*, which belongs to the protozoan parasite subclass Coccidasina. Usually, the coccidia have complex life cycle. The life cyle of *T. gondii* consists of three stages; tachyzoites, bradyzoites and sporozoites (Fig. 1). The size and shape are similar to red blood cells and crescent shape respectively in tachyzoites stage. The posterior and anterior ends are round and pointed. Various organelles are enclosed in outer covering called pellicle. The bradyzoites are slightly different in shape compared to tachyzoites, as the nucleus in tachyzoites is situated in the center of the cells, while in bradyzoites nucleus is located slightly towards the posterior end. The bradyzoites are more slender than tachyzoites and less affected by proteolytic enzymes. The cysts present inside the tissue in the form of bradyzoites possibly are not virulent and can persist for the long period in the host body (Dubey 2010).

Life cycle

The development of rapidly multiplying tachyzoites and slowly multiplication of bradyzoites occurs in asexual cycle. During acute infection, penetration of tachyzoites causes rupturing of host cells, leading to exposure of tachyzoites into the blood stream. As immunity is developed against tachyzoites, they are retained in tissue and grow into bradyzoites with very slow multiplication rate and maintain the infection in the host. The microscopic cysts can be observed frequently in the skeletal muscles and brain tissues of the host during quiescent stage. Parasites keep their size and shape, but multiplication rate decreases and parasites undergo quiescent phase (Ajioka et al. 2001). The cysts present in meat (muscles) are main cause of infection in humans.

Those animals which fail to survive in acute infection of tachyzoites can be demonstrated in tissue sections of various affected organs, acetic fluid and through lung impression. The sexual cycle of *T. gondii* befall in interepithelial cells of definitive host (feline) that results in production of Toxoplasma oocysts. In cats, primary infection is followed by shedding of oocysts in feces for days. Then the oocysts spread in environment in the form of spores for the next 1-5 days. The sporulation time depends on the environmental factors including humidity, temperature and aeration. These spores (oocysts) are very resistant and remain infective for long time. The size of oocysts (spores) is 11 ×13 um (diameter) and each two sporocysts contain four sporozoites (Dubey and Beattie 1988). Sporulated oocysts penetrates the intestinal lining after the ingestion by the susceptible host (animal), followed by change into tachyzoites and cause infection. These cysts can persist for life time in the body of humans, sheep, goats and pigs after exposure (Dubey & Beattie 1988). Toxoplasma usually does not cause the clinical symptoms in deer, camelids and cattle but causes severe disease in marsupials, new world monkeys and other animals including hares (*Lepus europaeus*; *L. timidus*) (Gustafsson and Uggla, 1994), the Pallas cat (Brown et al. 2005), the arctic fox (Sørensen et al. 2005), some birds and marine mammals (Dubey 2010).

Oral transmission

The causal agent of Toxoplasmosis, *T. gondii,* usually is transmitted through oral rout when tissue cysts or oocysts are eaten accidently (Weiss and Dubey 2009). People living in the countries where undercooked meat-eating habits are common are more prone to Toxoplasmosis because this disease is related to ingestion of raw or under-cooked contaminated meat like pork, poultry, lamb etc. After raw material, contaminated hands with tissue cysts, is the potential factor in the transmission of the causal agent. Contact with contaminated things like knives, utensils or cutting board with raw meat leads to transmission of causal agent from hand to mouth. A study in Europe revealed that 60% transmission of Toxoplasma infection is through meat consumption and only 20% is through contact with contaminated soil. It means that

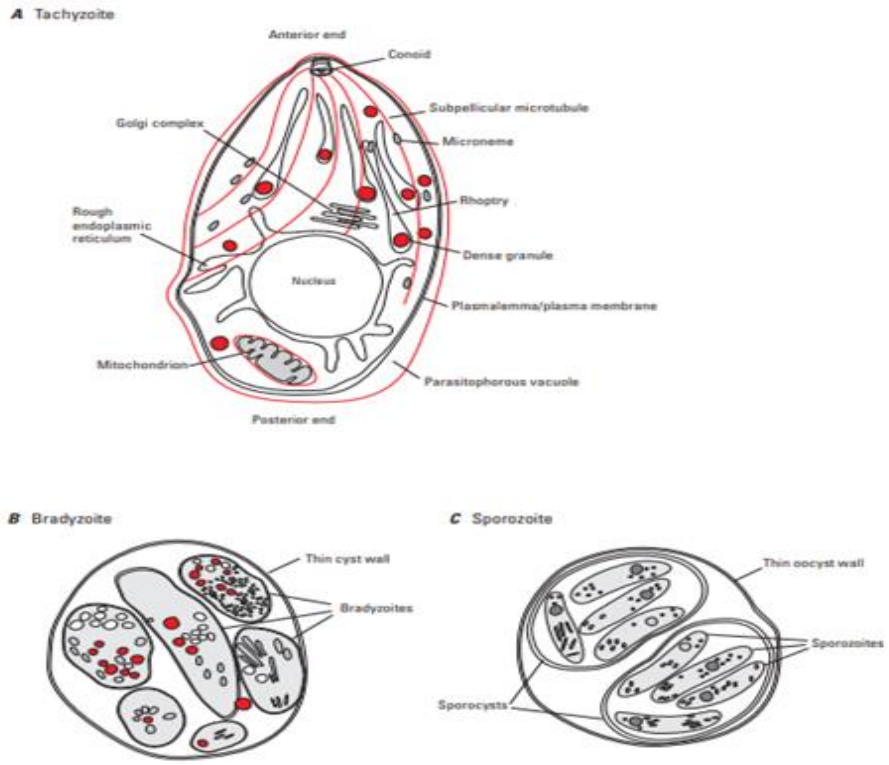

Fig. 1: Infectious stages of *T. gondii*: A, tachyzoite, B, bradyzoite, and C, sporozoite (Ajioka et al. 2001).

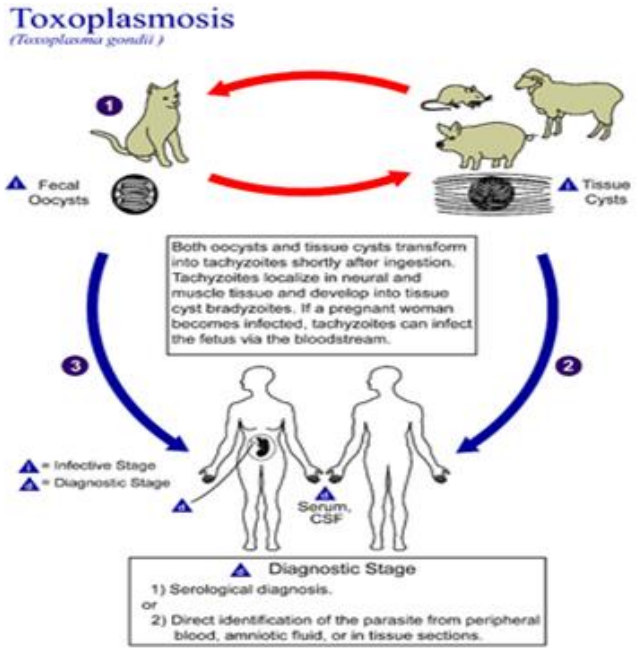

Fig. 2: Modes of transmission of Toxoplasmosis.

gardening related activities are also responsible for *T. gondii* transmission (Cook et al. 2000). Other transmission factors include raw vegetables and fruit contaminated with cat feces (Jones and Dubey 2012). As *T. gondii* is only secreted in cat feces, that's why contamination with cat feces is the only way of transmission. After work in garden or cleaning cat litter box, unwashed hand contact to mouth, can be a potential risk factor for infection. Potential causal agent can survive for months in environment, so direct contact to cat feces or children's sand pits also ultimately leads to oral transmission of *T. gondii* (Dubey 2017). Not only solid food stuff, liquid untreated food stuff is also responsible for the transmission of *T. gondii* like consumption of foods prepared through untreated water, directly drinking unfiltered water, unpasteurized milk and milk products. Eating birds and rodents can cause disease in cats and causal agent is secreted in their feces, which remains for months after disease development. After ingestion of contaminated food by cats, the pathogen shedding starts after third day of infection and may continue for months. Without sporulation, the excreted oocysts are not infectious. After sporulation process, these oocysts becomes potential infectious. Besides mammals and birds, human beings also serve as intermediate host in Toxoplasmosis and are actively involved in the transmission of infection. Transmission and pathogenicity of *T. gondii* is also dependent upon the type of species involved (Assadi-Rad et al. 1995).

Through organ transplantation

Organ transplantation always requires immune suppression of the organ recipient. If the donor of an organ is serum positive for Toxoplasmosis or recently infected, it is quite possible that the disease may be transmitted through transplantation and the recipient is at risk of developing the disease; without screening for Toxoplasmosis the recipient may the develop disease. Similarly, hematogenous stem cells transplantation requires longer period of immuno-suppression, so there are high chances of developing Toxoplasmosis in the recipient without screening.

Risk of Toxoplasmosis is also high in lungs and heart transplantation because of immunosuppression and

striated muscles active response in heart making process. As these muscles contain cysts, so there is high risk of transmission to other organs, cells and tissues (Coster 2013). This risk of disease development can be reduced by screening both donor and recipient for Toxoplasmosis prior to organ transplantation.

Congenital transmission

Toxoplasmosis also has character of vertical transmission from mother to fetus. Mothers infected with this disease during pregnancy can transmit causal agent to their fetuses through placenta. In acute cases, clinical signs may include neural signs, hepatomegaly, lymphadenopathy and interstitial pneuminia. After postmortem examination, enlarged liver, lymph nodes and spleen, are observed and later pale foci have been observed (Coster 2013).

Environmental Factors

Environmental factors also affect the survival, occurrence and transmission of *T. gondii*. Occurrence of *T. gondii* has also been observed in Canadian act that is too extreme low temperature for its survival. As the temperature increases, its survival rate also increases. As precipitation or snow melting process increases, presence of *T. gondii* oocyst also increases. Oocyst migration among birds, insects and rodents can have a huge impact on *T. gondii* distribution, as these animals can serve as vectors and reservoirs. Degradation of natural environment by urbanization also affects the distribution patterns and increases the *T. gondii* transmission.

Clinical signs

The incubation period of Toxoplasma infection is 5 to 23 days. Symptoms includes elevated liver enzyme, lymphocytosis, flue, prolonged fever, lymphadenopathy and weakness. Immunocompetent patients are more prone to this disease and with above symptoms, disseminated disease or chorioretinitis can also occur. Severe illness like pneumonitis, and fatal encephalitis can also develop in immunocompromised patients. Infants with congenital Toxoplasmosis are often asymptomatic, but systematic symptoms, neurological disorder and eye disease may develop. At later stage of life visual impairment, learning disabilities, and cognitive deficits may also develop (Montoya and Liesenfeld 2004). Child hearing problems may also develop in congenital Toxoplasmosis and intellectual disability with sensor neural hearing loss are also reported in 30% newborn children. Fig. 2 illustrates modes of transmission of Toxoplasmosis.

Clinical diagnosis

Human Toxoplasmosis can be diagnosed by serological, molecular or histological methods. These methods are sometimes used in combination to enhance the efficiency of diagnosis (Dubey 2002). As Toxoplasmosis mimics its clinical signs with many other infections; its signs are closely related to central nervous system lymphoma. Diagnosis of Toxoplasmosis cannot be made on the basis of clinical signs and symptoms, because these signs depict symptoms of many other infections. As a result, trial and therapy technique can be used. Folic acid, sulfadiazine and pyrimethamine are used as therapeutic agents.

The causal agent may also be detected in cerebrospinal fluid, amniotic fluid and blood through polymerase chain reaction (Switaj et al. 2005). Sometimes the potential agent hides in the host body and cannot be detected through these tests.

Serological testing

Through serological testing, antibodies against *T. gondii* can be detected in the blood stream. Different methods can be used for antibodies detection like indirect hemagglutination assay, direct hemagglutination assay, Sabin Feldman dye test (DT), indirect immune fluorescent assay (IFA), latex agglutination test (LAT), immunosorbent agglutination assay and enzyme linked immunosorbent assay (ELISA) (Dubey 2002).

In IFA and dye test, color change of tachyzoite is observed under microscope. Agglutination based serological test depends on the agglutination; and agglutination of red blood cells, latext particles and Toxoplasma tachyzoites is observed in indirect hemagglutination test, latex agglutination test and direct agglutination test, respectively. ELISA based diagnosis depends on the change in color of substrates with the degree of availability of antibodies. After infection with Toxoplasmosis, antibodies are developed within one to two week, peak within two months and decline with various rates (Montoya 2002). As IgG antibodies against Toxoplasma generally persist for longer period, so their occurrence always remain persistent in blood stream (Jones et al. 2014). The DT, IFA test, DAT and ELISA are outlined below and the IFA test is given in more detail.

The dye test (Sabin and Feldman 1948) is also called the gold standard test for diagnosis of Toxoplasmosis in humans by detecting antibodies in serum. In this test, the patient's serum is incubated with live Toxoplasma tachyzoites and necessary complement like accessary factors at 37°C for 1 hour. After incubation, methylene blue is added. If antibodies are present in the patient serum, these antibodies enhance the permeability of parasite membrane, the cytoplasm leaks out and tachyzoites do not incorporate the dye, test appears colorless which indicates Toxoplasmosis positive. If the antibodies are not present in the serum, parasite membrane permeability remains intact, and tachyzoites incorporate the dye color. So, the test color appears, showing that the result is Toxoplasmosis negative. This test is only applicable for Toxoplasmosis diagnosis in humans, and not in any other species. This test procedure is potentially hazardous because live pathogen is used. So, high level of care, and technical staff is required to perform this test. It should be noted that for the

production of tachyzoites laboratory cannot be used due to welfare and ethical reasons. Animal cell lines can be used for this purpose.

The IFA test (Munday and Corbould 1971) is used to detect the presence of antibodies against *T. gondii* in the patient's serum. This test is quite simple and widely used for the diagnosis of Toxoplasmosis. This test is performed by mixing killed Toxoplasma tachyzoites with fluorescent antiserum antibodies and diluted test serum, and incubated. After incubation, results are seen under fluorescent microscope. This test is not too expensive, as fluorescent antibodies are available commercially for all animals. Test kits are easily available and inexpensive. Test method is also simple and results can be seen by eyes but availability of fluorescent microscope is necessary. As results can be read by eyes, so variation in results may occur. Sometimes cross reactivity may occur with other factors like rheumatoid factors, so there is difficult to find specific conjugation.

The DAT (Desmonts and Remington 1980) is both specific and sensitive. The process of this test is simple. A U shaped microtiter plate is used for this test. Formalinized Toxoplasma tachyzoites are added to this titer plate and then diluted test sera is applied. Positive test sample will produce agglutination in the well, while negative test shows button formation of precipitated tachyzoites at the bottom of the well. This test is simple and easy to perform but requires high amount of antigen. Test kits are easily available commercially. Sera treatment with mercaptoethanol is compulsory to avoid non-specific antibody false positive result. Dubey and Desmonts (1987) modified the DAT test and named it as modified agglutination test (MAT). This test is very important and extensively used for the diagnosis of *T. gondii* in all animal species. Disadvantage of this test is that it can give false positive result in the early stage of infection or if performed on canine sera. Latex agglutination test is also simple and kits are easily available but this test has low sensitivity as compared to IFA and MAT.

ELISA based serological test is also useful in the diagnosis of Toxoplasmosis infection. The original method of ELISA (Voller et al. 1976) uses antigen prepared from Toxoplasma RH strain tachyzoites, embedded inside the bottom of microtiter plate. Test can be performed by adding test sera in titer well, followed by anti-specie antibodies linked with enzymes like horseradish peroxidase-labelled anti-ovine-IgG. This system changes its color after adding their substrates if conjugates are attached with antibodies. If there are antibodies in the serum, antigen-antibody complex formation occurs, that provides attachment opportunity to conjugate enzymes that ultimately leads to change in substrate color. This color change can be detected through spectrophotometer at the absorbance specific for the substrate used. This test is simple, easy to perform and can be used for large number of samples. ELISA kits are commercially available for Toxoplasmosis parasite diagnosis. But spectrophotometer requirement is mandatory for this test. This test design has advantage that it can be applied for large number of samples.

ELISA modified test named "kinetics ELISA (KELA)" has been developed (Werre et al. 2002). This modified test actually measures the reaction rate of bounded enzymes and substrate that results in change of substrate color. After 45 seconds intervals, three different optical densities are measured and graphically represented in the form of slope. Both tests KELA system and ELISA have high correlation between them and these tests are most important as diagnostic tool.

ELISA affinity and specificity for Toxoplasma has been improved by using recombinant antigen (Johnson and Illana 1991). Specific antigen of Toxoplasma parasite has been developed in sheep (Lekutis et al. 2001) but these tests are not in routine use (Sager et al. 2003; Tenter et al. 1992).

There is an urgent need to distinguish a current or new infection (acute infection) from long term (chronic) infection. Conventional ELISA test may permit the detection and discrimination between chronic and acute infections by identifying Toxoplasma specific IgG, IgM and IgA antibodies. In an assay avidity of IgG antibody for *T. gondii* P30 antigen has been developed in sheep. It was found that avidity increased over the period of time post infection (Sager et al. 2003). IgM antibodies cannot be used for the chronic infection. These antibodies can be used for the detection of chronic infection (Jones et al. 2014).

Congenital diagnosis

Congenital diagnosis of Toxoplasmosis is crucial and can be performed at every developmental stage. In prenatal developmental stage, diagnosis of Toxoplasmosis can be made through ultrasonic examination and amniotic fluid testing. Diagnosis methods at neonatal developmental stage include molecular testing of cord blood and placenta, clinical examination and comparative mother-child serological test at birth. At early childhood, diagnosis based on ophthalmic and neurological examination is better and same is true for serology survey during early years of life (Sterkers et al. 2011). Serological diagnosis at three-week intervals is necessary during pregnancy (Sensini 2006).

Molecular Methods

Diagnosis of Toxoplasmosis can also be made by using different clinical specimens including blood, tissue biopsy, amniotic fluid and cerebrospinal fluid, through using molecular methods. PCR based diagnosis methods have been developed and most affective technique is nested-PCR, followed by PCR product hybridization technique (Lin et al. 2000). The major limitations of these techniques are that they do not provide quantitative data and they are also time-consuming (Lin et al. 2000).

Methods involved in detection of pathogen by gene expression, gene regulation, use real time PCR. In this technique, Taq DNA polymerase activity to cleave 5' nucleotides is used (Lin et al. 2000). During the extension phase of PCR assay, fluorescent labeled probes are cleaved and a second dye 6-carboxy-tetramethyl-rhodamine

quenches the intact probe fluorescence (Lin et al. 2000). During PCR assay, hybridized probe cleavage activity increases the fluorescence proportional to PCR product formed, which can be monitored by a detector (Lin et al. 2000).
Immunostaining can also be used for detection of Toxoplasmosis. Lymph nodes affected by Toxoplasma have characteristic changes, including scattered epithelioid histiocytes, poorly demarcated reactive germinal centers and clusters of monocytoid B cells.

Treatment

Most widely used drugs for the treatment of Toxoplasmosis are pyrimethamine and Sulfadiazine (Chirgwin et al. 2002). These drugs are affective in the acute stage of disease, when organism is multiplying rapidly, but do not eradicate the organism completely from the body. In subclinical cases, these drugs show little affect, but in mice sulfonamides have shown complete eradication of tissue cyst. Other drugs useful in Toxoplasmosis include spiramycine, pyrimethamine, diclazuil, atovaquone and clindamycin.

Vaccines

There is a dire need for an effective vaccine to protect humans and animals from cyst production, especially in cats at different stage of development. It is unfortune that there is no effective and safe vaccine available against Toxoplasma infection. Only live-attenuated Toxoplasma (Toxovax®) vaccine is available for limited use in veterinary with little success (Buxton and Innes, 1995). This vaccine has protected Toxoplasma infected sheep from abortion but is not always safe and effective. Various DNAs form proteins, a virulence factor of parasite, are strong vaccine candidates but have shown no protection or limited protection yet. One-week pregnant rats were given irradiated tachyzoites orally following challenge with organisms, but there was no protection in dams and pups. However, immunization protected the birth rate and litter size (Camossi et al. 2014).

Prevention measures

The causal agent of Toxoplasmosis, *T. gondii,* present in meat, can be killed by soap and water (Dubey and Beattie 1988; Lopez et al. 2000). Washing hands contaminated with *T. gondii* after handling of raw meat is essential to prevent Toxoplasmosis infection. Washing all things that come in contact with raw meat like knives, sink top, cutting boards with soap and water will kill the parasite and prevent Toxoplasmosis.
Extreme heat and cold can also be used to kill organisms in meat. Heating meat at 67°C for 4 minutes kills the tissue cyst in meat (Dubey 2001). Cooling meat to -13°C for 3 days also kills *T. gondii* (Kotula et al. 1991). Exposure to gamma rays to 400 grays is also helpful in killing the *T. gondii* (Dubey and Thayer 1994). It is recommended that heating any animal meat to 67°C will kill the *T. gondii* and tasting should be avoided while cocking. As *T. gondii* presence is associated with contaminated soil, raw meat and cats, so pregnant women should avoid possible exposure to these objects, which will decrease the potential risk of Toxoplasmosis.
Cats play a key role in the transmission of *T. gondii*. Most effective and efficient method to minimize its transmission is adoption of good hygiene practices. Cooked food or dry canned food-based diet will prevent the infection of pet cats and control oocyst secretion. Cleaning cat box and emptying litter box decreases the oocyst load and pregnant women should avoid doing all these functions.
Gardeners should wear gardening gloves that will prevent from direct hand contact with cat faeces buried in soil. Fruit and vegetables washing with clean water will also decrease the risk of Toxoplasmosis.
Awareness development among pregnant women through education will protect them from harmful effects of Toxoplasmosis (Foulon et al. 1994, 2000). There is no environmental monitoring method available that can detect *T. gondii* oocysts in environment. To control the transmission of Toxoplasmosis in animals, preventive measure should be adopted to avoid direct contact of cats with other animals.
On farm, preventive measures include cat population controlling through spying programs. Affective rearing practices for domestic animals such as pigs, like confinement rearing to avoid their contact with cats, will limit the transmission risk among animals. In the same way, pregnant goats and sheep should be confined to limit their contact with cats. *T. gondii* infection in zoo animals can be prevented by housing all the animals separately, especial marsupials, New World monkeys and cats. Spread of infection through captive felid and domestic cats can be controlled by controlling their uncooked meat feeds.
Cleaning and disinfection of cages of cats will decrease the risk of oocyst transmission. Control of feral cats within zoos is also important.
In domestic poultry operations, risk of oocyst prevalence can be controlled by limiting presence of the potential source of infective tachyzoites like rodents, cats and coprophagic arthropods in the vicinity of rearing facility. For disinfection of the rearing facilities, ammonia is used, followed by drying at 55°C (Springer 1991). The combination of disinfection and other preventive measures will also limit the risks like use of serological testing and postmortem lesions analysis to determine the cause of bird death. Avoiding overcrowding is also helpful in limiting the disease transmission (Sanger 1971). *T. gondii* oocysts can resist the harsh environmental conditions and survive for long periods, even years (Dabritz et al. 2006, 2007). Over and uncontrolled population of feral cats in the United States makes the elimination of oocysts from the environment impossible.

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GIARDIASIS IN HUMAN AND ANIMALS

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INTRODUCTION

Giardia has been known since the times of Antonie van Leeuwenhoek's during 1681 but its public health significance was acknowledged during late 20th century. *Giardia* is known as the main cause of diarrhea now-a-days and is considered as major concern for the health authorities. There are estimated 280 million infections of giardiasis diagnosed every year (Einarsson et al. 2016). It affects the socio-economic condition of people residing in developing countries. Therefore, it is included in "Neglected Disease Initiative" of World Health Organization (Savioli et al. 2006).

Giardia is a unicellular flagellate, belonging to the phylum Protozoa, order Sarcomastigophora and family Mastigophora. Giardiasis is a chronic issue that infects the intestine of the hosts, including mammals, having a specific target on duodenum and ultimately causing bowel diarrhea (Ballweber et al. 2010). The recognized species of the genus include *G. agilis* infecting amphibians, *G. pasittaci* and *G. ardeae* infecting birds, *G. muris* infecting rodents and *G. duodenalis* (syn. *G. intestinalis, G. lamblia*) infecting human and other mammals (Thompson et al. 2012).

Giasrdiasis is considered as a common parasitic infection of companion animals. It also causes substantial pathological changes in laboratory animals like mice. In both human and animals, the disease results in weight loss, diarrhea (either acute or chronic), hypersensitivity and nausea like condition. Asymptomatic infection is also reported in many instances (Geurden and Olson 2011).

The research done so far on giardiasis has been mainly focused on its molecular characterization in various animals in order to identify the reservoir host and calculate the zoonotic risk of disease. At present, identification of specific genotype in animals and humans is possible due to the development of molecular markers (Durigan et al. 2018).

Giardiasis is an important disease, affecting both human and animals, and having millions of cases every year. As per significance of the protozoa, this chapter briefly summarizes the parasite prevalence across the world, pathogenesis in the host and control strategies with a view to reduce the infection rate.

History and evolutionary biology

Giardia is considered as a primitive eukaryote due to lack of several typical eukaryotic organelles like mitochondria and peroxisome. Phylogenetic analysis of gene and protein sequence also confirms its position in the evolutionary tree. Early classification of *Giardia* was based on characteristics of ribosomal RNAs (Van Keulen et al. 1993), but later it was classified on the basis of conserved proteins, like elongation factor (Hashimoto et al. 1994). *Giardia* was typically considered as the most derived member of its order, based on its life history and morphology. Furthermore, phylogenetic analysis of order Diplomonadida further confirmed *Giardia* as the most derived genus of its order (Siddall et al. 1992).

Furthermore, due to large differences in G+C composition of *Giardia* and other eukaryotes and large branch attraction effects, its position in other eukaryotes has been questioned (Dacks et al. 2002). Evolutionary history of *Giardia* is also difficult to trace due to its lateral gene transfer, as in anaerobic metabolism of organism many genes having been acquired from prokaryotes are involved (Andersson et al. 2003). According to the most recent molecular evidences, *Giardia* is classified as a highly derived organism. It is then suggested that it is derived by introns acquisition in genome during eukaryotic evolution, followed by spliceosomal peptides and intron detection in *Giardia* (Nixon et al. 2002). Further sequencing also supported the previous findings and identified the trans-spliced and cis-spliced intron in the genome, having exon dispersal across the genome, with a single transcript being produced by trans-splicing (Kamikawa et al. 2011; Roy et al. 2012).

There are various eukaryotic similarities found in *Giardia* upon genomic data analysis like RNA regulation pathways, such as micro RNAs and RNA slicing (Zhang et al. 2009), processing machinery for sequences encoded for eukaryotic RNA (Chen et al. 2011), meiosis-specific genes (Ramesh et al. 2005) and presence of nucleoli (Jiménez-Garcia et al. 2008). Presence of mitosomes (mitochondrial remnant) has also been demonstrated in *Giardia* and various other amitochondriate protists (Tovar et al. 2003). Upon phylogenetic analysis of genes coded for type-II DNA topoisomerase, it was found that *Giardia* is derived from mitochondrial kinetoplastids and amitochondriate is regarded as polyphyletic. Furthermore, studies also demonstrated that *Giardia* acquired mitochondria from eukaryotes and multiple evolution of organelles has also occurred (He et al. 2005).

The detailed understanding regarding evolution of *Giardia* could be gained by analyzing its genomic data. Due to loss or reduction of metabolic pathways, *Giardia* genome is thought to be in compact form. According to a study, 40%

genes in its genome were found duplicated. Upon phylogenetic analysis, these duplicated genes were found to be encoded for surface protein similar to the placental mammals (Sun et al. 2010).

Taxonomy of *Giardia*

The members of the genus *Giardia* are flagellated protozoa, characterized by the presence of diploid nucleus, unique adhesive disc on ventral surface, absence of peroxisomes and mitochondria (Morrison et al. 2007). The unique feature of *Giardia* which differentiates it from other member of family is the ventral disc, which also helps in its attachment with brush border of villi. Ventral disc is supported by cytoskeleton of microfilaments, microtubules and associated fibrous structures and mainly composed of protein tubulin (subunit a and b) and gairdins (Ankarklev et al. 2010).

According to old classification (1980), there are seven phyla placed under subkingdom Protozoa based on morphology. The zoonotic parasites mainly belong to five phyla i.e., Myxozoa, Ciliophora, Apicomplexa, Sarcomastigophora (containing both Sarcodina and Mastigophora) and Microspora. *Giardia* is a member of Phylum Sarcomastigophora, Subphylum Mastigophora, Class Zoomastigophorea, Order Diplomonadida and Family Hexamitidae. According to the most recent classification, Protozoa is recognized as a kingdom containing 13 phyla based on molecular sequence evidence. Former phylum Mastigophora is further divided into Percolozoa, Metamonada, Euglenozoa and Parabasalia. *Giardia* belongs to the Phylum Metamonada, Subphylum Trichozoa, Class Trepomonadea, Order Giardiida and Family Giardiidae (Morrison et al. 2007; Plutzer et al. 2010).

Giardia Species

There are six species of the genus *Giardia* accepted so far, including *G. psittaci, G. ardeae, G. agilis, G. microti, G. muris* and *G. duodenalis*. *Giardia duodenalis* is the only species which is able to infect humans and animals both. Another species has also been reported in reptiles, which resembles *G. duodenalis* in appearance, but it lacks median bodies and contains binucleated cyst. Therefore, it is characterized as *G. varani* (Cacciò et al. 2005). According to a previous study conducted on cultured and wild marine and freshwater fish in Australia, assemblage A and B (zoonotic nature) and assemblage E (artiodactyl-specific) of *G. duodenalis* and *G. microti* have been identified. It was not clear from the study that whether fish acted as mechanical host or actually got infection (Yang et al. 2010). The host range of *Giardia* species is very wide and *G. duodenalis* shows a great public health significance as well (Monis et al. 2009).

Giardia duodenalis, G. intestinalis and *G. lamblia* are the multiple names refer to the same organism in literature. *G. duodenalis* and *G. intestinalis* mostly infect livestock, companion animals and humans. In medical field, *G. lamblia* is used to discuss the giardiasis impact on humans (Xiao et al. 2008). *G. duodenalis* is considered as the only species which can infect humans, pets and livestock and now it is also considered as multiple species complex (Thomas et al. 2008).

There are multiple assemblages found within *G. duodenalis* morphological group which include assemblage A, B, C, D, E, F and G. These all appear to be associated with single species of mammalian host. Historically, allozyme analysis showed that two genetic assemblages, including A and B, are referred as human isolates (Thompson et al. 2004). Multigenic sequence analysis also confirms assemblage A and B to be referred as human isolates, assemblage E to be isolated from artiodactyls, assemblage G from rodents, assemblage C and D from dogs and assemblage F from cats (Cacciò et al. 2005).

Prevalence

Giardiasis affects the humans, as well as animals, across the globe. According to an estimate, around 280 million people across the globe are diagnosed with giardiasis every year, with higher infection in developing countries (Squire et al. 2017). The correct incidence of disease is unknown in many parts of the world due to unreported/undetected cases. In China, almost 28.5 million human cases have been reported every year (Feng et al. 2011). According to Ballweber et al. (2010), the prevalence of giardiasis varies according to different studies, regions, diagnostic methods, symptomatic or asymptomatic, age of the animal and housing conditions. *G. duodenalis* assemblages which mainly cause infection in humans include assemblage A and B (Ballweber et al. 2010). The prevalence of *Giardia duodenalis* in various animal species and humans reported in different studies has been listed in Table 1.

In cats, infection of giardia is often asymptomatic and self-limiting. Therefore, few studies have done on cats regarding presence of *Giardia*. According to these studies, the infection rate in cats in Europe is around 20.3% (Epe et al. 2010), 5.3% in UK (Gow et al. 2009), 10.8 to 44.4% in USA (Fayer et al. 2006; Garrett et al. 2006; Vasilopulos et al. 2007), 5.9% in Brazil (Coelho et al. 2009), 13.6% in Netherlands (Overgaauw et al. 2009), 19% in Chile (Lopez et al. 2006), 4.1% in Canada (Santin et al. 2006), 15.8 to 37% in Italy (Papini et al. 2007) and 2.0% in Australia (Palmer et al. 2008). The variation in infection rate of giardiasis is attributed to differences in age, diagnostic techniques and symptomatic stage of the animal. In many cases, the prevalence rate differs when diagnosed with multiple diagnostic methods i.e., microscopy, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR) or Immunofluorescence Assay (IFA).

Life Cycle

The life cycle of Giardia is simple and direct in nature. It involves two stages; i) the trophozoite stage that is replicative in nature and get attached to brush border of villi epithelium, causing damage to duodenum that

Table 1: Prevalence of *Giardia duodenalis* in multiple animals and humans across the world

Host species	Location	Detection Method	Prevalence (%)	Reference
Ruminants (sheep, goat, cattle, buffalo)	Islamabad, Pakistan	Microscopy	16.0	(Imran et al. 2013)
Dogs	Romania	ELISA	34.6	(Mircean et al. 2012)
Human	UK	Microscopy	30.0	(Waldram et al. 2017)
Human children	Portugal	Microscopy	1.9	(Júlio et al. 2012)
		ELISA	6.8	
Cattle	Ningxia, China	PCR	2.12	(Huang et al. 2014)
Cattle	Beijing, China	PCR	1.09	(Li et al. 2016)
Sheep	Belgium	IFA	36.0	(Geurden et al. 2008)
Sheep	Italy	Microscopy	1.5	(Giangaspero et al. 2005)
Goat	Spain	ELISA	42.0	(Ruiz et al. 2008)
Goat	Brazil	Microscopy	14.0	(Bomfim et al. 2005)
Sheep	Australia	Microscopy	9.0	(Ryan et al. 2005)
Cattle	Denmark	IFA	24.0	(Maddox-Hyttel et al. 2006)
Cattle	Norway	IFA	49.0	(Hamnes et al. 2006)
Cattle	USA	PCR	40.0	(Trout et al. 2004)
Dog	Guangzhou, China	Microscopy	8.61	(Li et al. 2012)
		PCR	11.0	
Human	Ethiopia	Microscopy	13.8	(Wegayehu et al. 2013)
Cattle			2.3	
Human	Lebanon	PCR	28.5	(Osman et al. 2016)
Cattle	Northeast China	PCR	7.9	(Liu et al. 2015)
Human	Cuba	PCR	22.8	(Puebla et al. 2014)
Beef Calves	USA	PCR	33.5	(Santin et al. 2011)
Human	Pakistan	Microscopy	2.75	(Naz et al. 2018)
		ELISA	9.5	
Human	Pakistan	Microscopy	59.54	(Haq et al. 2015)
Human	Pakistan	Microscopy	15.0	(Khan et al. 2018)

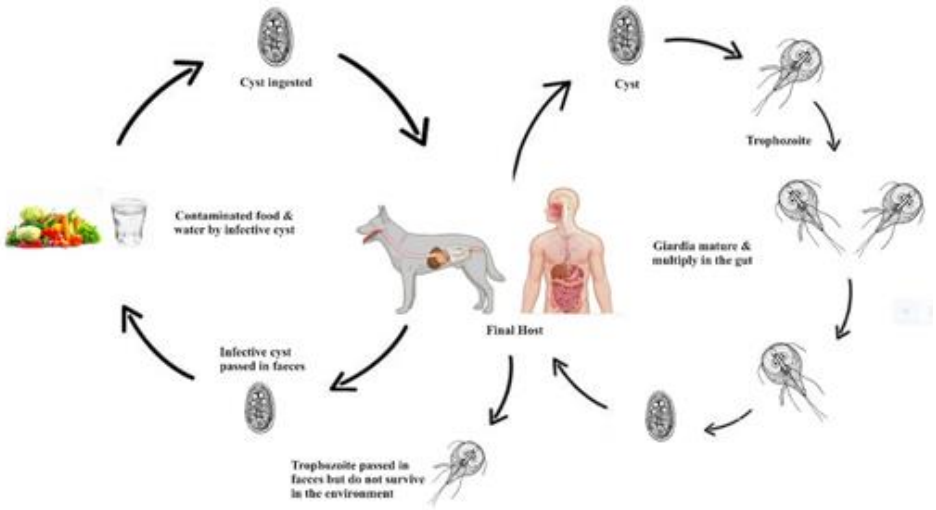

Fig. 1: General stages of life cycle of *Giardia*.

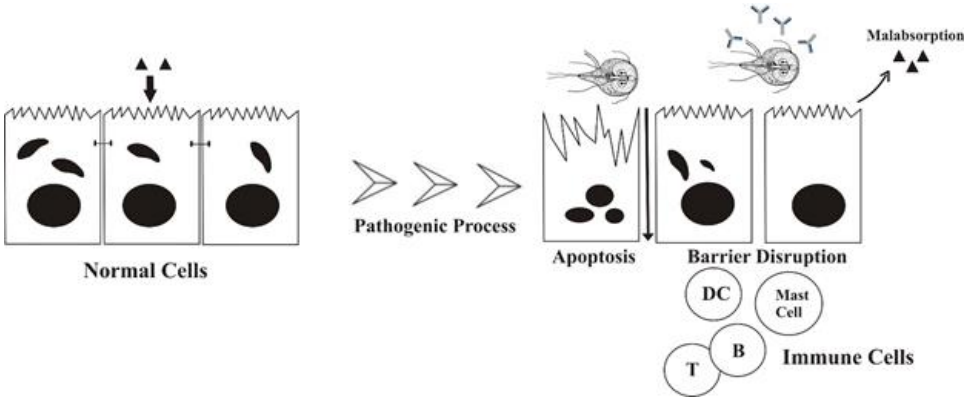

Fig. 2: Pathogenic process during *Giardia* infection.

ultimately leads to mucousal layer sloughing, atrophy of villi and finally increases crypt cell proliferation, and ii) the cyst stage which is infective in nature and passes out in faeces (Fig. 1). Trophozoite ultimately increases mucus secretion due to hyper-secretion of goblet cells (Sazalli et al. 2016).

The transmission of disease is possible directly through feaco-oral route, which may involve direct human to human contact, human to animal contact and animal to animal contact, and indirectly through consumption of contaminated food and water. Acidic environment in the stomach provides suitable stimuli for excystation (trophozoite to cyst conversion) in duodenum (Capári et al. 2013).

Trophozoite undergoes multiple mitotic divisions and then the condition of bile in small intestine converts it into environment resistant cyst. These cysts are shed in feces and able to survive in environment for months. Cysts are infectious in nature and contaminate the environment, leading to the contamination of food and water (Feng et al. 2011). Around 10-100 cysts are considered as the infecting dose in humans. Drinking of contaminated water is a main factor behind major outbreaks of giardiasis. According to a survey, a total of 199 protozoal outbreaks were recorded during the period of 2004-2010 and *Giardia* was responsible for causing 70 outbreaks (35%) (Baldursson et al. 2011).

Clinical Signs and Pathogenesis

The infection ranges from asymptomatic giardiasis to acute or chronic infection. It causes a wide range of infection in domestic, as well as companion, animals and is transmissible to humans. Giardiasis causes potential pathogenic changes in the host. The symptomatic infection appears after 1 to 2 weeks of cyst ingestion and persists for 3 to 4 days (Farthing et al. 1997). The pathognomonic sign of giardiasis is diarrhea with foul smelling, but it may also accompany with nausea, flatulence, weight loss, urticaria, itching and epigastric cramps. Symptoms of disease appear due to the dysfuntioning of small intestine because of the villus atrophy, malabsorption of fat, lactose, electrolytes, vitamin A, D-xylose and vitamin B12, immaturity of enterocytes, luminal enzyme deficiency and decrease in surface area of brush border. Due to malabsorption of electrolytes and nutrients, osmotic gradient is created in intestine, resulting in water accumulation, rapid peristalsis and ultimately diarrhea (Einarsson et al. 2016).

Severe form of giardiasis is usually observed in infants and children in developing countries, where it is associated with poor nutrition status, failure to thrive syndrome, poor cognitive function and retarded growth (Berkman et al. 2002). Sometime giardiasis sequelae may result into a chronic infection. According to a study, in Norway 10% of individuals had persistent infection of giardiasis with mean duration of 7 month after waterborne outbreak of the disease and among them 5% developed fatigue syndrome afterward (Naess et al. 2012). Post-infectious reactive arthritis, irritable bowel syndrome and allergic reactions are also demonstrated from several studies in literature (Wensaas et al. 2012). The pathogenic process of disease is contributable to both ends i.e., parasite and host. The trophozoite of *Giardia* is highly motile and gets attached with the enterocytes in upper portion of small intestine, resulting in establishment of infection (Cotton et al. 2011). The attachment with enterocytes is accomplished by ventral adhesive disc and movement by flagella present on *Giardia* surface. The adhesive disc also protects parasite from elimination by peristaltic movements. In host intestine, there is an active release of products of immune response, bile salts, proteases and lipases which attack on trophozoite to remove it from the intestine. The parasite protects itself by process of antigenic variation (Carranza et al. 2010).

The dense coat of variable surface proteins (VSP) is present on the surface of trophozoite. Single VSP is dominated during the course of infection. Due to on-off switching of gene expressing VSP, different VSP are expressed during infection, resulting in parasite escape from host immune response. Till date, there are no identical VSP sequences observed among three strains (Jerlström-Hultqvist et al. 2010). Additionally, the involvement of proteins having toxin like activities are also hypothesized but no such protein is identified so far. The four main parasitic proteins that are involved in pathogenesis include elongation factor 1-α, arginine deiminase (ADI), α-enolase and ornithine carbamoyl transferase (OCT) (Skarin et al. 2011). In specific, ADI and OCT interfere in the synthesis of nitric oxide, which is usually cytotoxic for the parasite, resulting in inhibition of innate response by the host. These proteins cause damage to L-arginine, which is needed by the epithelial cells of the host for synthesizing nitric oxide with the help of nitric oxide synthetase (Rópolo et al. 2010). The epithelial cell damage in giardiasis is the key pathogenic change. Patients suffering from chronic disease exhibit a significant damage to the enterocytes, which has also been observed in *in vitro* experiments as well (Troeger et al. 2007). Proapoptotic caspase-3 and 9 appear to induce the process of apoptosis by increasing the pro-apoptotic Bax which ultimately decreases the anti-apoptotic Bc1-2 expression, resulting in the cleavage of poly-ADP ribose polymerase (PARP). After initiation of apoptosis, *Giardia* trophozoite weakens the junctions between enterocytes by breakdown of proteins. During the process of apoptosis, claudin-1, F-actin, α-actinin and zonula-occludens-1 (ZO-1) are relocated to cytosol (Cotton et al. 2011).

Due to caspase-3 inhibition, the relocation of F-actin and ZO-1 is stopped, which indicates a direct relationship between enterocytes barrier function and apoptosis induced by *Giardia* trophozoite. The breakdown in the cells barrier results in the entrance of electrolytes in the sub-mucosal space after bypassing the normal epithelial cells uptake. Nutrient malabsorption occurs due to paracellular uptake of electrolytes, which leads to the activation of innate immune response (Solaymani-Mohammadi et al. 2010). The response of host to parasite also plays a significant role in pathogenesis of the disease. All kinds of proteins secreted from the parasite, including

VSP and major disc protein, are recognized by host sera which is also demonstrated in experimental infection to mice as well and indicates its importance regarding antibody-mediated Giardia immunity. According to a study, IgA antibodies have been recovered after induction of giardiasis to a murine model, indicating its importance in the development of protective immunity (Singer et al. 2000).

The role of T-cells in protection against giardiasis has also been demonstrated in literature. In a study, T-cell deficient mice and patients suffering from immunodeficient syndromes developed chronic giardiasis, indicating the importance of T-cells. Furthermore, there is CD8 and lymphocytes dependent shortening of microvilli, resulting in decreased water, nutrients and electrolytes absorption, as demonstrated in murine model (Scott et al. 2004).

Similarly, in animals, giardiasis results in the decreased crypt to villus ratio and deficiency of brush border enzymes. The severe infection in animals results in the mal-absorptive diarrhea and low weight gain. Diarrhea is a pathognomonic sign for diagnosis similarly as in humans. The disease in calves ranges from acute diarrhea to chronic and intermittent sings. In a study, after experimental infection with assemblage B of *Giardia*, the malabsorptive diarrhea in lambs and goat kids resulted (Aloisio et al. 2006). In another study, decreased feed efficiency and weight gain was noticed in lambs after experimental infection with *Giardia* (Geurden et al. 2010). Therefore, *Giardia* is considered as a potential cause of diarrhea in production animals, resulting in decreased production and economic losses (Geurden et al. 2006).

Cats and dogs exhibit asymptomatic infection of giardiasis, having more severe infection in immature animals. Sometime, infection may causethe acute diarrhea in very young animals but adults remain asymptomatic during the infection. Clinical signs in cats are particularly uncommon (Ballweber et al. 2010).

Diagnosis of *Giardiasis*

Giardiasis is usually diagnosed on the basis of clinical symptoms, environmental condition and by exclusion of other infectious diseases. Its diagnosis is not straightforward due to vagueness of clinical symptoms. Traditionally, Giardiasis is confirmed by identification of trophozoites and cysts in faecal samples through microscopy either by floatation or sedimentation methods (Cama et al. 2015). In the chronic phase of infection, the cyst stage of parasites is released from the host intermittently. Therefore, multiple sampling is sometimes necessary for consecutive three days. In case of animals, young animals of 2-4 weeks age are recommended to be included in sampling method, as they exhibit peak excretion of cysts without displaying clinical disease. Due to no increase in antibody titer after infection of *Giardia*, serum antibody test is not recommended for diagnosis (O'Handley et al. 2003). Apart from microscopy, there are many other methods which can be used for correct diagnosis of the infection. These are biochemical, serological, immunological and molecular methods. Molecular characterization is preferred due to high specificity and sensitivity (Thompson et al. 2004).

Microscopic Examination

Both cysts and trophozoites are considered as diagnostic stages for detection of giardiasis. These can either be detected through direct microscopy of fecal smear or after treating with floatation solutions i.e., sodium nitrate, zinc sulfate or sucrose. The specificity of diagnostic test is improved when performed with standard protocols (Dryden et al. 2006). Fat malabsorption and diarrhea are the characteristic features of giardiasis, which can interfere in the process of floatation after treating with sucrose, but this problem can be addressed by treating the sample with chloroform. Sometimes, trophozoite stage can be detected from diarrheic samples but immediate microscopic observation is required to observe trophozoites due to their characteristic movement. Alternatively, microscopic observation of the cyst is preferred over trophozoite detection which is mostly present in faeces (Elmi et al. 2017). Staining is usually done to observe the cyst under microscope. The most frequently used stains for giardiasis include Iodine, Zeil Nelson stain and modified Acid-Fast stain. Parasitic cyst and fecal debris can easily be separated by using floatation technique. Separation is done on the basis of difference of specific gravity. High specific gravity liquids, such as NaCl, $NaNO_3$, $ZnSO_4$, are used as floatation solutions. In comparison, sedimentation method requires centrifugation to obtain parasitic cyst from the sediment, which makes the process of diagnosis difficult. The cyst stage of parasite is preserved in 10% formalin solution. (Smith et al. 2011). The main advantage of microscopic method is its limited cost, but it requires skilled and experienced person for identification. Moreover, microscopy is less sensitive than immunological techniques (Geurden et al. 2004). The chances of false positive results are more in microscopy due to small size of *Giardia* cyst, which is sometimes confused with pseudoparasites (yeast, paricles) (Dryden et al. 2006).

Antigen Detection

There are many immunological methods commercially available for the detection of parasitic antigen, including rapid solid phase qualitative immunochromatography assay, immunofluorescent assay (IFA) and enzyme linked immunosorbent assay (ELISA) (Garcia et al. 2003). These techniques were originally developed for diagnosis of infection in humans. Monoclonal antibodies are used against protein of cyst wall in ELISA and IFA techniques. EPG score of 1000 could easily be detected by immunofluorescence assay (Vidal et al. 2005). For diagnosis of infection in calves, immunological techniques are considered as more specific and sensitive than microscopy (Geurden et al. 2004). Similarly, in dogs IFA is found more sensitive technique for diagnosing giardiasis (Geurden et al. 2008). The main disadvantage of using any

immunological technique is its elevated cost due to expensive laboratory instruments and trained personnel.
The alternative technique for diagnosis is the immunochromatographic assay, which enables field-based diagnosis of infection within 15 minutes. In this method, monoclonal antibodies are used against parasitic cyst or trophozoite stage (Garrett et al. 2006). In human medicine, various assays including rapid membrane assay and dip-stick etc. have become commercialized for quick detection of infection. Similarly, SNAP® Giardia test is available in veterinary science for detection of infection in animals, particularly in dogs (Geurden et al. 2008). Speed® Giardia is an immunochromatographic assay commercially available for detection of infection in production animals (Geurden et al. 2010). Fecal ELISA kits are also commercially available for cats and dogs.

Polymerase Chain Reaction

Polymerase chain reaction is a molecular technique used for species identification and genotyping of Giardia. This technique has limited diagnostic uses and mostly used in research laboratories for sub-typing purpose, in order to determine assemblage or sub-assemblages of Giardia (Hooshyar et al. 2017). For molecular studies of Giardia species, the target gene sequences, including genes encoding small subunit (SSU) ribosomal RNA, glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi) and β-giardin genes (a protein in the adhesive disk of Giardia) (Hooshyar et al. 2019). The most commonly used gene for genotyping and diagnosis of Giardia is 18S rDNA (Read et al. 2004). PCR is highly sensitive test, as it can detect even 1 cyst in the sample (Amar et al. 2002). DNA inhibition may interfere in correct diagnosis. Furthermore, DNA extraction from faeces also needs to be standardized (Da Silva et al. 1999). Currently, PCR is extensively used in diagnosis of human infection but has very limited use in veterinary science due to the need of expensive instruments. It is yet to be evaluated as a diagnostic assay in production animals (Hooshyar et al. 2017). Moreover, another technique that can be used for diagnosing giardiasis is Fluorescent *in situ* hybridization (FISH), in which targeted sequences within RNA or DNA can be detected specifically with the use of fluorescent-labelled probes or oligonucleotides (Amann et al. 1995).

Zoonotic Transmission

Both host specific and zoonotic *Giardia duodenalis* can be harbored by the animals and are morphologically identical. Therefore, molecular typing is needed to trace the transmission of parasites (Feng et al. 2011).
Zoonotic transmission of Giardia from cattle to humans has been documented in numerous studies. The major way of transmission is through direct contact between cattle and humans. In addition, zoonotic transmission may also occur due to contaminated surface and ground water (Budu-Amoako et al. 2012). Calves play a major role in zoonotic transmission as they can shed up to 105-106 cysts per gram of faeces. Mostly the infection in cattle is caused by assemblage E of *Giardia duodenalis* and assemblage A infection occurs rarely and only in young animals (Mark-Carew et al. 2013).
There is very limited data available regarding zoonotic transmission of giardiasis from cattle to humans. In a study conducted in India, genotype A1 was identified from both cattle and workers from the same farm. However, cattle are mostly infected with sub-assemblage AI of Giardia, while humans are mostly infected with sub-assemblage AII of parasite (Xiao et al. 2008), indicating zoonotic transmission of parasite from cattle to farm workers (Khan et al. 2011).
The role of dogs in zoonotic transmission of Giardia is very important and is a subject of intense investigation. The dogs in urban areas of developed countries play a vital role in zoonotic transmission (Ballweber et al. 2010). People living in cities often have a close association with one another and sometimes they may live with pets i.e. dogs and cats. They consider them as their true and loyal friends, but some other benefits are also seen, like emotional and physiological attachment and social development. Canines and felines can also serve as a reservoir for a large number of zoonotic parasites like Giardia. These parasites can enter into the body of host through multiple routes, like mucosa and faeco-oral route. Humans serve as the final host for more than 100 parasites. It has been revealed that 85% of adult people have parasitism in their bodies (Martinez-Moreno et al. 2007). Cats and dogs are also proved to adopt non-zoonotic host-specific assemblages of Giardia after molecular surveys. Although, dogs and cats are infected with assemblage A and B of Giardia, but it is very difficult to access the frequency of zoonotic transmission without obtaining data from the owner (Feng et al. 2011).
There are numerous studies which were performed on a specific focus, involving both pet animals and humans and gave very interesting results. A study performed in tea growing area of India (Assam) indicated identical genotypes circulating between humans and dogs. The molecular evidence of this was not very convincing, however epidemiological data showed a significant relationship between prevalence of Giardia in human and household dogs (Traub et al. 2004). The zoonotic species of Giardia was also reported in both dogs and humans of Temple communities of Bangkok (Inpankaew et al. 2007) and indigenous communities in North Canada (Salb et al. 2008). Alternatively, another study which was performed in endemic regions of Peru showed different assemblages of Giardia in human and dogs, indicating the zoonotic transmission from dogs to humans is very uncommon (Cooper et al. 2010). Therefore, it is a matter of intense research to identify the zoonotic potential of Giardia from dogs to humans.
Cross transmission of Giardia between different hosts is possible. Wild animals living in aquatic environment can also be source of spread of giardiasis to human. In 1980 in Canada giardiasis outbreak occurred in beavers after consuming municipal water. The isolated cysts of Giardia from beavers were found identical to the cysts found in gerbils and later on it was confirmed by growing these

cysts under *in vitro* condition in laboratory (Appelbee et al. 2002). That's why giardiasis is referred as "beaver fever" in Canada. According to a study, cyst from human sources can also cause infection to beavers but needed in large quantity. Although it remained unclear that either beavers were the source of infection or they got infected by cysts from human source (Erlandsen et al. 1988).

The transmission of *Giardia duodenalis* is also possible from monkeys to humans. According to a study performed on rhesus monkey (*Macaca mulatta*) in China, assemblage A and B were found in the monkeys living in public parks. Assemblage AII is the main source of human infection and some strains of assemblage B were also detected from human sources in China, indicating the possibility of zoonotic transmission from monkeys (Ye et al. 2012).

Giardia can also be transmitted to humans from wild primates. A study conducted at Uganda indicated high degree of cross transmission of *G. duodenalis* among wild primates, livestock and humans at locations where humans and livestock interact at high degree with wildlife (Ghariebet al. 2016).

Treatment and Control

There are several compounds with known *in-vitro* and *in-vivo* efficacy against Giardia, and this has also been demonstrated in laboratory animals. There is no published data available regarding good efficacy of any treatment against giardiasis in sheep and goats. Although different studies were conducted on ruminants to evaluate the efficacious treatment against the disease (Gultekin et al. 2016; Karademir et al. 2016; Santin 2020), but no licensed treatment is available in ruminants yet. However, several compounds were evaluated and found to be effective in dogs and cats.

Chemotherapeutic Treatment

In human medicine, nitro-imidazoles (NZs), including furazolidone, metronidazole, are frequently used to treat giardiasis. Although, there are chances of side effects from these compounds, yet these show very good efficacy in humans against giardiasis. Metronidazole is even considered as carcinogenic in nature. Furthermore, there are also chances of resistance development against both metronidazole and furazolidone (Harris et al. 2001).

In veterinary medicine, dimetridazole and metronidazole are used against giardiasis in companion animals and ruminants and exhibit very good efficacy. In cats, metronidazole and dimetridazole is used for a period of 15 days in order to achieve cyst reduction. In some countries, the use of nitro-imidazoles in farm animals is contra-indicated (Scorza et al. 2004).

Furthermore, nitazoxanide has proved to be a promising drug against giardiasis in *in-vitro* trials (Cedillo-Rivera et al. 2002). According to Zygner et al. (2008), azithromycin is also effective against giardiasis in companion animals but only few numbers of animals were included in the trial.

Benzimidazoles

Benzimidazole compounds (BZs) belong to the class of broad spectrum anthelmintics and can be used against giardiasis due to their broad safety margins and less toxicity. In *in-vitro* trials, BZs have also proved to be more efficacious than metronidazole against Giardia. Tubulin is the structure found in the trophozoite cytoskeleton and BZs interfere in its polymerization, resulting in inhibition of all activities of ventral disc and median body. As a result, trophozoite will not be able to get attached and colonized in intestine. BZs seem to be ineffective against flagellar tubulin due to their different structure. BZs also get attached with giardin which is a Giardia-specific protein present in the ventral disc (Shaharyar et al. 2017).

In calves, use of albendazole and fenbendazole in combination showed promising results regarding decrease in cyst excretion. The total dose of benzimidazole used against giardiasis (5-20 mg/kg/day for three consecutive days) is higher than that used against helminth infection (Leitsch et al. 2015). According to another study, BZs showed very limited cyst suppressing effect against giardiasis in calves under field condition. This might be due to high infection pressure, less efficacious treatment or rapid re-infection. Due to less efficacy, it was concluded that calves need continuous treatment with BZs for long period of time in low dosage (O'handley et al. 2000). However, the continuous treatment with BZs may lead to a resistant development in animal.

In dogs, fenbendazole, oxfendazole and albendazole are used against giardiasis and show very good results regarding cyst elimination and alleviation of clinical symptoms. The recommended treatment is for consecutive three days along with good hygiene practices. Furthermore, there are few reports of albendazole causing bone marrow depletion and carcinogenic effects, specifically in pregnant bitches (Chon et al. 2005). In cats, fenbendazole is not recommended against sole giardiasis infection due to its less efficacy to remove cysts but it shows good efficacy against co-infection of Giardia and Cryptosporidium (Keith et al. 2003).

Pyrantel-febantel-praziquantel combination

Various studies have shown that combined use of pyrantel, febantel and praziquantel in dogs and cats resulted in a significant reduction in cyst excretion (Payne et al. 2002; Scorza et al. 2006; Montoya et al. 2008; Bowman et al. 2009). Another study suggested that combination of pyrantel and febantel showed very promising results compared to febantel alone (Olson et al. 2009).

Paromomycin

Paromomycin is a broad-spectrum antibiotic, belonging to the amino-glycoside group and shows very good efficacy against giardiasis in humans (Wright et al. 2003). It inhibits the synthesis of proteins after binding with small subunit of rRNA, resulting in either destruction of Giardia

or withdrawal of its nutrients (Rossignol et al. 2010). It is well tolerated by calves due to prolonged absorption period from gastro-intestinal tract (Grinberg et al. 2002). The recommended dose in calves is 50-75 mg/kg/day for a period five consecutive days (Geurden et al. 2006).

Control

Although the treatment of giardiasis by chemotherapy showed good results, even then most of treated animals show re-infection after several days (2-3 weeks) due to contaminated environment (Geurden et al. 2006). The cysts of this parasite can survive for 7 weeks in soil and 1 week in feces, while the treatment is mostly done for three to days which is very short to prevent occurrence of re-infection in a contaminated environment. So, there is need of integrated control approach, including both treatment of infected individuals and cleansing of environment in order to minimize the chances of re-infection. Giardia cysts survive well after disinfectant treatment like chlorine. Ultraviolet irradiation, chlorine dioxide and ozone are included in the category of disinfectants which can be used in drinking water treatment for research purpose and strongly contra-indicated to be used in calf facility (Saleh et al. 2016). Alternatively, quaternary ammonia disinfection and heat treatment can be used safely in housing facilities. According to a study, when environment is disinfected with 10% ammonia along with treatment of animals with fenbendazole, the efficacy of treatment is improved, resulting in the reduction of excreted cysts from animals. This indicates that treatment efficacy can be improved effectively by breaking the transmission cycle of the parasite (Geurden et al. 2006).

As the chances of getting re-infection in companion animals from fecal contaminated limbs or fur increased in contaminated environment, so the practice of introducing the treated animals into a clean environment is emphasized. Animals should be washed after treatment and before introducing in to a clean environment (Uehlinger et al. 2007).

Good management is the key practice to achieve good curative results after every treatment regimen. There are several management practices that can prevent the occurrence of infection if applied correctly, including proper cleaning of housing facilities and limiting the number of animals per housing facility (Maddox-Hyttel et al. 2006). Indoor animals are more likely to get giardiasis infection than outdoor animals, indicating the importance of housing facilities management (Itoh et al. 2009). As Giardia cysts can persist for long period of time on moist areas, the management of manure is another alternative approach to reduce the infection load at farm level (Van Herk et al. 2004).

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MEATBORNE PARASITIC ZOONOSIS

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INTRODUCTION

Foodborne parasites seriously threaten human health in many parts of the world. Besides affecting human health, parasites adversely affect the export of animal products from countries with a disease to other countries. Parasitic diseases affect meat and milk production, and reproductive performance of the animals they infect, and thus, cause loss of millions of dollars by reducing the quality of the skin and fleece. Therefore, foodborne zoonoses can serve as a commercial barrier among countries. The prevalence of foodborne parasites can vary among countries and even between different regions of the same country. Factors contributing to this variation include differences in animal breeding systems, demographic structure, and nutritional habits (Macpherson et al. 2000; Slifko et al. 2000; Gupta et al. 2018; Zarlenga and Gamble 2019).

Parasitic diseases continue to be an important public health problem in both developed and developing countries. Data from the World Health Organization (WHO) estimate that one of every four people worldwide is infected with parasites (WHO 2021). Due to the rapid increase in the world population, more protein sources are needed every day. In addition, as seen during the COVID-19 epidemic, the food supply chain becomes of paramount importance in pandemics that affect the whole world at the same time. Therefore, it is clear that food safety will continue to be an important issue in the future.

Foods of animal origin play an important role in providing people with balanced and adequate nutrition (Dhaliwal and Juyal 2013). According to the Food and Agriculture Organization of the United Nations (FAO), at least 40-50% of daily protein needs of humans should be obtained from foods of animal origin for a balanced diet. The FAO has recommended that 44 grams of the daily protein need out of 90 grams should be of animal origin. However, the worldwide daily consumption of animal protein per person is approximately 32 grams. Based on this data, it seems that many people do not consume enough animal protein.

Meat is the most accessible animal protein source. People meet protein needs by eating the meat of various animals, including game animals. However, the meat can cause viral and bacterial diseases, as well as directly or indirectly transmit various parasitic diseases, to humans if it are not subjected to a proper and meticulous examination.

Compared to other foodborne parasitic diseases, the number of those transmitted by meat is small, but their consequences in humans can be much more severe (Zhou et al. 2008; Bhatia et al. 2010; Dhaliwal and Juyal 2013). For instance, various growth stages of the *Toxoplasma gondii*, which are found in the muscles and edible internal organs of infected animals, are transmitted to humans through meat that is eaten raw or undercooked. The prevalence of diseases with meat borne zoonoses is higher in underdeveloped or developing countries, where meat is generally consumed unprocessed, than in developed countries (Gupta et al. 2018). In such countries, animals are usually slaughtered outside slaughterhouses, and the meat of animals slaughtered in slaughterhouses is typically not adequately inspected by veterinarians. Therefore, meat carrying the infective forms of parasites quickly enters public circulation and is consumed. In addition, people's lack of knowledge about parasitic diseases transmitted from meat accelerates this spread.

In recent years, various tests with high specificity and sensitivity have been developed to facilitate the diagnosis of parasitic diseases in humans and animals. In addition, important developments have been made in the effective treatment of people, who contract these diseases. Further, strict legal regulations regarding meat inspection have been implemented, especially in European countries. Despite these developments, various precautions have not completely prevented the transmission of parasitic diseases by meat. Accordingly, more effective measures must be taken for safer meat consumption and to raise people's awareness about these diseases and their means of transmission.

In the next section, parasitic zoonoses transmitted directly to humans through meat will be outlined. However, diseases such as Echinococcosis, which are transmitted to definitive hosts through meat or other means and then infect humans, will not be discussed.

Parasitic diseases transmitted to humans by consuming the meat of various animals are Taeniasis, Trichinellosis, Toxoplasmosis, and Sarcocystosis (Gamble 1998; Ünüvar 2018; Liu 2019; Zarlenga and Gamble 2019). The parasitic factors that cause these diseases are outlined in Table 1.

TAENIASIS

Three main types of *Taenia* infect humans; these include *Taenia solium, Taenia saginata,* and *Taenia asiatica*. Although *T. asiatica* was initially considered a strain or

subspecies of *T. saginata*, subsequent morphological and genetic studies have revealed that it is a different species. While adult forms of these species live in the small intestines of humans, metacestode forms are seen in muscles or internal organs of animals that are intermediate hosts (Ünüvar 2018; Liu 2019; Zarlenga and Gamble 2019). The larva of *T. saginata* is called *Cysticercus bovis* and is located in the tongue, diaphragm, heart, and striated muscles of cattle, which serve as intermediate hosts. *Cysticercus cellulosae*, the larva of *T. solium*, is mainly seen in the muscles of pigs. However, humans can be both the definitive host and intermediate host for *T. solium*, and these larvae can develop in the muscles, under the skin, or in the brain. *Cysticercus viscerotropica*, the larva of *T. asiatica*, mostly develops in the liver of domestic and wild pigs, but can sometimes be found in the lungs (Li et al. 2007; Liu 2019; Zarlenga and Gamble 2019). Some of the main characteristics of the *Taenia* species that infect humans are summarized in Table 2 (Liu 2019).

Table 1: Main meatborne parasitic zoonoses

Parasites group	Genus	Species	Disease	References
Helminth	*Taenia*	*Taenia saginata*	*Taeniosis*	• Dubey (1986; 2015)
		Taenia solium	*Taeniosis/Cysticercosis*	• Bhatia et al. (2010)
		Taenia asiatica	*Taeniosis*	• Dhaliwal and Juyal (2013)
	Trichinella	*Trichinella spiralis* and any other species	*Trichinellosis*	• Ünüvar (2018) • Ortega (2019)
Protozoon	*Toxoplasma*	*Toxoplasma gondii*	*Toxoplasmosis*	• Liu (2019)
	Sarcocystis	*Sarcocystis suihomonis* *Sarcocystis hominis*	*Sarcocystosis*	• Zarlenga and Gamble (2019)

Table 2: Some characteristics of *Taenia* species that infect humans.

Species	Definitive host	Intermediate host	Adult cestode	Metacestode	Prevalence
Taenia solium	Man	Domestic and wild pig	• Its length is between 2-8 m. • There are 1000 proglottids in its strobila.	*Cysticercus cellulosae* • Its size is 5–8 × 3–6 mm. • It is located in the tongue, diaphragm, masseter and other muscles.	Cosmopolitan. Common in Latin America, Africa, Mexica, Russia, India, Asia.
Taenia saginata	Man	Cattle	• Its length is between 4-12 m. • There are 1000-2000 proglottids in its strobila.	*Cysticercus bovis* • It is size is 6–10 × 4–6 mm. • It is located in the tongue, diaphragm, masseter and other muscles. • Sometimes it can be found in the liver and lungs.	Cosmopolitan. Common in Africa, Asia, Europe and America.
Taenia asiatica	Man	• Domestic and wild pig • Rarely cattle and goats	• Its length is 3.5 m. • There are 700-900 proglottids in its strobila.	*Cysticercus viscerotropica* • It is size is 2 × 2 mm. • It is located in the liver and lungs.	Its prevalence is more limited. It is seen in countries such as Taiwan, South Korea, Indonesia, Japan, etc.

T. solium TAENIASIS

This species, known as "Pig tapeworm," is an important public health problem, causing Neurocysticercosis in people living in areas where pig breeding is common. Around 50 million people worldwide are estimated to be affected by this disease. Death occurs in 1% of the individuals affected by the disease, and reports indicate that approximately 50,000 people worldwide die from this disease every year. Therefore, WHO describes Neurocysticercosis as an important neglected tropical disease (Schantz et al. 1993; Zarlenga and Gamble 2019).

Epidemiology

T. solium, which is a worldwide common helmintozoonosis, poses a serious public health problem, especially in underdeveloped and developing countries (Tsang and Wilson 1995; Bern et al. 1999; Murrell 2005; Juyal et al. 2008; Yanagida et al. 2012). *T. solium* is more prevalent in areas where pig farming is widespread, people are in close contact with pigs, and pork is consumed raw or undercooked. Humans can be intermediate hosts for this parasite, which is of great importance in epidemiology. People can consume *T. solium* eggs via contaminated food or water, or autoinfections can occur.

In such cases, the eggs may travel to the brain, causing Neurocysticercosis, or they can settle in other tissues and organs, such as muscle tissue and subcutaneously (Bruschi and Gomez-Morales 2017; Ünüvar 2018; Liu 2019; Zarlenga and Gamble 2019). Neurocysticercosis is one of the most frequent preventable causes of epilepsy in developing countries and is a long-term infection that adversely affects patients' quality of life (Flisser et al. 2005).

T. solium infections can be seen in many countries, and are more common in Latin America, Mexico, India, Africa, non-Muslim Asian countries, China, Nepal, Russia, Southern and Eastern European countries, and Slavic countries (Geerts 1993; Schantz et al. 1998; Liu 2019; Zarlenga and Gamble 2019). Distinguishing *T. solium* eggs from other *Taenia* species in coprological examinations is very difficult. For this reason, determining the prevalence in humans precisely and accurately is not possible. Increasing illegal human migration in recent years has caused infection to spread rapidly.

Morphology

Adult forms of *T. solium* live in the small intestine of humans. Due to the presence of hooks in the rostellum, the parasite is called "armed tapeworm." Its length varies between 2-8 m. There are approximately 1000 proglottids in its strobilas, and 22-32 hooks in two rows in its scolex. Each gravid proglottid contains approximately 40,000 eggs. *T. Solium* is a long-lived parasite and can survive in the human intestine for more than 10 years. *C. cellulosae* are about 3x5 mm and filled with a cloudy liquid. Eggs are spherical or slightly oval, brownish, and 40-48 mμ in size (Saygı 1999; Liu 2019).

Transmission

Humans are infected by eating the raw or undercooked cysticerci-containing meat of intermediate host (pigs). Infection can also occur by ingesting external eggs, and *C. cellulosae* can develop. Intermediate host pigs are infected by consuming food and water contaminated with eggs.

Life Cycle

The only known final host of *T. solium* is humans. Pigs normally serve as intermediate hosts. However, sometimes in pig-breeding regions, the metacestode form can be found in dogs. Eggs leave the host when proglottids break off from the parasite and are excreted in human feces. The breakdown of the proglottids in the external environment releases and spreads the eggs. When pigs consume the eggs, they open in the small intestine, releasing oncospheres. The oncospheres enter the mesenteric veins in the intestinal wall and move through the bloodstream, eventually settling in muscles or internal organs. Then, they develop into cysticerci, the larval shape, in about two months. In the early period, a fibrous capsule develops around the *C. cellulosae* as a result of the host's response. Under normal conditions, the cysticercus, which is 3x5 mm in size, can reach a size that can contain 60 ml of liquid in its brain and subdural areas (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019). The most common muscles, where *C. cellulosae* are found in pigs, are the heart, masseter, diaphragm, and tongue muscles. Due to their appearance in the pork meat, cysticerci are also called "pork measles" (Saygı 1999).

People often become infected by eating raw or undercooked pork meat that carries *C. cellulosae*. Larvae separate from muscles or other tissues in the stomach, adhere to the wall of small intestine and evaginate in the upper parts of the small intestine; adult parasites develop in 5-12 weeks (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019). Humans can also be an intermediate host for this parasite. Infections in this way occurs by consuming foods contaminated with *T. solium* eggs or by transmitting eggs from the hand to the mouth. Eggs that open in the small intestines of humans, enter the blood vessels as they do in pigs, and lodge under the skin or in the muscles, eyes, tongue, and most importantly, the brain. Another form of transmission in humans is autoinfection. In humans, who carry this parasite in their small intestines, the eggs can enter the stomach through reverse peristalsis. After the oncospheres are released, they become cysticerci by traveling to various organs and tissues. Cysticerci that settle in the brain or on its surface cause neurocysticercosis (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019).

Clinical Signs

Although infected people usually have only one parasite, occasionally more parasites are found, and in some cases more than 25 parasites have been found (Saygı 1999; Zarlenga and Gamble 2019). The hooks in the scolexes of *T. Solium* cause damage to the intestinal tissue where they attach, and consequently cause inflammation and pain. Intestines typically are not obstructed. However, intestinal perforation, peritonitis, and gall bladder inflammation may occur (Zarlenga and Gamble 2019). The clinical signs of adult *T. solium* infections are similar to those of*T. saginata* infections. When a single parasite is found, typically no significant symptoms occur. Infection may not be noticeable, until a proglottid is seen being ejected from the anus of an individual carrying the parasite. The most common clinical symptoms are colic, abdominal pain, nausea, vomiting, and chronic dyspepsia. Abdominal pain and nausea may be seen in children, especially in the morning, and improve when a small amount of food is eaten. In addition to these symptoms, anal itching, diarrhea, constipation, weakness, anorexia, anemia, and rarely fever may be observed in infected people (Saygı 1999; Liu 2019). In addition, blood eosinophil counts can increase by up to 28%.

The main pathogenic effects in humans are caused by the larval stages, which can settle in various tissues and organs. Mild infections, in which cysticerci are not found in vital areas and the larvae are not yet developed, typically display no remarkable symptoms in humans. Cysticerci can cause neurocysticercosis, ophthalmic cysticercosis, subcutaneous cysticercosis, and muscular or lingual cysticercosis, depending on where they are located (Cardenas et al. 1992; Garcia et al. 2003; Elias et al. 2005). Clinical symptoms can also vary according to the organ infected and number of cysticerci. The most severe infection and symptoms occur in the central nervous system (CNS). The most common symptom of cysticerci

located in the brain is epileptic seizures, which start when the larvae first settle. The death of these larvae is increased by cysting and tearing. Another common symptom is hydrocephalus, caused by blockage of the cerebrospinal fluid (CSF). In addition, mental disorders and meningeal irritations may occur, depending on the site where larvae are located in the CNS (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019).

In ophthalmic cysticercosis, the cysticerci are usually located in the retina, anterior, and posterior camera of the eye. These larvae, especially those pressing on the optic nerve, can cause visual field loss and deficits in vision. Larvae that settle in the retina can cause hemorrhage and retinal tears (Saygı 1999; Liu 2019). In subcutaneous cysticercosis, the cysts resemble small lipomas that can be easily palpated. In muscular cysticercosis, the larvae settle in voluntary muscles and generally do not produce symptoms, as they continue their development. Sometimes they can cause myositis and therefore, eosinophilia and fever.

In addition, swelling, atrophy, and fibrosis may occur in the muscles. However, cysticerci that die in muscle tissue do not cause any symptoms, if they are calcified (Liu 2019; Zarlenga and Gamble 2019). These metacestodes, which cause serious life-threatening diseases in humans, do not cause any significant clinical symptom in their intermediate pig hosts. In some exceptional cases, tongue paralysis and convulsions may be observed in pigs. However, the presence of cysticerci in pigs spoils the aesthetics of the meat and thus has important economic impacts.

Diagnosis

The most commonly used conventional and reliable method for the diagnosis of mature *T. solium* in humans is stool examination. For this purpose, the stool is examined macroscopically for discharged proglottids and microscopically for eggs. In microscopic examination, eggs are sought by using flotation methods. However, it is not possible to distinguish *Taenia* eggs seen in the stool examination at the species level, so the species responsible for the infection cannot be determined by fecal examination. The only known way to determine to the causative species is to obtain the gravid proglottids that were thrown off individually or in groups and to count the uterine side branches in these rings, which are the only known feature for pre-diagnosis. If the scolex can be found after treatment, the presence of four large suckers and hooked rostellum also helps to distinguish the parasite from *T. saginata*. Serological techniques are used for diagnosis, while more advanced techniques such as PCR are used to differentiate species (Allan et al. 1996; Saygı 1999; Liu 2019; Zarlenga and Gamble 2019). The definitive diagnosis of cysticercosis can also present difficulties due to the tendency of parasite to settle in brain tissues, where routine biopsy is not possible. For this reason, serological tests of antibodies against cysticercosis and imaging techniques, such as CT, MRI, and x-rays are used in the diagnosis of human cysticercosis (Schantz et al. 1993; Saygı 1999).

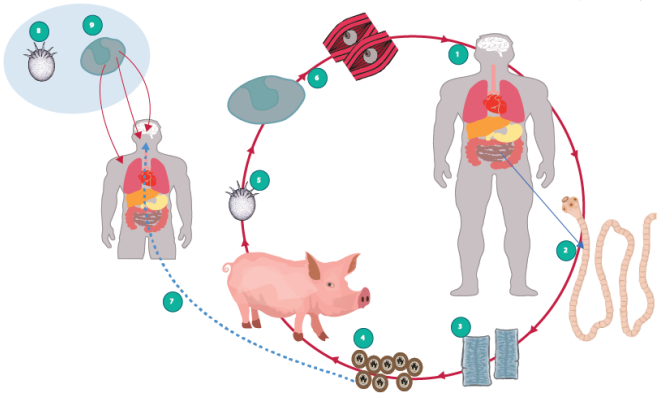

Fig. 1: Life cycle of *T. solium* (Illustrated by B Senlik)
1: Humans infected by ingesting raw or undercooked infected meat. **2:** Adult tapeworm developed and attached in small intestine **3:** Gravid proglottids and sometimes eggs in faeces passed in environment **4:** Eggs released from proglottids in the environment are taken by intermediate host pigs with contaminated water and food. **5:** After the oncospheres inside the egg is released, it gradually settles in the tongue, diaphragm and other muscles of the pigs. **6:** In these muscles, they become fluid-filled cysticercus. **7:** In humans, infection can also occur by oral ingestion of eggs that have been passed into the environment, or sometimes by the development of eggs in their own intestines as a result of auto infection. **8:** In this case, the released oncospheres go to various body parts and settle. **9:** The oncospheres, which are located in organs such as eyes, brain, tongue, muscles and under the skin, develop here and form cysticerci.

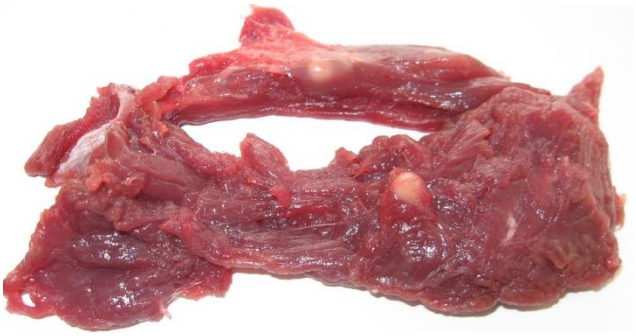

Fig. 2: *C. cellulosae* in pig muscles (from B. Şenlik).

Calcification of *C. cellulosae* helps to determine its localization in the central nervous system and muscles. Brain scans, using CT and MRI, can show the lesion and allow a pre-diagnosis before an operation. An x-ray examination of the soft tissue of the extremities can show the characteristic rice grains or calcified shadows, called “puffed rice” (Saygı 1999). Eye cysticercosis is usually diagnosed by looking at the morphology and movements of the larval form (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019).

In pigs, the diagnosis of *C. cellulosae* is made by meat examination. The diagnosis can be made in living animals by examining the tongue for cysticerci. However, the reported sensitivity of tongue examination is low. After cutting the meat, the muscles and organs, where the larvae can be found, should be carefully examined by cutting the sections. In particular, the diaphragm, intercostal, heart, tongue, and masseter muscles should be examined for cysticerci. Successful diagnostic results

have also been obtained by ELISA, using some fractions obtained by purification of cysticercus cyst fluid and excretion products of the parasite.

Treatment

Niclosamide is the anthelmintic of first choice for the treatment of adult parasites in humans. This drug acts by causing the parasite to separate from the intestinal mucosa by necrosis of the scolex and prevents the parasites from sugar absorption. It is taken orally at a dose of 50 mg/kg in children and a dose of 2.0 g in adults. Praziquantel is used orally at a dose of 5-10 mg/kg as a single dose. If albendazole is preferred, it should be administered orally, 400 mg once a day for three consecutive days. Another option in the treatment of adult parasites is tribendimidine. This drug is also taken orally and is used at a dose of 200 mg at a time in individuals younger than 15 years old, and 400 mg at a time in individuals older than 15 years (Liu 2019; Zarlenga and Gamble 2019).

The treatment of Neurocysticercosis depends on the symptoms of the disease, the location of the infection in the nerve tissue, and the number of cysts. Conservative treatment is preferred in most patients. Albendazole gives better results than praziquantel. Albendazole 10-15 mg /kg/day is used for 8-28 days, while praziquantel is used 50 mg/kg/day (divided into three doses) for 15 days. Stool proglottids of individuals treated for adult parasites should be sought for three days after treatment. In addition, stool examinations should be performed 30 days and 90 days after treatment, and the eggs of the parasite should be sought (Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

Cysticerci that die after anthelmintic treatment can cause inflammatory reactions in the brain. Therefore, corticosteroids should be used in addition to anthelmintic therapy to reduce the inflammatory reaction and increased intracranial pressure. For this purpose, patients should be given 60 mg prednisone or 6 mg dexamethasone orally once a day. Corticosteroid therapy should be initiated three days before anthelmintic administration and continued until one week after administration (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019). In addition, anticonvulsant drugs can be used to help treat seizures that may occur. If symptoms persist despite medical treatment and the patient does not improve, surgical treatment should be advised (Liu 2019; Zarlenga and Gamble 2019). Surgical treatment is more beneficial in orbital cysticercosis. In muscular cysticercosis, positive results can be obtained from praziquantel.

It is not possible to eliminate all cysticerci with anthelmintics in pigs that are intermediate hosts. For this reason, anthelmintic treatment is generally not used in pigs.

Control and Prevention

In order to successfully combat this disease, the life cycle of the parasite between pigs and humans must be broken. For this purpose, people carrying the parasite should be treated with an appropriate anthelmintic. It should be borne in mind that an infected person can infect thousands of pigs. Especially in the period when large amounts of proglottids are thrown off after treatment, contamination of the environment by human feces should be prevented, and feces and contaminated water should not be used for irrigation of agricultural areas. Having hygienic toilets alone is not enough to prevent the disease. Accordingly, chemical agents that can inactivate the parasite eggs should be used in the areas where the infection is intense. In addition, attention should be paid to personal hygiene and hand hygiene to prevent the ingestion of parasite eggs spread to the environment by humans. Lettuce, parsley, cress or other leafy vegetables and salad ingredients should not be eaten without washing, as they may be contaminated with eggs (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

People should be prevented from consuming pork with *C. cellulosae*. Meat should be meticulously examined, and pig carcasses should be checked by veterinarians for cysticerci after slaughter. If a large number of cysticerci are found in the carcass, the entire carcass must be destroyed. If small numbers are found, consumption should be permitted provided that the cysticerci are subjected to processes that will destroy them. Meat should be cooked at 80°C or at high temperature to reach an internal temperature of 56°C. Keeping meat at -10°C for 14 days also ensures the inactivation of cysticerci in pork (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

T. saginata TAENIASIS

This cestode species that infects humans is known as “bovine tapeworm.” It is widely seen in regions and countries where cattle are raised in open areas. Although cattle are their intermediate hosts, *Cysticercus bovis*, the metacestode form of the parasite, can sometimes be seen in deer, antelope, buffalo and llama (Liu 2019; Zarlenga and Gamble 2019).

Epidemiology

T. saginata infections are common in countries and regions, where cattle are raised freely. In addition, the prevalence is high in regions where raw or undercooked beef consumption is high. Exotic diets, which have gained popularity in recent years, encourage people to consume raw meat. Tourism, illegal immigration, and illegal crossings between countries also contribute to the spread of the infection. Another factor driving the spread of infection is the increase in animals and animal products trade between countries (Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019). Parasite eggs, that enter the environment through human feces, are highly resistant to drought and can maintain their infectivity for a long time, even at low temperatures. For example, eggs can remain infective for 154 days in the

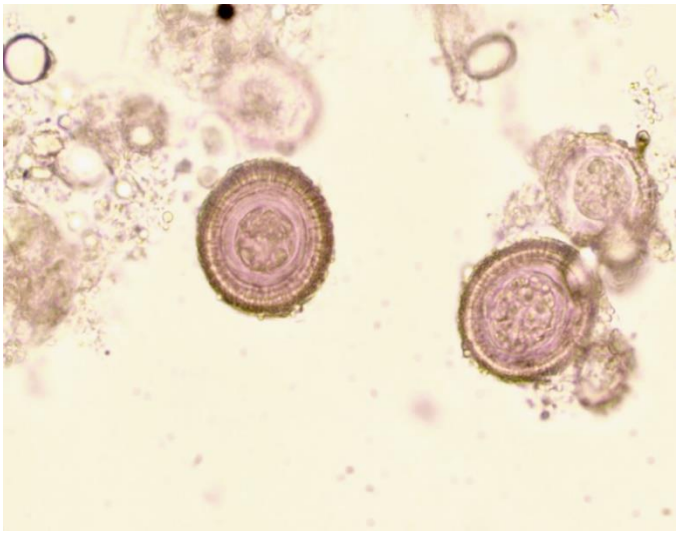

Fig. 3: The appearance of Taenia spp eggs in stool examination (from B. Şenlik).

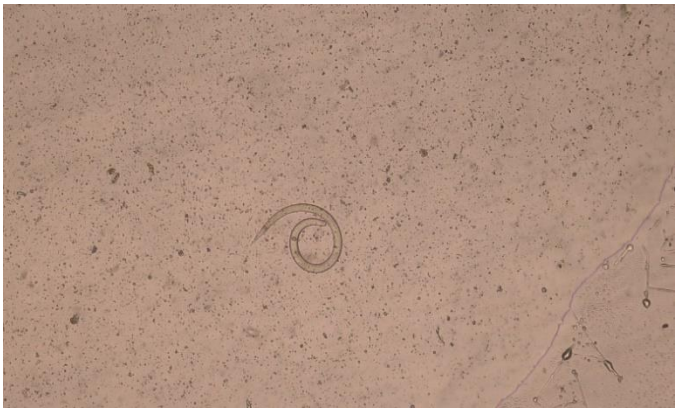

Fig. 4: The appearance of Trichinella spp. larvae in digestion method (from B. Şenlik).

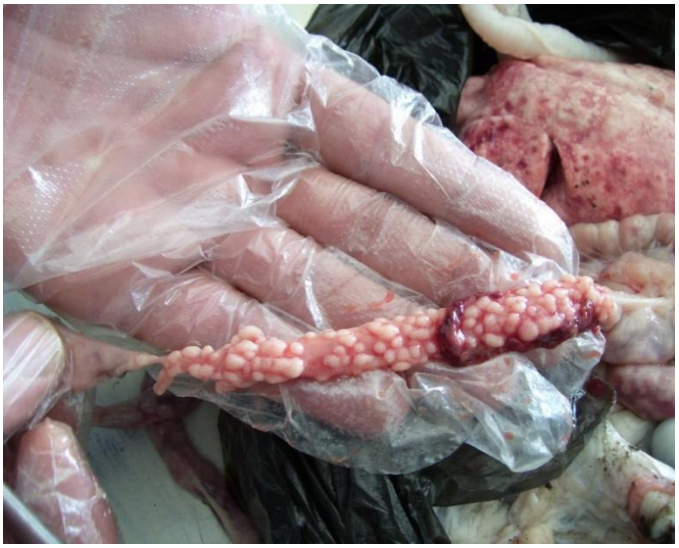

Fig. 5: *Sarcocystis* cysts in sheep esophagus (from B. Şenlik).

pasture and for more than 70 days in stool. This parasite is common in Africa, the Middle East, Asia, Europe, Latin America, Ethiopia, the Philippines, and Zaire (Liu 2019; Zarlenga and Gamble 2019).

Morphology

The mature form of *T. saginata* is seen in the small intestine of humans, while the larvae form (*Cysticercus bovis*) is seen in cattle. The length of the parasite varies between 4-12 m and sometimes can reach 25 m. The strobila bears 1000-2000 proglottids. Each gravid proglottids contains approximately 100,000 eggs. Since the scolex does not contain rostellum and hooks, it is called "unarmed cestod." Cysticerci are 4x6 mm in size and liquid-filled (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019). Eggs are spherical or slightly oval and similar in size to other *Taenia* eggs. It is not possible to distinguish these eggs from other *Taenia* eggs under the light microscope. Eggs are surrounded by a thin capsule, which disintegrates when eggs leave the proglottids. An average of 720,000 eggs are laid in proglottids per day (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

Transmission

People are infected by this parasite through eating raw or undercooked beef contaminated with cysticerci, while cattle are infected by ingesting eggs via contaminated feed and water.

Life Cycle

The final hosts of *T. saginata* are humans, and their intermediate hosts are domestic cattle. However, sometimes animals such as deer, antelope, llamas and giraffes can also be intermediate hosts (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

After *T. saginata* eggs are ingested by cattle, oncospheres released in the duodenum pass through the blood vessels or lymph channels by piercing the intestinal wall. These eggs circulate through the bloodstream and eventually settle in the tongue, neck, heart, shoulder, and thigh muscles, where they change into the larval form of *C. bovis*. Cysticerci can sometimes develop in organs, such as the lungs, liver, esophagus, brain, and lymph nodes. The development of *C. bovis* begins about 10 days after the oncospheres settle. During this time, cysticerci are seen as small, white lesions with a diameter of 1-2 mm. During the 12-15 weeks after infection, these cysticerci continue to develop and reach a mature length of 6-9 mm. About 8-10 weeks after the eggs are ingested by cattle, these cysticerci become infective and can remain viable in the tissues of intermediate hosts for more than a year (Saygı 1999; Liu 2019; Zarlenga and Gamble 2019).

Humans are infected by eating the raw or undercooked cysticerci-bearing tissues and organs of intermediate host animals. When these cysticerci in the muscles and organs are eaten by humans, they are separated from the scolex and neck sac by evagination in the jejunum and cling to the intestines with their hooks. While the activity of gastric and intestinal fluids is effective on the evagination

of the cysticerci in the gastrointestinal system of humans, the speed of the intestinal passage also affects this event.
In the development process, proglottids begin to form from the neck area of these young parasites clinging to the intestines. An average of 4-8 proglottids per day form from the neck area and are added to the strobila. Adult cestodes develop 8-10 weeks after the ingestion of cysticerci by humans. Gravid proglottids detached from the strobila fall off the anus due to their own movements. *C. bovis* does not develop in humans except in very rare cases (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).

Clinical Signs

People infected with *T. saginata* usually develop a parasite, but sometimes more than one parasite may develop. In people carrying the adult parasite, the infection is usually mild and asymptomatic. The most common symptom that bothers people the most is the throwing of the proglottids broken off from the parasite's strobila. About 5-10 minutes before the proglottids start to be thrown, the patient feels a sensation in their rectum and the proglottids come out of the anus with a feeling of shivering (Saygı 1999). The patient may feel uneasy during this process. Proglottids have a higher rate of being thrown during the daytime, when an individual is physically more active. Other clinical symptoms seen in infected individuals include abdominal pain, nausea, vomiting, weakness, weight loss, anorexia, constipation, diarrhea, excitement, and most significantly, anal itching. Abdominal pain is in the form of "hunger pain" and mostly eases after eating (Zarlenga and Gamble 2019). Some patients may also experience dizziness and headache. The severity of the clinical symptoms may vary, depending on the burden of infection (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble 2019).
Adult *T. saginata* in the intestine causes traumatic and irritative effects. Proglottids detached from the strobila can occasionally cause obstruction in the pancreas and bile ducts or obstruct lumen of the appendix and cause acute appendicitis (Liu 2019; Zarlenga and Gamble 2019).
C. bovis does not usually cause clinical symptoms in cattle. In experimental studies, it was observed that it caused fever and stiffness in the muscles. In addition, it has been reported that glycogen synthesis is negatively affected in the skeletal muscles and liver in infected cattle, and embolism may also be seen (Saygı 1999).

Diagnosis

Since *T. saginata* infections in humans do not have a characteristic clinical presentation, diagnosis is based on laboratory findings. Diagnosis can be made by stool examination, the cellophane band technique, serological tests, and searching for coproantigens in the stool (Gottstein et al. 1991). Stool examinations sometimes may be insufficient to reveal infections. Because there is no uterine hole in the proglottids of the parasite, the eggs cannot be released before the proglottids are broken. In such cases, the egg may not be detected in the stool. As in *T. solium* infections, eggs detected in the stool cannot be distinguished by classical examination under light microscopy. Eggs can only be distinguished from one another using molecular techniques (Liu 2019; Zarlenga and Gamble 2019). Although antibodies are formed against this parasite in humans, there is no specific test that can be used for diagnostic purposes. Since *T. saginata* does not cause a life-threatening clinical condition in humans, it is sufficient to detect proglottids in the anal area or in the feces.
The diagnosis of *C. bovis* in cattle can usually be made at a necropsy, or during the meat examination performed in the slaughterhouse (Saygı 1999; Liu 2019). Examination should start with the masseter muscles. For this purpose, sections should be made parallel to the mandible, then the neck muscles should be examined. Subsequently, the tongue, diaphragm and heart should be examined. It should be kept in mind that cysticerci can be overlooked during normal meat examination in mild infections, since it is not possible to cut all the muscles of the body for examination. For this reason, meat examination should be carried out carefully and meticulously in accordance with rules. Some serological methods, such as ELISA, can be used for diagnosis in cattle. The specificity of the ELISA method with monoclonal antibodies has been found to be very high, and it has been shown that this method allows the detection of infection in cattle with 50 cysticerci (Harrison et al. 1989; Brandt et al. 1992).

Treatment

Treatment of *T. saginata*-infected people is of paramount importance, as they are a source of infection for cattle in their area (Liu 2019; Zarlenga and Gamble 2019). Anthelmintics, such as niclosamide, praziquantel and albendazole, used for the treatment of *T. solium* infections can be used. Corticosteroids or anticonvulsants are unnecessary in *T. saginata* taeniasis, since cysticerci are not formed in humans. However, in order to reduce complaints such as vomiting and nausea, auxiliary drugs can be used in treatment. Treatment of cattle carrying *C. bovis* is typically not applicable. Especially in cattle with a large number of larvae, it is not possible to destroy all of them. However, when needed, praziquantel and albendazole can be used.

Control and Prevention

Since people are the main source of infection, stool must be disposed of properly during and after treatment. In the absence of hygienic toilets in underdeveloped countries or regions, the proglottids discarded with feces should be collected and burned in a fire or boiled in water. In addition, contamination of the environment by human excreta should be prevented by installing appropriate sewage systems, and sewage water should not be used for irrigation of agricultural areas (Saygı 1999; Bruschi and Gomez-Morales 2017; Liu 2019; Zarlenga and Gamble

2019). Contamination of the pastures grazed by cattle with sewage waste should be prevented. Social activities such as excursions, trekking, camping and picnics enable *T. saginata* eggs to easily spread to the environment. For this reason, infection of the surrounding cattle with *T. saginata* eggs should be prevented by installing hygienic toilets at resting areas, waterfront, picnic, and recreation areas. In addition to these measures, the meat of slaughtered animals should be examined by veterinarians for cysticerci.

Cysticerci die when beef is cooked to an internal temperature of 56°C for 20 minutes or when the infected carcass is frozen at -10°C for 10-14 days. In addition, cysticerci are sensitive to the salt, and become inactivated in meat containing at least 2% salt. If meat is kept in 20% salt brine for 21 days, cysticerci lose their vitality (Saygı 1999; Erol 2007; Zarlenga and Gamble 2019).

The habit of eating raw meatballs plays an important role in the spread of the disease. Therefore, if eaten raw, meatballs must be made from sheep meat. Additionally, public awareness should be raised about the transmission routes and importance of the disease.

T. asiatica TAENIASIS

T. asiatica, which was first detected in indigenous people living in mountainous regions in Taiwan, was initially likened to *T. saginata* (Zarlenga and Gamble 2019). However, later studies revealed that this species is morphologically and genetically different from *T. saginata* (Liu 2019; Zarlenga and Gamble 2019). Although the intermediate host of this species is the pig, a cysticercus is not formed in humans as in *T. solium* infections (Zarlenga and Gamble 2019). This species is also known as “Asian tapeworm” or “Taiwan tapeworm.”

Epidemiology

Compared to the other two *Taenia* species, *T. asiatica* is spread in a more limited area. This parasite can be seen in many Asian countries, such as Korea, Indonesia, Taiwan, Thailand, China, and Japan (Schantz et al. 1998; Anantaphruti et al. 2007; Liu 2019; Zarlenga and Gamble 2019). The intermediate hosts of this species are domestic and wild pigs. Unlike other species, the metacestode form of the parasite prefers the liver and lungs of the intermediate host. Therefore, eating the raw or undercooked internal organs of infected pigs contributes to the spread of the disease (Liu 2019; Zarlenga and Gamble 2019).

Morphology

The scolex of *T. asiatica* is spheroidal and approximately 0.8 mm wide. There are no hooks on the rostellum, which has a pointed appearance. The strobila of an adult cestode contains 700-900 proglottids and is approximately 3.5 m in length. Each gravid proglottids contains an average of 80,000 eggs. The size of the metacestode, *C. viscerotropica*, which develops in the liver of domestic and wild pigs, is smaller than the larvae of other species, measuring up to 2x2 mm (Liu 2019; Zarlenga and Gamble 2019). The structure of the gravid proglottids is similar to those of *T. saginata.* Similarly, the spherical eggs are similar in shape to the eggs of other *Taenia* species.

Transmission

People ingest *C. visceratropica* in the raw or undercooked liver and lungs of pigs. Pigs consume the eggs via contaminated water and feed.

Life Cycle

The definitive host of this cestode is humans and its intermediate hosts are domestic and wild pigs. While the adult parasite settles in the small intestines of humans, the metacestode form develops in the liver and lungs of intermediate hosts. Experiments have demonstrated that cattle, goats, rodents and monkeys can be infected in addition to pigs (Zarlenga and Gamble 2019). Most of the larvae in the liver settle in the parenchyma in pigs, while some grow on the surface. Experimental studies have also shown that the omentum, colon serosa, diaphragm, abdominal muscles, gall bladder, and peritoneal cavity are alternative locations of larvae. The life cycle is generally the same as that of the other species. *C. viscerotropica* develops in the liver approximately four weeks after pigs ingest eggs released from proglottids excreted in human feces. Larvae consumed by eating raw or undercooked infected pig liver are evaginated in human small intestines and become adult parasites after 2.5-4.0 months. Adult parasites in infected individuals can live for more than two years (Galán-Puchades and Fuentes 2013; 2014; Liu 2019; Zarlenga and Gamble 2019).

Clinical Signs

As in infections caused by other *Taenia* species, clinical symptoms are generally not observed in *T. asiatica* infections. Infected individuals may not be aware that they are carrying the parasite until they start throwing the proglottids. However, in some cases, where the number of parasites is high, clinical symptoms may be apparent. Infected individuals may experience emotional trauma during the exit of the proglottids from the anus, even if they do not show symptoms (Liu 2019; Zarlenga and Gamble 2019). Other than the discharge of the proglottids, other common symptoms include nausea, dizziness, headache, loss of appetite, constipation, hunger, hunger pain in the abdomen, diarrhea, and vomiting. Less frequently, symptoms such as muscle pain, stomach pain, drowsiness, convulsions, anxiety, skin itching, and respiratory disturbance may may be seen (Galán-Puchades and Fuentes 2013; 2014; Liu 2019).

Although necrosis, hepatocyte degeneration, and granulomatous reactions occur in the liver of intermediate host, no typical clinical symptoms of the disease occur in these animals.

Treatment

Few treatments exist specifically for *T. asiatica*. Successful results have been obtained from oral administration of praziquantel in a single dose of 150 mg. Anthelmintics, such as niclosamide and albendazole, which are used in the treatment of other *Taenia* types, can also be used in *T. asiatica* infections (Zarlenga and Gamble 2019).

Control and Prevention

The best way to prevent this disease is to avoid eating pig livers and viscera contaminated with *C. viscerotropica*. As in *C. cellulosae* infections, *C. viscerotropica* from the viscera of hunted animals or domesticated pigs should be inactivated by high temperature cooking or freezing. In addition, infected people should be treated and feces should be prevented from contaminating the environment.

TRICHINELLOSIS

Trichinellosis can be transmitted among mammals and humans, and may be fatal. Eating raw or undercooked meat of pigs, bears, horses, seals and other omnivorous animals infected with *Trichinella spiralis* and various species of the Trichinellidae family transmits the disease to humans (Gajadhar et al. 2009; Bruschi and Gomez-Morales 2017; Zarlenga and Gamble 2019; Pozio and Bruschi 2019).

T. spiralis was first identified by Baget during an autopsy in England in 1835. The first clinical case of Trichinellosis was reported by Zenker in 1860, when larvae inside a capsule were detected in the arm muscles of a young girl, who died of a typhoid-like disease (Zarlenga and Gamble 2019).

Etiological Agents

The genus *Trichinella* is a complex, consisting of 12 species and many genotypes spread over a wide geography. *Trichinella* species, which cannot be morphologically distinguished from one another, are divided into two groups on the basis of their ability to form a capsule in the host muscles. The species encapsulated in the host muscle cells are *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni* and Trichinella T6, T8, T9 and T12. Species that do not form capsules in host muscle cells are *T. pseudospiralis, T. papuae* and *T. zimbabwensis*. Encapsulated *Trichinella* species infect mammals. Among the unencapsulated species, *T. pseudospiralis* infects mammals and birds, while *T. papuae* and *T. zimbabwensis* are also seen in mammals and reptiles (Gajadhar et al. 2009; Pozio and Bruschi 2019; Zarlenga and Gamble 2019).

Epidemiology

Human infections are caused by eating raw or undercooked meat of animals that harbor larvae in their muscles, especially pigs. In addition, meat products prepared from the meat of these animals also contribute to contamination. Trichinellosis, a highly dangerous zoonosis, is estimated to affect up to 11 million people worldwide (Dupouy-Camet 2000). Infections are widespread, especially where there is little or no control of rodents such as mice and rats, animals are not under the control of a veterinarian, pigs are fed with animal tissues, or animal carcasses are discarded randomly. Transmission between animals occurs by eating meat infected with *Trichinella* larvae as a result of cannibalism or carnivorism. Wild mammals in nature act as reservoirs for these parasites. These animals are important in the transition of infections from the rural to the domestic cycle. The role of carnivorous birds and reptiles, such as crocodiles and lizards, in the natural cycle of these parasites is not yet fully understood (Pozio and Bruschi 2019). In the epidemiology of the disease, mice are the main carriers. These animals eat carrion or each other, causing the infection to continue in mouse colonies.

Trichinella species can be seen in humans and in many animal species. This nematode can infect more than 150 mammal species, such as domestic or wild boar, rat, mouse, bear, horse, seal, walrus, dog, fox, jackal, and wolf, but is rarely seen in birds. *Trichinella* species have been detected in domestic animals, mainly pigs, in 43 countries, and in wild animals in 66 countries. Trichinellosis has been reported in humans in 55 countries worldwide. On the other hand, there is no information about *Trichinella* infections in 92 countries (Pozio and Bruschi 2019). *Trichinella* infections have been found in domestic and wild pigs in Germany, Austria, France and Italy. While the larvae are seen in striated muscles, adult forms are embedded in the small intestine mucosa. Both the mature form and its larvae can be found in the same host (Rommel et al. 2000).

The distribution of the species varies according to geographical regions and countries, with some species are seen in some countries to a limited extent. For example, the spread of *T. nelsoni* is limited to Southeast Africa. *T. spiralis* and *T. pseudospiralis* are cosmopolitan species. Serious clinical symptoms, and even death, were seen in the first human case of *T. pseudospiralis* in 1993 (Campbell 1988; Macpherson et al. 2000).

As a zoonotic disease for about 150 years, Trichinellosis has seriously threatened public health in both developed and developing countries. In the European Union countries, 420 million dollars are spent for the mandatory examination of 190 million butchered pigs each year, and 1 billion dollars is spent for operations, such as freezing and cooking meat in the US (Pozio 1998).

Trichinella species are the largest known intracellular parasites. Among the *Trichinella* species, *T. spiralis* is the most known and the most common type of infection. Larvae of this species are encapsulated in fibers of striated muscles and preserve their vitality for years. Larvae can survive for 39 years in humans and 11 years in pigs.

Transmission

Pigs become infected by eating infected mice, other animals, or trichinated pig waste. Cannibalism between

pigs also causes the spread of the disease. Animals such as sheep, cattle, and horses can be a source of infection due to the use of animal proteins as a feed for grass-eating animals (Rommel et al. 2000).
The main source of human infections is pork infected with larvae. Larvae in pigs are commonly found in the diaphragm, tongue, masseter, and intercostal muscles. Raw and insufficiently cooked pork and pork sausages are seen as the most important causes for the spread of the infection. Human consumption of the never frozen, raw, or undercooked meat of animals such as horse, bear, walrus, dog, or fox can also lead to infection (Prasad 2010).

Life Cycle

Adults of the genus *Trichinella* settle in the small intestine, while the infective larvae settle in the muscles of the same host. These parasites do not have a free life stage.
When the meat of infected animals is eaten by another host, L1 larvae are released in a few hours with the help of digestive enzymes in the stomach and pass into the small intestine. Here, they become adults in 2-4 days. However, if the host has a hypersensitivity to the parasite or diarrhea, the larvae in the small intestine, as well as the adults that have developed, will be excreted with feces. Reinfection can occur if food contaminated with feces containing larvae or adult parasites excreted in this way are eaten by a carnivorous, omnivorous, or herbivorous animal (Gajadhar et al. 2009; Bruschi and Gomez-Morales 2017; Pozio and Bruschi 2019; Zarlenga and Gamble 2019).
Adult parasites in the small intestine of the host settle among the villi. The male and female parasites mate, and then the males die. Females enter the Lieberkühn glands and Peyer's patches, and three days after mating begin to give birth to L1 larvae. A female can give birth to 1000-10,000 larvae during her life time. These larvae enter the lymph channels and come to the left vena cava cranialis through the ductus thoracicus, and then spread to all organs and tissues via circulation. Larvae generally prefer muscle groups with high blood flow, so they mostly settle in the diaphragm, tongue, larynx, eye, masseter, intercostal, abdominal, and hip muscles. Although larvae can be found in the liver, pancreas, and kidneys, only the larvae that go to the striated muscles survive, and those that go to other organs and tissues die. L1 larvae that reach the striated muscles develop rapidly by settling in the sarcolemma of the muscle fibers. If the infection has occurred with a capsule-forming species, the larvae are encircled by a capsule formed by tissue histiocytes in the area (Pozio and Bruschi 2019; Zarlenga and Gamble 2019). Later, they develop within the capsule and reach a characteristic size and appearance. The capsule, which was previously open at both ends, closes completely and surrounds the larvae. These events are completed in the seventh week of infection and the larvae become infective.
In the later stages of the infection, the muscle fibers with the capsule degenerate and these foci with the larvae begin to become calcified after 5-6 months. However, despite this, the larvae can survive in the striated muscles for years by resisting the immune response of the host. Even after the host dies, the larvae can survive for a long time. Larvae that have settled in muscle cells do not show any further development and continue their cycle when the meat containing larvae is eaten by another host (Gajadhar et al. 2009; Bruschi and Gomez-Morales 2017; Pozio and Bruschi 2019; Zarlenga and Gamble 2019).

Clinical Signs in Humans

An incubation period begins with the ingestion of *Trichinella* larvae by humans, and symptoms appear at the end of this period. The incubation period varies from 2 to 45 days, depending on the type of meat consumed (raw or undercooked), the consumption frequency, the number of live larvae ingested, the region where the larvae are settled, the type or genotype of the parasite, and host immunity. A short incubation period indicates that the prognosis of the disease is poor. The incubation period is usually short in severe cases of Trichinellosis (Pozio and Bruschi 2019).
In the first week of the infection, gastrointestinal symptoms, such as hyperemia in the small intestines, catarrhal enteritis and minor wounds, nausea, vomiting, diarrhea, and abdominal pain are observed. Stool is mostly soft, greenish-brown in color, and sometimes contains mucus. However, there is no blood. Electrolyte imbalance and hypoproteinemia occur in patients due to fluid loss. One week later, edema occurs on the eyelids and face along with persistent headache, dizziness, and insomnia. Edema sometimes covers the whole face (Pozio and Bruschi 2019; Zarlenga and Gamble 2019). In severe cases, edema can spread to arms and legs. Symmetrically shaped edema heals within 5-6 days after corticosteroid treatment. In addition, hyperemia and bleeding occur in the host, and eye itching and lacrimation are observed. Patients with such symptoms typically avoid light. Other common symptoms of the disease are severe sweating and high fever (40-41°C). Sometimes, fever can take up to three weeks depending on the severity of the infection. Tachycardia occurs due to the increase in body temperature. In addition, cough, pulmonary edema, and diaphragm pain occur in relation to the respiratory system. The patient may experience dehydration, weakness, and cachexia, as well as dizziness, headache, and ascites (Gajadhar et al. 2009; Pozio and Bruschi 2019; Zarlenga and Gamble 2019).
Following these symptoms, patients may experience muscular pain, which is especially seen in the neck, arms, and legs. Therefore, the patient does not want to move. In addition, the pain can manifest itself severely in the diaphragm, masseter, intercostal, and orbital muscles. Myalgia continues for 2-3 weeks. In severe infections, fatalities may occur as a result of paralysis of the respiratory muscles, especially the diaphragm. Eosinophilia of 60-85% is seen in patients and leukocytosis may develop.

In severe cases, complications related to various tissues and organs, such as encephalitis, myocarditis, dizziness, visual impairment, and dyspnea, occur within the first two weeks. In mild and moderate cases and in patients who are not treated appropriately, there is a possibility of complications in later periods (Pozio and Bruschi 2019; Zarlenga and Gamble 2019).
Chronic Trichinellosis can develop after acute cases that are not treated well or leave sequelae. In such patients, pain may not go away, quality of life may be impaired, and paranoia may occur (Pozio and Bruschi 2019).

Clinical Signs in Animals

Trichinellosis is generally mild and does not cause clinical symptoms in animals. In severe infections, adult parasites can cause enteritis, while larvae reaching the muscles can cause acute myositis, fever, and eosinophilia (Macpherson et al. 2000).

Diagnosis

Although eosinophilia, leukocytosis, and sediment increase are helpful in diagnosis, definitive diagnosis is not possible in live animals. However, a diagnosis can be made by examining muscle samples taken from suspected animals during meat examination in slaughterhouses.
Two main methods are used for the direct detection of *Trichinella* in the meat: trichinoscopy and the digestion method (Şenlik 2011). The appropriate method must be chosen to get reliable results. For example, there is no capsule around *T. pseudospiralis* and *T. papuae.* Species without capsules are very difficult to detect by trichinoscopy. For this reason, all meat samples should be examined by both methods (Şenlik 2011). *Trichinella* larvae prefer different muscles in different animal species. Therefore, samples should be taken from these muscles for both routine meat examination and epidemiological studies. Samples should be taken from the tongue and diaphragm muscles of animal species in which the preferred locations of larvae are unknown (Şenlik 2011).
For this reason, the amount of sample that should be taken varies according to animal species. In addition to these two methods, the DNA of the parasite can be detected in meat samples by multiplex PCR (Pozio and Bruschi 2019).
Differential diagnosis is important for appropriate and timely treatment in humans. Fever with orbital and facial edema can occur in cases of glomerulonephritis, dermatomyositis, allergic reactions, or intoxications. In addition, neck stiffness, headache, confusion, and diseases with excessive arousal should be ruled out. Even in atypical cases, with high fever and eosinophilia but without specific findings, other parasitic diseases such as Fasciolosis and Toxocariosis should be considered. Serological tests can also be used to diagnose Trichinellosis cases in humans. ELISA used for this purpose shows a high sensitivity and specificity. The definitive diagnosis of the disease in humans is made by examining biopsy samples by parasitological and histological methods, preferably taken from the deltoid muscles. If this is not possible, a 0.2-0.5 g muscle sample should be taken from any part of the body. The muscle samples can be examined by trichinoscopy or the digestion technique, or multiplex PCR can be used to identify the DNA of the parasite (Pozio and Bruschi 2019; Zarlenga and Gamble 2019).

Treatment

Since definitive diagnosis cannot be made in living animals, treatment is not possible in usual practice. However, in experimental studies, anthelmintics, such as mebendazole, flubendazole, albendazole, fenbendazole, cambendazole, and thiabendazole, were found to be effective against both adult and larval stages of the parasite. Flubendazole is effective against both larvae and mature parasites when administered by adding to pig feed for two weeks at doses of 30-125 ppm. Febantel is effective at a dose of 20 mg/kg and albendazole at a dose of 10 mg/kg against larvae (Rommel et al. 2000).
In humans, benzimidazole compounds are used against parasites and corticosteroids are used to eliminate symptoms. Mebendazole is the most commonly used benzimidazole. Anthelmintics should be used within one week of infection to destroy adult parasites in the intestines and to prevent the larvae from spawning and going to the muscles. However, it is not possible to determine when the infection started. Therefore, in practice, mebendazole or other anthelmintics should be used within 48 hours after the suspected meat has been eaten. Mebendazole is used at a total daily dose of 5 mg/kg and divided into two doses. The drug should be used for 10-15 days when treatment is started after the clinical picture has taken shape, then a 5-day break should be taken. Then it should be used again for 10-15 days. This treatment protocol should be continued for 4-6 weeks. Mebendazole can also be used at a dose of 20-25 mg/kg in treatment. However, it should be kept in mind that allergic reactions may occur and liver enzymes may increase when used in high doses (Pozio and Bruschi 2019). Mebendazole should not be used in pregnant women in the first trimester. Pyrantel at a daily dose of 10 mg/kg and levamisole at a dose of 2.5 mg/kg against adult parasites are also effective (Pozio and Bruschi 2019).
In addition to anthelmintic therapy, corticosteroids can be used to alleviate patients' symptoms and accelerate recovery. For this purpose, prednisolone can be administered with a daily dose of 30-60 mg for 10-14 days (Pozio and Bruschi 2019).

Control and Prevention

As mentioned earlier, pigs are the main source of Trichinellosis in humans. People become infected through eating raw or undercooked pork or meat products containing pork, such as salami and sausages. In certain parts of the world, wild pigs, polar bears, various seal species, horses and some fur animals are also blamed for human Trichinellosis.

Preventive measures that can be taken on pig farms include separating dead animals from the herd immediately, ensuring no garbage is kept near the farm, controlling rats, and informing consumers when necessary (Gajadhar et al. 2006).
The most critical point in control is the inspection of pork meat before sending to market. The methods identified in the diagnosis section should be used to determine whether meat is infected. If positive samples are found as a result of the tests, the carcasses must be destroyed. Epidemiological screening should be used at farms, where pigs with positive muscle samples originated to identify other infected pigs, which should be removed from the herd (Gamble 1998; Gamble et al. 1996). High temperature cooking, as well as freezing of meat, have a lethal effect on the larvae in the muscles. Thus, meat must be subjected to the following procedures before being offered for consumption in endemic regions (Pozio and Bruschi 2019; Zarlenga and Gamble 2019). The main inactivation processes that can be used for this purpose are as follows.

1. Cooking

Cooking the meat at 77°C for at least 10 minutes inactivates the larvae (Erol 2007).

2. Freezing

Cold shocking should be done in deep freezers, so that the internal temperature of meat is -40°C. Different freezing procedures are used in different countries and regions. For example, in the United States, meat that is 15 cm thick is kept at -15°C for 3 weeks, while meat that is 69 cm thick is kept at -15°C for 4 weeks. On the other hand, in European Union countries, meat is frozen at -25°C for 10-20 days, depending on its thickness. However, not all species are inactivated by freezing. For instance, *T. nativa*, which causes Trichinellosis in polar bears, is resistant to freezing. It has been reported that meat retains its infectivity even months after freezing (Erol 2007).

3. Irradiation

In countries where this method is permitted, gamma rays at a dose of 0.3 kGy can be applied (Erol 2007).

TOXOPLASMOSIS

Toxoplasmosis is one of the most important parasitic zoonoses of humans and can be seen in almost every part of the world. The disease is caused by *Toxoplasma gondii*, an obligate intracellular protozoan.

Hosts

The final host of the parasite is domestic and wild cats. Many warm-blooded animal species serve as intermediate hosts, including poultry and humans. *T. gondii* is found inside the cell in muscle tissue and organs such as the heart, brain, and liver of humans and many intermediate host animals such as sheep, goat, cattle, pigs, and chickens (Dubey and Beattie 1988).

Epidemiology

In terms of human and veterinary medicine, *T. gondii* is a very important protozoonosis, widely seen in humans and animals all over the world (Dubey and Beattie 1988; Fayer 1994). The disease can be found in all continents except Antarctica. It is estimated that 500 million people worldwide are infected with *T. gondii*, although they may be asymptomatic. More than 50% of women of childbearing age are reported to be infected with Toxoplasmosis in Western Europe, Africa, South America, and Central America. Seropositivity rate of Toxoplasmosis among adults in Europe ranges from 10-80%, and in Central Europe it ranges from 30-45% in women of childbearing age. In HIV patients, the prevalence is higher, between 50-75% (Fayer 1994; Parija 2009). The prevalence of this disease, which is more common in animals intended for slaughter, can reach up to 90% in some regions.
The prevalence of *T. gondii* infections in humans and animals varies by country, and even among different geographic regions of the same country. Many factors, such as feeding habits, animal breeding systems, hunting, culture, and cleaning habits, affect the spread of the disease.
Environmental factors may also affect the spread of *T. gondii* infection. The rate of infection is higher in hot climates and regions with low humidity, while infection is less common in cold climates and mountainous and dry areas. For example, in Latin America and Africa, which are very suitable for the development of oocysts, the prevalence of the disease can reach up to 90%.

Morphology

Non-sporulated oocysts excreted with feces in cats are 10x12 µm in diameter, while the diameter of sporulated oocysts is 11x13 µm. Sporocysts are 6-8 µm in size. The tachizoids found in the intermediate host are crescent shaped and range in size from 2x4 to 4x8 µm. The size of the bradyzoites found in the tissue cysts of intermediate hosts is 1.5x7 µm. Young cysts are 5 µm in size, while those in the brain can reach up to 70 µm. The length of the cysts in the muscles can reach 100 µm. The thickness of the wall of the cyst, which is not divided into sections, is less than 1 µm (Dumanlı and Aktaş 2015).

Transmission

The most important reservoirs for human infections are livestock infected with Toxoplasmosis. Transmission occurs by oral ingestion of any of the infective forms of the parasite, such as tachizoid, bradyzoite, or oocyst (Parija 2009). Humans become infected by (1) eating raw or undercooked meat of intermediate hosts such as sheep, goat, or pigs that contain tachizoids or bradyzoites; (2) by

ingesting sporulated oocysts obtained by contact with cats via drinking water or food; or (3) congenitally. Intermediate host livestock or other animals take the disease by ingesting feed or water contaminated with sporulated oocysts shed by cats. Cats become infected by eating tachizoids and bradyzoites found in the tissues of intermediate hosts, infected organs and meat, and by ingesting sporulated oocysts with contaminated food and water.

Life Cycle

Cats, which are the final hosts, can also be intermediate hosts. After infective forms ingested by cats enter the intestinal epithelium, they pass the periods of Merogony and gametogonia and produce oocysts within 3-10 days. In infected cats, oocysts are excreted with feces for 1-2 weeks. These oocysts, which are shed to the outside environment without sporulation, sporulate in a short time in environments with suitable temperature and humidity (Joynson and Wreghitt 2005; Shakespeare 2009; Dumanlı and Aktaş 2015). The sporulated oocysts, taken up by the intermediate hosts, are broken down by digestive enzymes and the sporozoites are released. Later, these sporozoites go to cells of reticuloendothelial system (RES), become tachizoids and multiply to form pseudocysts. When the tachizoid number reaches 8-16, the cell disintegrates and infect new cells. This period is the acute form of the infection. Tachizoids released from pseudocysts grow and multiply in endothelial cells by going to various organs and forming tissue cysts containing bradyzoites. These cysts are mainly found in the brain, heart, skeletal muscles, eye and, to a lesser extent, other organs and tissues. Thousands of bradyzoites can be found in these cysts, which can reach 100 µm in diameter. The host cannot resist these cysts with an adequate immune response, so they can survive in intermediate hosts for many years or even for life. In some intermediate hosts such as cattle, these cysts lose their vitality by calcification after a certain period of time. People and carnivorous animals, that eat the meat of intermediate hosts infected in this way, also become infected (Dumanlı and Aktaş 2015).

Carnivorous animals are infected by eating various tissues and organs of intermediate hosts that contain tachizoids and bradyzoites, as well as by drinking water or eating food contaminated with oocysts. Herbivorous animals such as sheep, goats, and cattle are infected by ingesting oocysts with foodstuffs such as weeds, hay, fodder, and water. Cats become infected by eating the tissues and organs of intermediate hosts carrying tachizoids and bradyzoites or by hunting and eating animals such as mice and rats. Cats can also become infected when they ingest oocysts with contaminated food (Urquhart et al. 1996; Dominique et al. 2008; Prasad 2010). Tissue cysts can survive throughout the life of the intermediate host. Tachizoids found in body secretions, such as milk, cannot survive long in the external environment. On the other hand, oocysts, the non-host form of the parasite, can survive for 2-3 weeks in the external environment under appropriate humidity and temperature (Urquhart et al. 1996; Dominique et al. 2008; Prasad 2010).

Although sheep and pork pose a high risk for human infections, beef and poultry meat have a lower risk of infection (Dubey 1986; Dubey et al. 1986). In humans or animals with low immunity, the risk of Toxoplasmosis is very high and infection progresses more seriously.

Clinical Signs

Clinical symptoms that occur in infected humans and animals are quite different. Sometimes no symptoms occur, while sometimes several clinical pictures can be encountered.

Clinical Signs in Humans

The disease is asymptomatic in more than 80% of healthy individuals with strong immunity. However, in people with weakened immunity, the disease is severe and sometimes death can occur. Symptoms usually occur within 7-14 days of ingestion. The disease can affect the functions of many vital organs, such as the brain, eyes, liver, lungs, and heart (Hohlfeld et al. 1989; Prasad 2010; Ortega 2019; Souza and Damatta 2019). In symptomatic cases, high fever, cervical lymphadenopathy, retinochoroiditis, sore throat, fatigue, and chronic muscle aches can be seen.

Toxoplasmic encephalitis usually occurs in individuals with suppressed or weakened immunity, as in patients with HIV. The most common symptoms in such patients are high fever, headache, memory loss, dementia, slowed psychomotor movements, and behavioral changes (Hohlfeld et al. 1989; Prasad 2010; Souza and Damatta 2019). In the ocular form of the disease, intraocular pressure increases, and inflammation and blurred vision may occur.

Pregnant women are always at risk for Toxoplasmosis. Infections that occur in the first and second trimesters of pregnancy may result in abortion and stillbirth, and congenital sequelae may occur in the fetus. If the infection is acquired in the third trimester, normal delivery may occur in patients without acute symptoms, but acute chorioretinitis may develop as the child grows. In addition, congenital Toxoplasmosis can cause blindness, mental retardation, multiple organ failure, and hydrocephalus (Hohlfeld et al. 1989; Prasad 2010).

Clinical Signs in Animals

Toxoplasmosis is generally asymptomatic in animals, though it may cause pregnant animals to abort (Dhaliwal and Juyal 2013; Dumanlı and Aktaş 2015). Sheep are the intermediate hosts mostly affected by Toxoplasmosis in clinical and economic terms. If contamination occurs during pregnancy in sheep that were not previously infected, tachizoids pass to the offspring through the placenta and cause congenital Toxoplasmosis. Infections in the early stages of pregnancy (up to 45-55 days) result in the death of the fetus with abortion. However, since

the fetus is very small, the abortion is often not noticed. If the infection occurs towards the middle of pregnancy (up to the 90th day), abortion occurs. If the dead fetus is not discarded, it becomes mummified in the uterus. If the infection occurs in the last stage of pregnancy (up to the 120th day), the offspring can be born and appear normal, or may be born weak and thin and die shortly thereafter (Şenlik 2017). Clinical signs are rarely seen in cats. The main symptoms seen in symptomatic cats include enteritis, pneumonia, enlargement of mesenteric lymph nodes, encephalitis, and chronic interstitial nephritis (Dumanlı and Aktaş 2015).

Diagnosis

Early diagnosis is of great importance in pregnant women, immunocompromised individuals, and patients with chorioretinitis. Various serological tests, molecular methods, and rapid commercial kits can be used for the diagnosis of Toxoplasmosis in humans. The most commonly used serological tests for diagnosis are the dye test, IgG avidity test, ELISA, agglutination, indirect hemagglutination, and indirect fluorescent antibody test (Urquhart et al. 1996; Joynson and Wreghitt 2005; Ortega 2019; Souza and Damatta 2019. Although serological tests are very effective in revealing prenatal infections, they can sometimes be insufficient. In such cases, the diagnosis can be confirmed by performing PCR on blood samples. Molecular methods can detect the DNA of a single tachizoid. Apart from serological and molecular methods, imaging methods, such as CT and MRI, can be used. Although the sensitivity of these methods is low, they can reveal calcifications and hydrocephalus in the brain, especially in congenital Toxoplasmosis (Souza and Damatta 2019).
Many direct and indirect methods are used for the diagnosis of Toxoplasmosis in intermediate host animals. In direct diagnosis, crust, exudates, and smears made from the internal organs of the fetus are prepared, stained with Giemsa and examined under the microscope. Diagnosis can also be made through histological examination of sections of the internal organs of fetus under microscope. White colored focal necrosis of 2 mm diameter in cotyledons of the fetal membranes discarded in sheep abortions are pathognomic for Toxoplasmosis. In indirect methods, antibodies developed against the parasite or antigens of the parasite are sought. In addition, molecular methods, such as PCR, can be used. Oocysts can be searched for by examining feces in cats by the flotation method or by using rapid commercial kits. However, it is very difficult to distinguish oocysts detected in stool examination from other protozoan oocysts, such as *Hammondia* (Prasad 2010; Dhaliwal and Juyal 2013; Şenlik 2017).

Treatment

The easiest way to control the disease in humans is to treat infected individuals. However, implementing control measures worldwide is difficult. Currently available drugs are only effective on tachizoids. Tissue cysts are less sensitive to existing drugs due to their metabolic activities (Urquhart et al. 1996; Ortega 2019; Souza and Damatta 2019). Pyrimethamine and sulfadiazine are generally preferred in the treatment of acute Toxoplasmosis infections. These drugs act by disrupting folic acid synthesis in the parasite. However, these drugs may have toxic effects on host cells and disrupt folic acid synthesis. Other protein synthesis inhibitors that can be used for treatment are spiramycin, clindamycin and clarithromycin. Spiramycin is preferred in the treatment of pregnant women because it passes through the placenta at a lower rate (Souza and Damatta 2019).
In animals, there is no effective treatment for the disease. Therefore, control measures are essential. However, experimental studies have shown that spiramycin, piritrexin, roxithromycin, clindamycin, cyclosporin A, atovaquone, azithromycin, ponazuril and triazine may be effective (McCabe 2001).

Control and Prevention

The immune system of the host plays the most important role in Toxoplasmosis infections. Although there are usually no symptoms in people with a normal immune system, the disease progresses and can result in death in individuals with a weak or suppressed immune system. Especially in cancer and AIDS patients, Toxoplasmosis has a very severe course and can be fatal. For this reason, pregnant women and individuals with a weak immune system should not have close contact with cats or cat litter and should avoid contact with soil and raw meat. Children should be prevented from touching cat feces in playgrounds and parks (Dhaliwal and Juyal 2013).
In order to prevent contamination from infected meat, it must be subjected to various processes before consumption, as described below:

Cooking

Raw or undercooked meat, especially raw meatballs, should not be consumed. Meats should be cooked at least 3-5 minutes at the temperature of 66°C (Dubey et al. 1990). In addition, tasting meat while making homemade sausages or salami, or while seasoning it, should be avoided.

Freezing

Freezing meats at -12°C kills tissue cysts. Even freezing meat overnight in a domestic freezer is sufficient to inactivate most of the tissue cysts in the meat.

Irradiation

Irradiation at 50 krads or high-pressure processing at 400 MPa are effective in killing tissue cysts (Dubey and Thayer 1994).

Water sanitation

Surface water that has not been subjected to any treatment should not be used for drinking and washing vegetables and fruit. Water obtained from streams, rivers, lakes, and rivers should never be used as drinking water without boiling (Erol 2007).
The following prevention methods should also be used.
i) To prevent infection of zoo animals with *T. gondii*, brooms, shovels, and all equipment used should be autoclaved or heated to 70°C for at least 10 minutes.
ii) Cats are the key animals in preventing Toxoplasmosis in intermediate hosts, as cats are the final hosts of the parasite. Cats should not be given raw meat and offal, and they should not be permitted to hunt and feed on birds, mice, and rats (Şenlik 2017).
iii) Other measures that can be taken regarding cats are as follows:
• Cat feces should be removed daily.
• Cat litter, sand, and feces collected for disposal should never be kept in the kitchen.
• Containers in which cats defecate should be washed thoroughly with hot water after cleaning and disinfected at least once a week.
• Cats should be prevented from contaminating parks and lawns where children play.
• Cats should be prevented from entering the feed stores of other animals and pets.
There is not yet any vaccine available for use in humans. A vaccine called "Toxovax," which was developed to prevent the infection in sheep, is used in England, France, and New Zealand. This vaccine is a live vaccine containing tachizoids produced in cell culture and provides protection for 18 months after administration (Dubey 1998; Şenlik 2017; Souza and Damatta 2019).

SARCOCYSTOSIS

Etiological Agent

The final hosts of the *Sarcocystis* species are cats, dogs, and humans. Its intermediate hosts are mammals, poultry, reptiles, and humans. Humans are intermediate hosts for some species and definitive hosts for others. *Sarcocystis* species, which are widely seen in the world, show a heteroxene development. There are about 200 species in the *Sarcocystis* genus that can infect mammals, poultry, reptiles, and fish (Sing et al. 2019). However, only two of them are zoonotic and use humans as the final host. These species are *Sarcocystis hominis* and *Sarcocystis suihominis*.

Morphology

In *Sarcocystis* species, the size of the oocysts varies from 7-18 x 15-30 µm and their walls are quite thin. The size of the sporocysts ranges from 8-10 x 15-19 µm. There are 2 sporocysts inside the oocysts and 4 sporozoites in each sporocyst. The length of the banana-shaped sporozoites is 7-10 µm. The size of tissue cysts can vary from a few millimeters to a few centimeters, depending on the species and the intermediate host. Some, called macrocysts, are quite large and can be seen with the naked eye. Others, called microcysts, are very small and cannot be seen with the naked eye. The interior of mature cysts is divided into compartments and is filled with 14 µm long banana-shaped bradyzoites (also called cystozoites) (Sevgili 2015).

Transmission

Sarcocystis species need two hosts to complete their development. Definitive hosts become infected by eating the raw or undercooked meat of intermediate hosts, such as cattle and pigs, which contain *Sarcocystis* cysts. Intermediate hosts become infected by orally ingesting oocysts and sporocysts shed with the feces of the definitive hosts (Fayer 2004; Sing et al. 2019).

Life Cycle

Oocysts or sporocysts shed by the final host (cats, dogs, and humans) arrive in the small intestine after ingestion by the intermediate host, a herbivorous or omnivorous animal. The sporozoites released there migrate to intestinal epithelial cells. The sporozoites then enter the capillary endothelial cells. After the merogony period, a large number of merozoites are formed. The first-generation merogony period takes place 15-16 days after the sporocysts are ingested. Merozoites formed as a result of first-generation merogony enter the venous and arterial cells of the internal organs and pass the second-generation merogony period there. Merozoites formed as a result of second-generation merogony enter the bloodstream and go to various organs and tissues. The most preferred tissue is muscles. There, they form macro- and microcysts by proliferating via endopolygeny. The cysts are composed of partitions that contain bradyzoites. Cysts mostly develop in striated muscles, tongue, diaphragm, heart, and esophagus. Occasionally, cysts can be found in the brain. Cysts take approximately 75 days after infection to develop in intermediate hosts. The end hosts can only become infected by eating cysts containing bradyzoites (Dhaliwal and Juyal 2013; Sevgili 2015).
When the final host eats meat containing cysts, proteolytic enzymes in the digestive system break down the cysts and bradyzoites are released. They enter intestinal epithelial cells, where they pass the gametogony period. During this period, micro- and macrogametes are formed. Then the gametes are fertilized to form the zygote. At the end of this development process, oocysts with two sporocysts are produced. The wall of oocysts formed during the development of the *Sarcocystis* species is weak and thin. Therefore, the oocyst fragments while still in the host's intestine and sporocysts can be released. Thus, sporocysts can be excreted with feces. The prepatent period can be 7-14 days, and the patent period can vary from one week to several months (Fayer 2004; Dhaliwal and Juyal 2013; Sevgili 2015).

Clinical Signs in Humans

Intestinal *Sarcocystis* infections in humans are usually asymptomatic. However, abdominal bloating, pain, nausea, vomiting, and diarrhea can occasionally occur in infected individuals. Humans sometimes serve as a random intermediate host for several *Sarcocystis* species, in which case muscular Sarcocystosis occurs. Mild muscular Sarcocystosis infections usually do not show any significant symptoms. However, in some patients, painful edema, erythema, fever, chronic myositis, myalgia, bronchospasm, lymphadenopathy, subcutaneous nodules, and eosinophilia can be seen (Sing et al. 2019).

Clinical Signs in Animals

Sarcocystis species, which are highly pathogenic for intermediate hosts, can sometimes cause death. The second period of merogony in the vascular endothelium of intermediate hosts is more pathogenic than cysts in the heart, nerve, and skeletal muscles. Severe acute infections often result in death. Cysts contain a toxin that is highly toxic to animals, such as rabbits, mice, and sparrows. In the case of cysts in intermediate hosts, myocarditis occurs in the heart muscles and myositis in other muscles. In addition, petechial bleeding occurs in the heart muscles and serosa during infections, and edema can occur in the lymph nodes. Macroscopic cysts in the muscles that are visible to the eye are considered to be less harmful. As time passes, macroscopic cysts become calcified. In cattle infections, symptoms such as anorexia, weakness, irregular fever, decreased milk yield, respiratory distress, and anemia are observed (Dhaliwal and Juyal 2013; Sevgili 2015). In chronic infections, weakening, edema under the chin, and exophthalmos may occur. Anorexia and weakening are seen in acute infections in sheep, while abortion can occur in severe infections (Sevgili 2015).

Diagnosis

Macrocysts between the muscles can easily be seen with the naked eye when examining the infected meat of intermediate host animals such as sheep, ducks, and rabbits. These visible cysts are 1-10 mm in size and white or ash in color. Cysts in other animals are microscopic, but can be seen in histological or pathological examination. When the disease is suspected in humans, biopsy material is examined from the blood vessels of various organs, including the kidney and heart. However, serological tests such as IFA and ELISA and molecular techniques such as PCR can also be used to diagnose infection in intermediate hosts (Sevgili 2015; Vasan and Tsuji 2009). In final host animals, stool examination is performed using the flotation method to determine the presence of sporulated oocysts or sporocysts (Fayer 2004).

Treatment

Since there are no symptoms to diagnose Sarcocystosis, the disease is usually hidden. Therefore, the use of medications for treatment is limited. Amprolium can be used at a dose of 100 mg/kg in the treatment of cats and dogs, which are end hosts. In addition, experimental studies have reported that halofuginone prevents severe infections (Sevgili 2015).

Control and Prevention

Humans can serve as both intermediate and final *Sarcocystis* hosts. To prevent infections in which humans are the final host, eating the raw or undercooked meat of animals such as sheep, goats, cattle, and pigs should be avoided. Meat suspected of being infected should be frozen or well-cooked to inactivate cysts. In meat cooked at 60-80°C for 15 minutes, bradyzoites lose their vitality. To prevent infection by *Sarcocystis* species in which humans are intermediate hosts, food and water contaminated with the feces of carnivorous or herbivorous animals should be avoided. Vegetables and fruit should be well washed. If water is not known to be uncontaminated, it should not be used for drinking. To protect intermediate host livestock from infection, contamination of shelters, feeders and water containers of these animals with the feces of the final hosts should be prevented. Animals such as cats and dogs should be prevented from entering such areas. To prevent infection in final host carnivores, these animals should not be given raw or undercooked meat or organs. Periodically, the stool of cats and dogs should be examined (Urquhart et al. 1996, Sevgili 2015, Ortega 2019, Sing et al. 2019).

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FLY BORNE DISEASES IN ANIMALS

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INTRODUCTION

In the Animal Kingdom, arthropods are organisms with external skeletons, a segmented body, and jointed legs, such as lobsters, spiders, centipedes, and insects. The segments can be fused or grouped into body regions and the appendages can be exaggerated, modified or missing. It has been estimated that there are between one and ten million species of arthropods (they constitute 85% of animal species) and are found practically in all habitats on the planet, so it is not surprising that many species are parasites on humans and farm animals (Bautista Garfias 2016).

Some arthropods, including flies, can also transmit different pathogens, such as fungi, bacteria, viruses and parasites to domestic animals and humans (Rodhain 2015). The relationship between environment, pathogens, arthropod vectors and hosts is essentially dynamic, which means that it constantly changes. This must be kept in mind when we refer to the global climate change and the unexpected outbreaks of known and unknown diseases, as it is the case of the COVID-19.

Flies as disease vectors

As previously indicated, flies (Insecta: Diptera) are able to transmit directly or indirectly pathogenic agents to farm animals, causing considerable economic losses. There are many families and species belonging to the Class Diptera, however, only a few genera and species such as: *Musca domestica*, *Glossina* spp., *Stomoxys calcitrans* and *Haematobia irritans*, *Tabanus* sp., which transmit parasites to farm animals, have been considered in this chapter. In this context, the comparative size of four different hematophagous flies (*Tabanus* spp., *Glossina* spp., *Stomoxys calcitrans*, and *Haematobia irritans*) has been shown in Fig. 1.

Musca domestica (house fly) (Diptera: Muscidae)

The house fly, *Musca domestica,* is a cosmopolitan insect, commonly associated with farm buildings destined to house poultry, pigs, sheep, goats, horses and cattle. It is also abundant in human settlements where the garbage produced by humans is left on the surface of the soil.

House flies are medium-sized Diptera, with their body length ranges from five to 12 mm, when they are adult. *Musca domestica* (Fig. 2) is a successful insect pest, associated to humans and domesticated animals and found all over the world. This insect transmits more than 100 different disease causing pathogens, including parasites, to humans and animals (Mehlhorn 2015; Issa 2019). Flies feed on a variety of organic materials originated from residues that could be present in the garbage or animal excreta. Solid food is first converted into a liquid (liquefied) with the saliva of flies and then they ingest the liquified material through the mouthparts that allow them to lick and suck. When the ambient temperature is between 35 and 40ºC, the complete life cycle takes between 10 and 14 days (Bautista Garfias 2016), as shown in Fig. 3.

The parasite genera that have been isolated from houseflies include *Ascaris, Entamoeba, Ancylostoma, Necator, Trichuris, Strongyloides, Metastrongylus, Haematopinus* (an insect), *Cryptosporidium, Giardia, Enterobius, Taenia, Hymenolepis* (Khamesipour et al. 2018) and *Habronema* (Buzzell et al. 2011).

Glossina spp. (tsetse fly) (Diptera: Glossinidae)

All species of the genus *Glossina* are hematophagous. There are 23 species and eight subspecies in the *Glossina* genus (Rogers et al. 1994). Adults (Fig. 4) range in length from six to 15 mm. Reproduction is viviparous through a process known as notrophic viviparity, in which the egg transforms into a larva inside the mother, which deposits the mature larva in an appropriate microhabitat (Fig. 5).

Flies of the genus *Glossina* are vectors of the parasite *Trypanosoma,* that causes African Trypanosomiasis in animals (Nagana in cattle) and humans (Sleeping sickness). Both males and females flies feed on blood and cause painful welts on their hosts. Flies of genus *Glossina* are found in Africa south of the Tropic of Cancer. The most common species of *Trypanosoma t*ransmitted by *Glossina* spp. are: *T. vivax, T. uniforme, T. congolense, T. simiae, T. brucei, T. gambiense, T. rhodesiense* (Jordan 1976).

Stomoxys calcitrans (Stable fly) (Dipetra: Muscidae)

The stable fly, *Stomoxys calcitrans,* is an hematophagous ectoparasite of a wide variety of domestic and wild animals that has a worldwide distribution (Zumpt 1973) (Figs. 6 and 7). Being the only species of *Stomoxys* in America, it is found from southern Canada, through the USA and México, and the rest of the countries in the American continent. Worldwide there are 18 species of *Stomoxys*, including *S. calcitrans*.

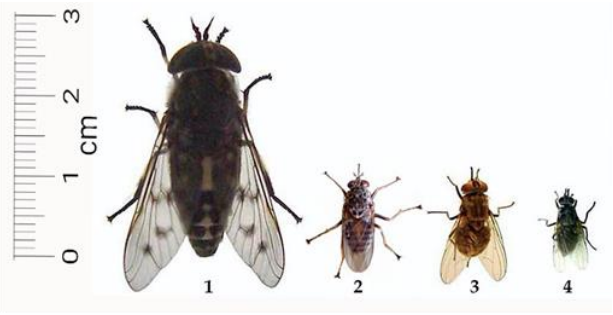

Fig. 1: Comparative size of some haematophagous flies. 1) *Tabanus* spp., 2) *Glossina* spp., 3) *Stomoxys calcitrans*, 4) *Haematobia irritans* (Figure made by Carlos Ramón Bautista-Garfias).

Fig. 2: *Musca domestica*. Details of the head are shown in the center (Figure made by Carlos Ramón Bautista-Garfias).

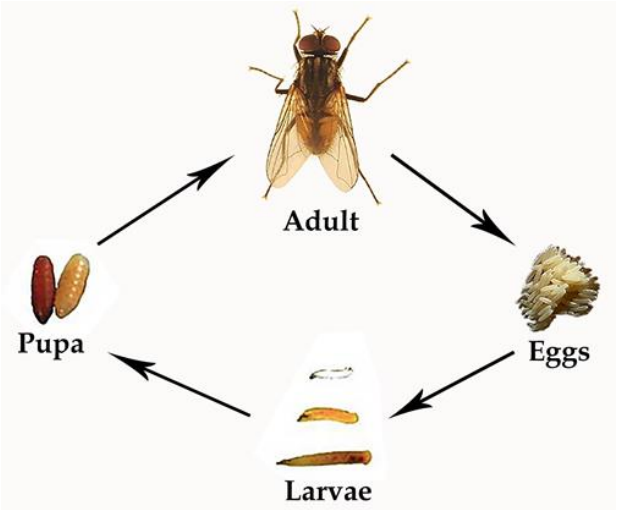

Fig. 3: Life cycle of *Musca domestica* (Figure made by Carlos Ramón Bautista-Garfias). Is is a holometabolous insect, which comprises egg, larva, pupa and adult stages. The life cycle is completed in seven to 10 days. Adults live for 15 to 28 days.

This genus is believed to have originated in Africa, where the largest number of species is currently found (Scholl et al. 2003). The stable fly, *S. calcitrans* mechanically transports *Anaplasma marginale* to cattle, which causes Anaplasmosis, an economically important livestock disease (Bautista-Garfias et al. 2018).

Haematobia irritans (Horn fly) (Diptera: Muscidae)

The haematophagous horn fly *Haematobia irritans* (Figs. 8 and 9) has also been implicated in the mechanical transmission of the rickettsia *Anaplasma marginale* to cattle in Mexico (Rodríguez et al. 2009) and in some areas of the world where there are no ticks (biological vectors) (Bautista-Garfias et al. 2018).

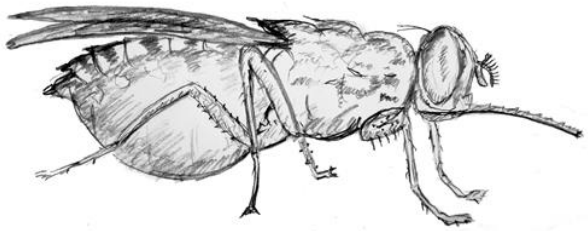

Fig. 4: *Glossina* spp. after a blood meal (Drawing by Carlos Ramón Bautista-Garfias).

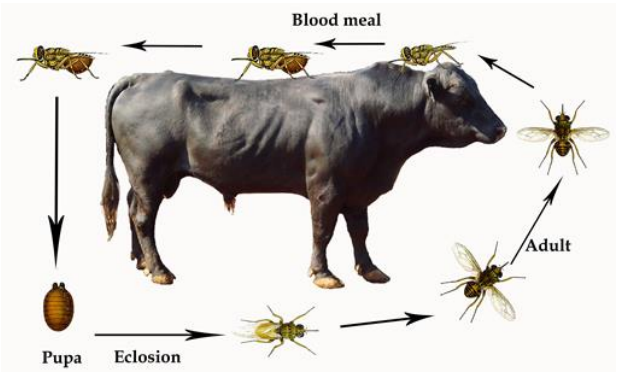

Fig. 5: Life cycle of *Glossina* spp. (Figure made by Carlos Ramón Bautista-Garfias). This fly is holometabolous, which comprises egg, larva, pupa and adult stages. The life cycle is completed in 38 days. Adult males may live **for** 15 to 21 days, while females can live for 30 to 120 days.

Fig. 6: *Stomoxys calcitrans* after a blood meal (Photograph by Carlos Ramón Bautista-Garfias).

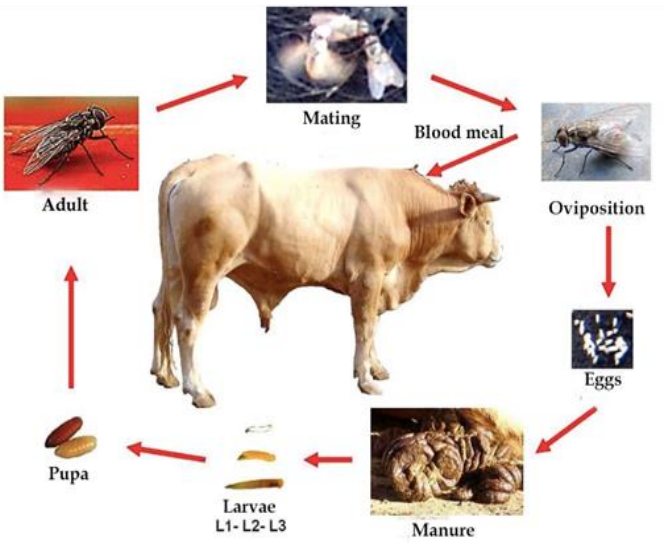

Fig. 7: Life cycle of *Stomoxys calcitrans* (Figure made by Carlos Ramón Bautista-Garfias). Is is a holometabolous fly which comprises egg, larva, pupa and adult stages. The life cycle is complete in 30 days. Adults live for 24 days (Bautista Garfias 2016).

Fig. 8: *Haematobia irritans*. Details of the head are shown in the center (Photograph by Carlos Ramón Bautista-Garfias).

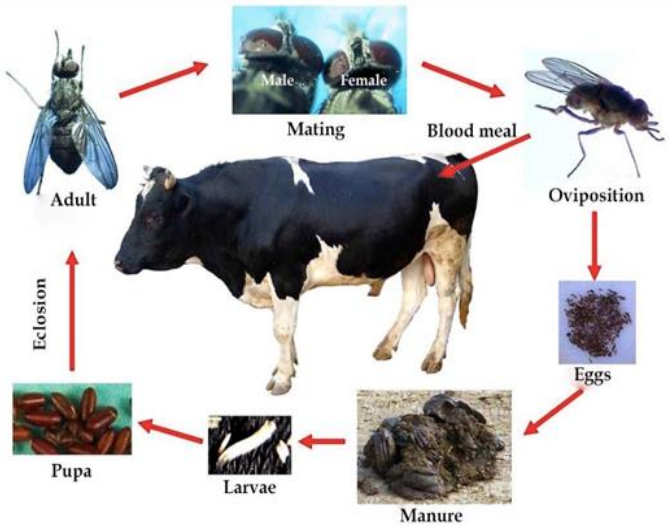

Fig. 9: Life cycle of *Haematobia irritans* (Figure made by Carlos Ramón Bautista-Garfias). Is is a holometabolous fly which comprises egg, larva, pupa and adult stages. The life cycle is complete in 10 to 20 days. Adults live for 6 to 7 days.

Fig. 10: *Tabanus* spp. (Photograph by Carlos Ramón Bautista-Garfias).

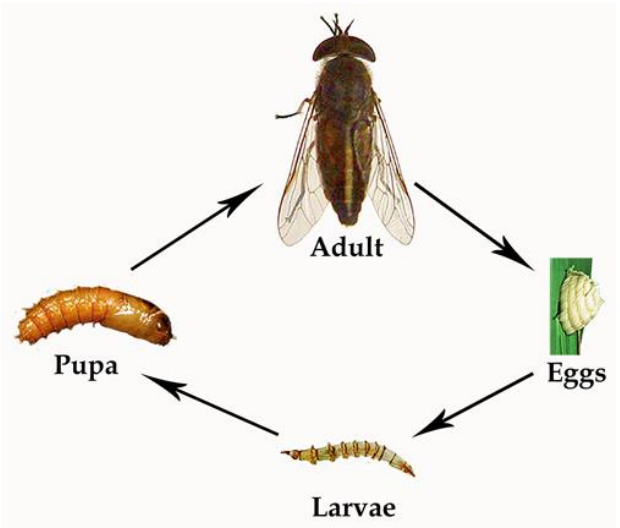

Fig. 11: Life cycle of *Tabanus* spp. (Figure made by Carlos Ramón Bautista-Garfias). This is a holometabolus insect which comprises egg, larva, pupa and adult stages. The life cycle is completed in 70 days to two years. Adult life span is 30 to 60 days. Twenty to thirty flies feeding for six hours are capable of taking 100 mL of blood.

Tabanus spp. (Horse flies) (Diptera: Tabanidae)

Another important group of hematophagous insects is members of the genus *Tabanus*, which transmit a wide variety of pathogenic organisms to the domestic animals (Baldacchino et al. 2014). There are approximately 3,000 species of this genus (Bautista Garfias 2016). These insects are very large as compared with other haematophagous flies (Figs. 1, 10 and 11). It has been demonstrated that *Tabanus* spp. can transmit *Trypanosoma evansi* (Silva et al. 1995) and *Anaplasma marginale* in cattle in some areas of the United States of America (Hawkins et al. 1982).

Anaplasmosis

Bovine Anaplasmosis is a major infectious disease of cattle, first described in 1910 by Sir Arnold Theiler as a different disease than the one produced by *Babesia* spp. parasites (Theiler 1910a, b, 1911). This is characterized by a mild to severe anemia, jaundice and weight loss in the infected animals that could end in the death of bovines.

In susceptible cattle, the disease may be present inadvertendly until is too late to treat (Rodríguez et al. 2009).

General overview of the importance in livestock productivity

The World Organization for Animal Health (Office International des Epizooties; OIE) has included Anaplasmosis in the list of bovine infectious diseases that are mandatory declared. To import cattle from an enzootic region, a certificate of health is required (Rodríguez et al. 2009; Almazán et al. 2018). This leads to local and international movement restrictions and translates into socioeconomical issues.

The disease causes losses reaching thousands of US dollars every year in non-enzootic areas, which frequently include death of animals over 2 years old (Ristic 1981; Kocan et al. 2010). The cost of this disease to the livestock production is beyond the cost of treatment; in USA the estimated average cost is $400 dollars per affected animal (Whitlock 2014; Zabel and Agusto 2018). Infected animals become carriers when they recover, and this represents a risk of cyclic reactivation of the disease, which signifies frequent expenses in treatment and loses of milk or beef production, besides the restriction that is imposed for introduction of genetically improved cattle to low-production crossbred cattle herds located in enzootic areas (Rodríguez et al. 2009).

In addition, the reproductive ability of recovered animals might be affected for long periods, situation that can affect enormously when the affected animal is of great genetic merit (Garry 2008). Mathematical modeling of the disease has shown that a producer can expect a 3.6% reduction in successful calving, 30% increase in the cull rate and 30% mortality in adults with clinical disease, when an outbreak occurs in an *Anaplasma*-free herd (Zabel and Agusto 2018).

Etiological agent

Bovine Anaplasmosis is caused by *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). This is an intraerythrocytic pathogen of 0.3 - 1 µm in diameter that can be observed under an optical microscope on blood smears stained with Giemsa or Wright methods (Ristic 1981). The intracellular organism can be observed in the border of red blood cells. The organism is host specific, causing disease in cattle, although it can infect other ruminants that serve as reservoirs (Kocan et al. 2010). The subspecies *A. centrale* may cause minor clinical signs and it is used for the preparation of live vaccine in Israel, Australia, Africa and some South American countries (Theiler 1911; Abdala et al. 1990; Bock and DeVos 2001).

A reclassification placed these organisms in the order of Rickettsiales (Dumler et al. 2001). Thus, two families were defined Rickettsiae and Anaplasmataceae. The first family comprehends obligated intracellular organisms that develop freely in the cytoplasm of eukaryotic cells, while the second family includes organisms that belong to four distinct groups: *Anaplasma, Ehrlichia, Wolbachia* and *Neorickettsia*. These organisms are also obligate intracellular parasites that infect eukaryotic cells within membrane-bound vacuoles formed in the cytoplasm of the host cells. These host cells may include erythrocytes, reticuloendothelial cells, bone marrow-derived phagocytic cells, endothelial cells and cells of insect, helminths and arthropods' reproductive tissues.

The genus *Anaplasma* includes the most pathogenic organisms like *A. marginale*, *A. marginale* subspecie *centrale* (mainly known just as *A. centrale*), *A. ovis* (that infects sheep), *A. bovis* (formerly *Ehrlichia bovis*), *A. platys* (formerly *Ehrlichia platys*) and *A. phagocytophilum* (which itself includes organisms previously known as *Ehrlichia equi*, *E. phagocytophila* and the previously unnamed causative agent of human granulocytic ehrlichiosis).

Under natural conditions, *A. marginale* infects the bovine erythrocytes and replicates within them, generating up to eight initial bodies that form a corpuscle or inclusion body. Each of the initial bodies exits the cell to infect another, causing more than 70% of red blood cells infected during an acute case. The prepatent period ranges from 7 to 60 days, with an average of 28 days, and the percentage of erythrocytes infected depends on the virulence of the strain (García-Ortíz et al. 2000) and the infective dose (Gale et al. 1996; Scoles et al. 2005). In the spleen, the infected cells are phagocytized by reticuloendothelial cells, resulting in anemia and icterus without hemoglobinemia or hemoglobinuria (Rodríguez et al. 2009; Kocan et al. 2010).

Diagnosis

The most significant signs in acute cases are the loss of appetite, apathy and fever (higher than 41°C). In severe cases, animals present breathing difficulties that can lead to death if there is no timely treatment with the correct drugs. The microscopically detection of inclusion bodies in blood smears (Fig. 12) is used to confirm the diagnosis without molecular or serological tests. However, microscopic evaluation could be difficult if the operators have lack of experience with *Anaplasma* detection in the edge of erythrocytes (Theiler 1910a; Ristic 1981; Rodríguez et al. 2009; Kocan et al. 2010).

Another clinical sign is the abortion in pregnant females during the last trimester of gestation (Quiroz-Castañeda et al. 2016; Zabel and Agusto 2018). Furthermore, there are some reports describing reductions in serum testosterone levels related to single or co-infections with *A. marginale*, *Babesia* spp. and *Trypanosoma vivax* (Camejo et al. 2016), adversely affecting fertility and reproductive performance.

Persistently infected cattle have life-long immunity and, generally, do not develop clinical disease, unless they are affected by concomitant or stress-related diseases (Rodríguez et al. 2008; Rodríguez et al. 2009). Moreover, the immune and nutritional status of bovines is of great importance in the host response to the infection (Ribeiro-Gasparini et al. 2013).

Recovered animals may remain as carriers for life. In such chronic cases, there could be production losses, whether in milk or muscular mass (Fig. 13), together with clinical signs of jaundice (Fig. 12). Cyclical peaks of rickettsemia are characterized by fever, low red blood cells count and the presence of *A. marginale* inclusion bodies in the edge of erythrocytes. The count of infected cells may increase in an arithmetical manner during 5 to 10 days. Complications

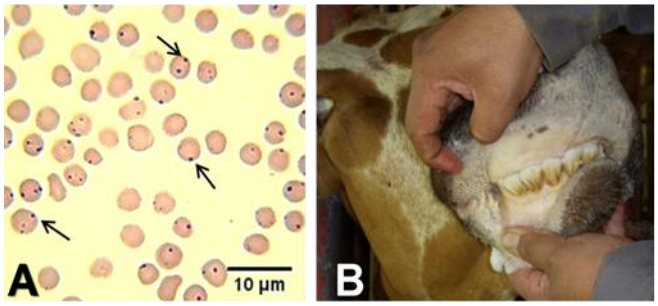

Fig. 12: *Anaplasma marginale* effects on cattle. A) Giemsa stained blood smear; infected erythrocytes with inclusion bodies of *A. marginale* (arrows). B) Jaundice observed in mouth mucosal tissue of a bovine (Designed by Salinas EE. Photograph by Anaplasmosis Research Unit CENID-SAI INIFAP).

and death may occur if other concomitant diseases are present in the herd, and mostly occurs when farmers introduce new cattle from non-enzootic areas to an enzootic area (Rodríguez et al. 2009; Quiroz-Castañeda et al. 2016).

Complementary test for diagnosis is an indirect or competitive enzyme immunoassay (ELISA) for detection of antibodies against *A. marginale*. The PCR or qPCR can also be used for the molecular detection of the pathogen DNA in blood samples (Torioni de Echaide et al. 1998; Carelli et al. 2007; Rodríguez et al. 2009; Bacanelli et al. 2014).

Epidemiology

The disease is enzootic in some areas of North, Central and South America, including the Caribbean Islands. Mediterranean countries have made some reports of this disease, as along-with some countries of Asia and Africa (Kocan et al. 2010). Some of the countries where Anaplasmosis detection has been made either from cattle's blood, ticks or flies and other mechanical flying vectors are shown in Fig. 13 (de la Fuente et al. 2005; Carelli et al. 2007; Rodríguez et al. 2009; Awad et al. 2011; Ait Hamou et al. 2012; Belkahia et al. 2015; El-Ashker et al. 2015; M'ghirbi et al. 2016; Quiroz-Castañeda et al. 2016; Yunik et al. 2016; Aktas et al. 2017; Gondard et al. 2017; Tana-Hernández et al. 2017; Rjeibi et al. 2017; Abdela et al. 2018; Cui et al. 2018; Aydin et al. 2019; Noaman et al. 2019; Kumar et al. 2019; Sprong et al. 2019; Zhou et al. 2019; Zhyldyz et al. 2019; Ringo et al. 2020).

Reports of outbreaks of Anaplasmosis are common in countries geographically located between the Tropic of Cancer and Tropic of Capricorn but not in some mountain regions and desert areas (Kocan et al. 2010). Although climate change, management mistakes and adaptations of the biological vector have made clear that the pathogen can cause outbreaks outside of this geographical range. An outbreak in southern Canada in 2008 showed the importance of disease monitoring and focused on the role of ticks and horse flies in the possible transmission of bovine Anaplasmosis (Yunik et al. 2016). More recently, Bursakov and Kuvalchuk (2019) reported the overall rate of hemoparasite infection in cattle in the Moscow region; and discovered that a remarkably high numbers of the animals were co-infected with *A. marginale* and *Theileria* spp.

Bovine Anaplasmosis prevalence has been reported in many countries, and varies from one study to another due to several factors, such as the sampling size, detection technique, the moment (in terms of infection) when the sample was taken, the immune state of the animals sampled and other factors such as age, breed, genre, and even the conservation of the samples. A short sample of studies made around the world is shown in Table 1, where the variation in prevalence among studies is evident.

There are well-defined enzootic regions, primarily due to life-long persistent infections in the recovered bovines. These animals remain as asymptomatic carriers, contributing to spread the disease to susceptible bovines. Besides, there are some regions where sharing grazing grounds with wild-life ruminants contributes to a wider distribution and a larger number of reservoirs for the pathogen (Rodríguez et al. 2009; Kocan et al. 2010).

As shown before, this disease has a worldwide distribution in tropical and subtropical regions related to the presence of its biological vectors. The distribution of Anaplasmosis has shown, and will continue to, change due to international trade movements of cattle and also as a result of global warming, which may influence the movement of the tick hosts (Kocan et al. 2010). In higher altitudes with temperate climate and no ticks present, the main sources of transmission of bovine Anaplasmosis are iatrogenic and mechanical vectors. The latter refers to contaminated objects inadequately used in several animals (like needles) (Reinbold et al. 2010) and, most important and difficult to control, different biting flies.

Transmission

The transmission of this disease from cow to calf can be common in herds located in enzootic areas. If the cow and the calf are raised in an enzootic area, the calf is born healthy with a low level of infection and this produces protective immunity (Zwart 1985; Gale et al. 1996; Vega et al. 2007; Garry 2008; Ribeiro-Gasparini et al. 2013). When pregnant cows are introduced from a non-enzootic area into an enzootic area, abortion may occur in the third trimester of pregnancy. It may affect the milk production and therefore, the productive value of the recently arrived cow. When the gestation ends normally, the calf may be born with the disease and may die after a few days, if it is not properly treated.

Fig. 13: Detection of bovine Anaplasmosis. Some countries where *Anaplasma marginale* has been detected are shown in orange. Several of the studies have taken place in small regions or with samples from small herds. There are few countries where an extensive epidemiological study has been made (Designed by Salinas EE, made by MapChart®).

Table 1: Prevalence of bovine Anaplasmosis in some countries.

Country	Type of analysis	Prevalence	Number of animals tested	References
India	PCR (*msp5*)	18.48%	211	Kumar et al. 2019
	Giemsa stained blood smear	6.64%		
Iran	PCR (*msp4*)	44.00%	200	Noaman et al. 2019
Sri Lanka	PCR (*msp5*)	32.70%	437	Zhyldyz et al. 2019
Rusia	RT-PCR (*msp4*)	58.00%	113	Bursakov and Kovalchuk 2019
Turkey	PCR (*msp1*)	6.00%	150	Aydin et al. 2019
	Giemsa stained blood smear	2.67%		
	nPCR (*msp4*)	31.00%	247	Aktas et al. 2017
	Giemsa stained blood smear	3.80%		
China	PCR (*msp5*)	2.61%	345	Zhou et al. 2019
	PCR (*msp4*)	25.71%	35	Cui et al. 2018
	Giemsa stained blood smear	80.00%		
South Korea	cELISA	7.00%	568	Seo et al. 2018
Ethiopia	Giemsa stained blood smear	5.10%	408	Abdela et al. 2018
Algeria	PCR (*msp4*)	11.10%	180	Rjeibi et al. 2017
Ecuador	PCR (*msp5*)	86.10%	151	Tana-Hernández et al. 2017
Tunisia	PCR (*msp4*)	24.70%	328	M'ghirbi et al. 2016
	RT-PCR (*msp1b*)	25.40%	232	Belkahia et al. 2015
	PCR (*msp4*)	4.70%	1035	Belkahia et al. 2015
Egypt	PCR (*msp1b*)	20.10%	164	El-Ashker et al. 2015
México	cELISA	67.47%	166	Castañeda-Ortiz et al. 2015
Pakistan	cELISA	31.05%	1050	Atif et al. 2013
Morocco	nPCR (*msp5*)	21.90%	668	Ait Hamou et al. 2012
Sudan	PCR (*msp4*)	6.10%	692	Awad et al. 2011
Bolivia	Giemsa stained blood smear	6.90%	160	Mercado et al. 2011
Brasil	cELISA	58.74%	223	Marangoni-Marana et al. 2009

Ticks are the biological vector. The transmission mechanism most commonly known was studied with *Rhipicephalus microplus*, which is also the most widely distributed cattle tick, and, like some species of *Dermacentor*, were reported to be a vector of the pathogen causing Anaplasmosis (Futse et al. 2003; Kocan et al. 2004; Scoles et al. 2005; Rodríguez-Vivas et al. 2006). The transmission by ticks is efficient, since the amount of rickettsia transmitted by ticks does not depend on the level of rickettsemia in the bovine at the moment of feeding.

The *A. marginale* replicates in the midgut epithelium and in the salivary glands of the tick (Lohr et al. 2002, Futse et al. 2003; Scoles et al. 2005). However, some strains of *A. marginale* are not transmitted by ticks but by flies or mosquitoes (Kocan et al. 2004; Scoles et al. 2005). The replication within the tick tissues and the intrastadial, transtadial and transovaric transmission makes arthropods the primary reservoir for the pathogen (Amaro et al. 2020).

The iatrogenic ways of transmission are not so common. These may include the repeated use of a single needle

among several bovines or the use of other fomites, including castration instruments, dehorning saws, tattooing instruments and ear tagging devices (Reinbold et al. 2010; Kocan et al. 2010).
Mechanical transmission by biting flies (similar to the use of contaminated instruments on several animals) explains the spread of the disease in the absence of ticks. There have been several reports, involving a large number of flies in transmission of Anaplasmosis, but just a few of them have been studied under natural conditions. In livestock, their direct effects are disturbance, skin lesions, reduction of feed intake, stress, blood loss, and a global immunosuppressive effect (Baldacchino et al. 2013). The common stable fly (*Stomoxys calcitrans*) is one of the species that can transmit the pathogen in the mechanical form. Scoles et al. (2005) determined by qPCR that the acquisition of *A. marginale* was low, even 20 minutes after feeding, and this value increased when the infection increased on the host.

Fig. 14: Transmission of bovine Anaplasmosis. A) Ticks, also the biological vector, B) Flies and C) Tabanids, both act as mechanical vectors. Iatrogenic ways of transmission that require the incorrect use of the same needle in two or more animals are D) application of antibiotics, vaccines or other drugs and E) sampling; F) the incorrect use or lack of sterilization of surgical equipment contaminated with infected blood. G) Susceptible bovine that may be infected by either of the ways mentioned before. Last way of transmission is H) from the cow to the calf (Designed by Salinas EE. Photograph by Anaplasmosis Research Unit CENID-SAI INIFAP).

The transmission of disease by stable flies fails, either because of desiccation or due to some inhibitor components on the saliva of the flies. Another explanation is that at least a portion of the infected erythrocytes remains on the mouthparts after the transmission feeding, also, 20 minutes after feeding the proportion of flies with measurable amounts of *A. marginale* on their mouthparts was reduced. Mechanical transmission by direct transfer of flies from an infected to a susceptible host is at least two orders of magnitude less efficient than tick-borne biological transmission.
This indicates that the transmission occurs within minutes due to the short period that *A. marginale* can survive in mechanical vectors and the quantity of biting flies present at the same time on an animal most be large enough to generate a high rickettsemia (Zwart 1985).

The second fly species is the horn fly (*Haematobia irritans*) which, besides the traumatic effect on the hide quality, causes stress by the infestation interfering with cattle feeding and this generates a reduction in meat and milk production. Flies leave the host only to move to other hosts or to lay eggs on fresh manure (Torres et al. 2012). In order to confirm the participation of this fly, some analysis has been made. In a study made in a tropical region in Mexico, male calves appeared to attract more flies than female calves, and lighter coat color of breed-crosses attracted fewer flies than black and red-black coat colors of other breeds and crosses.
Finally, temperature and rainfall were the environmental factors that influenced higher fly infestations (Rodríguez-Gallegos and Acosta-Rodríguez 2011). In Australia, a similarity in the distribution of the cattle tick and the buffalo fly (*Haematobia irritans exigua*) raised the question whether the presence of *A. marginale* could be more associated with the presence of flies than thought before. The experiments were made on spleenectomized calves giving an unrealistic scenery about the feeding and transmission potential, nonetheless the results of transmission were negative, indicating that not all species of Tabanids have a role in the epidemiology of Anaplasmosis (Allingham et al. 1994). Another study corroborated that *Haematobia* spp. was not important as a mechanical vector of *Anaplasma* spp. (Hornok et al. 2008). However, their contribution to a lack of welfare is notorious and it has to be analyzed in order to control the population of these flies.
The third genre most studied is the horse fly (*Tabanus bovinus and T. fuscicostatus*) which, besides the risk of transmission of pathogens and the blood feeding, produces a higher level of discomfort for the animals. Tabanid horse flies species may be more important for their mechanical role in transmission of *A. marginale* than the biological vector in the spreading of this pathogen in places were ticks are not abundant. Tabanid females suck blood only every 3–4 days, but their painful feeding is usually interrupted by host animals several times, allowing mechanical transfer of pathogens.
Although remaining able to inoculate *A. marginale* for at least 2 h during which *T. bovinus* can cover several km with its flight speed around 5 m/s, they tend to feed in the same herd until completing their blood meal (Foil 1989; Hornok et al. 2008). Scoles et al. (2008) showed that Tabanid-borne mechanical transmission is at the minimum two orders of magnitude less efficient than tick-borne biological transmission. Still, there are many places around the world where Tabanids species may be the principal carriers of *A. marginale*.
It has been reported that transmission by biting flies is less efficient than transmission by ticks, due its dependence on the rickettsemia level of the host (Scoles et al. 2005). However, transmission by flies is of great concern in those places where the population of flies is abundant and difficult to control and ticks are absent or they are not the principal vector. The latter is the case for certain areas of Africa, USA, Central and South America (Foil 1989; Figueroa et al. 1998; Coronado 2001; Baldacchino et al. 2013).

Control

The development of a worldwide protective vaccine and an effective antimicrobial for clearance of carrier bovines are two of the main goals for the Anaplasmosis research. However, current strategies and measures for control of Anaplasmosis are not very different from the ones employed in the past. In general, these strategies are based on the control of biological vector, prophylactic antibiotic administration, control of fomites and restrictions in animal trade between non-enzootic and enzootic areas. Simple management practices can decrease the incidence of Anaplasmosis within the herd, such as decreasing the number of movements for sanitary activities. This can be achieved by an adequate vaccination/deworming calendar with the objective of making the less possible movements to the herd (Almazán et al. 2018; Zabel and Agusto 2018). Education and training of personnel is important to achieve control of infectious diseases.

Control of horn flies mainly relies on the use of insecticides, but issues with resistance have increased interest in development of alternative means of control (Madhav et al. 2019). In parallel, inadequate use of acaricides for tick control have contributed to an increasing resistance against chemical components. However, there are no current guidelines to introduce any recommendation for farmers after resistance is detected (Estrada-Peña and Salman 2013). This situation generates a frequent administration of products, increasing the cost of control expenses, which also increase the production. Methods based on vector control may also affect the environment due to residues of the chemicals deposited on the soil and water (Rodríguez-Vivas et al. 2006; Almazán et al. 2018).

Another approach that has been developed is keeping cattle in an environment that is not favorable for survival and completion of the life cycle of ticks. However, this may represent additional costs that could be difficult to manage for some farmers. Integrated pest management combines sanitation practices, biological control and chemical agents to reduce tick and fly populations. Biological control of ticks and flies is highly recommended and is based on natural enemies (beetles, mites and parasitic wasps, or parasitoids) (Aubry and Geale 2011). The use of certain types of grass and bushes that tend to repel ticks has also been suggested. All these methods should be combined with rotational grazing (Almazán et al. 2018). The discovery of Bm86, a gut membrane protein found in the intestine of *R. microplus*, in the 80's resulted in the development of anti-tick vaccines. Cattle vaccinated with Bm86 experience a reduction in the number of engorging females, their weight, and reproductive capacity, leading to reduced tick numbers after several generations (Almazán et al. 2018). The discovery of vaccines against ticks is one of the most promising branches in the research of tick-borne and fly-borne diseases that could contribute to an improvement in environment care and animal welfare.

In the past, the treatment of acute bovine Anaplasmosis was based on a variety of chemotherapeutic agents, including arsenicals, antimalarials, antimony derivatives and dyes. These compounds had little or no chemotherapeutic effects. Due to the close relation of this disease with Babesiosis, imidocarb dipropionate was used in some places to treat animals suspected for *A. marginale* infection, although it has been restricted due to prolonged retention of the drug in edible tissues of food producing animals (Kocan et al. 2010).

The treatment of Anaplasmosis consists of the administration of tetracyclines (Swift and Thomas 1983). Although other antibiotics, including enrofloxacin, have been studied (Coetzee et al. 2005; Coetzee et al. 2006), none has been shown to clear persistent infections in carrier bovines. The dosage recommended by the OIE is based on five daily injections of oxytetracycline administered intravenously at 22 mg/kg body weight. These antibiotics have been used in a prophylactic approach when introducing naïve animals into an enzootic herd (Rodríguez et al. 2009; Quiroz-Castañeda et al. 2016; Almazán et al. 2018). Despite their efficiency in the control of the disease, repeated administration of tetracyclines constitutes a risk in itself, as it enhances the emergence of antibiotic resistance for many other microorganisms.

Live vaccines have been developed almost since the discovery of *A. marginale*, but this has restrictions in several countries due the inexistence of the vaccine agent *A. centrale* in their territories (Theiler 1911; Abdala et al. 1990). Inactivated strains and low virulence strains of *A. marginale* have been tested and showed some protection against homologous challenge but showed a low or no protection against heterologous challenge (Rodríguez et al. 2000; Vega et al. 2007; Rodríguez et al. 2008; Almazán et al. 2018). In addition, this method represents a risk of transmission of other microorganisms.

The use of recombinant highly immunogenic proteins has been evaluated with similar results, most probably due to the presence of great diversity among different strains. The genomics approach is now the one on the focus to develop a vaccine that could be safer and effective against all the strains of *A. marginale*. The success of new vaccines, using molecular technologies, for Anaplasmosis will depend on their cross protection between genotypes and to either mimic or redirect the host response during natural infections or block infection of host cells (Kocan et al. 2010; Almazán et al. 2018). An integrated approach is needed to prevent the vector infestation and the infection of the pathogen by the development of safer and effective vaccines. Research in the control of, and vaccination against, Anaplasmosis is still a great goal. When accomplished, it will contribute greatly to bovine health, food security and environment care with direct effect on human health.

African Trypanosomiasis

Generalities

Mainly known as American Trypanosomiasis and African Trypanosomiasis (Cevallos and Hernandez 2020), these

diseases belong to the family Trypanosomatidae and are characterized by an organelle called kinetoplastid. The genus Trypanosoma is characterized by the use of two hosts, one vertebrate and one invertebrate, to carry out its life cycle. The most studied species of this genus are *T. cruzi* and *T. brucei*. These are different subgenera and therefore, their biological aspects are different. The diseases for each of these infections are Chagas disease and sleeping sickness (America and Africa).

Chagas disease is a zoonosis caused by *T. cruzi*, which was described in 1909 by the Brazilian doctor Carlos Chagas and the doctor Salvador Mazza, naming it "Chagas-Mazza disease". This disease is localized only in America, mainly in the jungle, where triatomines, as well as various mammals including opossums, raccoons and rats, act as vectors.

This interaction has been mainly due to the invasion of rainforests by humans. These people can be bitten by infected bedbugs and start the transmission of the disease. Triatomines are “hemimetabolous”, they do not have a pupal stage and have an incomplete metamorphosis. The stages they go through include eggs, nymphs and adults; this stage development can last from six months to one year (Fig. 15).

Human African Trypanosomiasis is a disease caused by protozoan *Trypanosoma brucei* (gambiense and rhodesiense). In the case of the parasite *T.b gambiense*, it is considered a more chronic disease that is localized in West and Central Africa. This disease is transmitted by the bite of a fly, known as tsetse fly (*Glossina palpalis*, *G. tachinoides*, *G. morsitans*, *G. pallidipes*, *G. swynnertoni* and *G. fuscipes*) (Roche 2004). The classification of African Trypanosomes according to Cordon-Obras et al. (2013) is set out in Table 2.

In general, African Trypanosomes are reported to parasitise a large number of domestic and wild vertebrates (cattle, swine, equidae, giraffes), primates and carnivores (canids, felids, mustelids) (Isobe et al. 2003). Only two African Trypanosomes have been reported to be able to survive in human serum, these are *T.b gambiense* and *T.b rhodesiense*. They can cause disease in humans, which can lead to anemia, fever, edema and sometimes death. The tsetse fly is the only vector for *T.b gambiense* and *T.b rhodesiense*. A map, showing distribution of the African disease in humans is presented in Fig. 16 (Simarro et al. 2013).

T.b gambiense and *T.b rhodesiense* are distinguished by their geographical distribution. *T. rhodesiense* is found in East Africa and *T. gambiense* in West and Central Africa. (Rhodesia is the former name of Zimbabwe). Other differences that can be found between *T.b gambiense* and *T.b rhodesiense*, can be found in Table 3 described by Mark (1999).

Molecular data suggest that *T. rhodesiense* may be a variant of *T. brucei* in the host range. Molecular techniques also show that *T. gambiense* is relatively homogeneous across its wide distribution in central and West Africa.

Furthermore, a mutation in an ancestral *T. brucei* conferred human infectivity and thus represented the origin of *T. rhodesiense*. Therefore, *T. rhodesiense* can be considered as a variant of the host range of *T. brucei* (Gibson 2002).

Biology

The genus Trypanosoma has an elongated morphology, cell size varies from 18-42 µm in length and 1-3 µm in width. It also has the basic organelles of eukaryotic cells, the Golgi apparatus, ribosomes, mitochondria, nucleus and endoplasmic reticulum. In addition, the surface of the parasite is coated with proteins for protection from the immune response. In general, there are different morphological forms of Trypanosomes: trypomastigote, epimastigote, promastigote and amastigote. The differentiation of these forms is based on the position of the kinetoplast in relation to the nucleus, and by the presence or absence of the undulating membrane. These morphological forms can be seen in Fig. 17.

For *Trypanosoma cruzi* disease: The etiological agent is characterized by the presence of a single flagellum and a single mitochondrion. Three groups of *T. cruzi* strains have been differentiated. They are the TCI lineage, which is a wild strain and has been reported mainly in the highlands of Chile-Bolivia. TC2 is a domestic cycle. On the other hand, the African Trypanosimiasis genera are characterized by some variations in morphology and physiology. The subgenus Trypanozoom is characterized by a kinetoplast, which develops in the salivary glands and midgut of the vector (Fig. 18).

The genome of *T. brucei* is reported to have 11 chromosomes. Furthermore, the genome has already been sequenced and is available in several databases.

Life cycle of *Trypanosoma brucei gambiense*

The life cycle of *T. b. gambiense* begins when an infected tse tse fly injects metacyclic trypomastigotes into the skin tissue of a mammalian host. The parasites enter the lymphatic system and pass into the blood stream. Once inside the host, the parasites transform into trypomastigotes in the blood stream, travel to other sites throughout the body, and also enter body fluids, such as lymph and cerebrospinal fluid. Later, trypomastigotes

Table 2: The classification of African trypanosomes taken from Cordon-Obras (2013).

Taxon	Name	Descriptor
Genus	*Trypanosoma*	Gruby 1843
Subgenus	*Trypanozoom*	Lühe 1906
Specie	*Trypanosoma brucei brucei*	Plimmer and Bradford 1899
	Trypanosoma brucei gambiense	Dutton 1902
	Trypanosoma brucei rhodesiense	Stephens and fantham 1910
	Trypanosoma evansi	Balbiani 1888
	Trypanosoma equiperdum	Doflein 1901
Subgenus	*Nannomonas*	Hoare 1964
Specie	*Trypanosoma congolense*	Broden 1904
	Trypanosoma simiae	Bruce 1912
Subgenus	*Duttonella*	Chalmer 1918
Specie	*Trypanosoma vivax*	Zieman 1905

Table 3: Differences between *T.b gambiense* and *T.b rhodesiense*, described by Mark (1999).

Attribute	*T. rhodesiense*	*T. gambiense*
tse tse vector	*G. morsitans* group	*G. palpalis* group
ecology	dry bush, woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	human-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, some epidemics
disease progression	rapid, often fatal	slow (~1 yr) acute ⇒ chronic
parasitemia	high	low
asymptomatic carriers	rare	common

multiply by binary fission and circulate in the blood stream. By ingesting blood from an infected mammalian host, the tse tse fly becomes infected with trypomastigotes present in the blood stream. In the midgut of the tse tse fly, parasites are transformed into procyclic trypomastigotes and multiply by binary fission. The procyclic trypomastigotes leave the midgut, become epimastigotes and reach the salivary glands of the fly, multiply by binary fission and become metacyclic trypomastigotes (Fernandes-Rodríguez et al. 2014).

Diagnosis

The diagnosis of African Trypanosomiasis is established by direct techniques in the acute phase that are based on the parasitological examination of blood and cerebrospinal fluid. Indirect diagnostic techniques are used when the disease affects the central nervous system. A more specific and highly sensitive test is the polymerase chain reaction (PCR) that allows the identification of the genus, species, subspecies or type of parasites, depending on the cases. The highly specific primers or the sequencing of the PCR products allow the identification of *T. brucei* spp. zoonotic, which provides de "novo" information on the role of domestic and wild animals in the maintenance of some spotlights of sleeping sickness (Hopkins et al. 1998; Holland et al. 2001). On the other hand, there is also the serological test called indirect immunofluorecence and the detection of antibodies by ELISA, both tests are used routinely for the detection of antibodies against Trypanosoma in cattle (Greiner et al. 1997).

Control

There are various control methods for African Trypanosomiasis. The first one is to eliminate or eradicate the vectors (tse tse fly), mainly through destroying the places where they reproduce. On the other hand, insecticides, especially "organochlorines" have been widely used for the eradication of tse tse flies. The most widely used insecticides are dicloro difenil tricloroetano (DDT) and dieldrin, the former being the product used to eradicate the Zululand tse tse fly in South Africa; it would

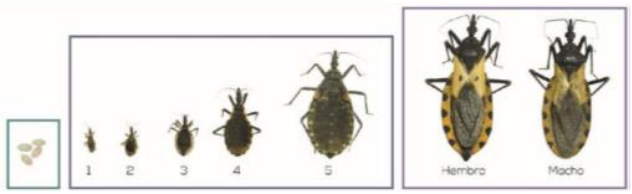

Fig. 15: Life cycle of triatomata. Figure taken from "Manual de vigilancia y control de la enfermedad de chagas en el Ecuador", 2020.

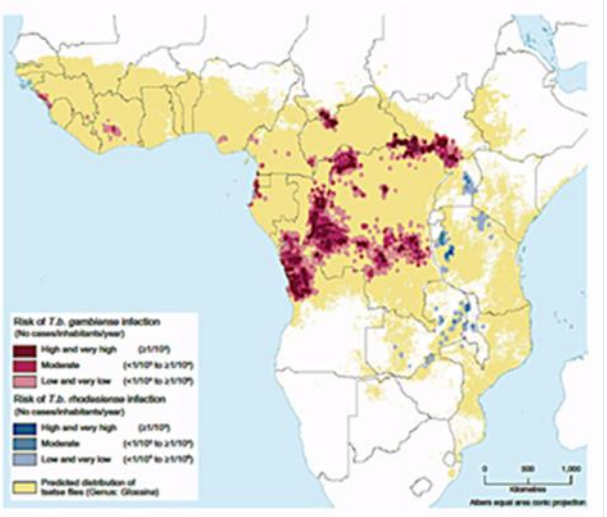

Fig. 16: Human African Trypanosomiasis distribution map from Simarro et al. (2013).

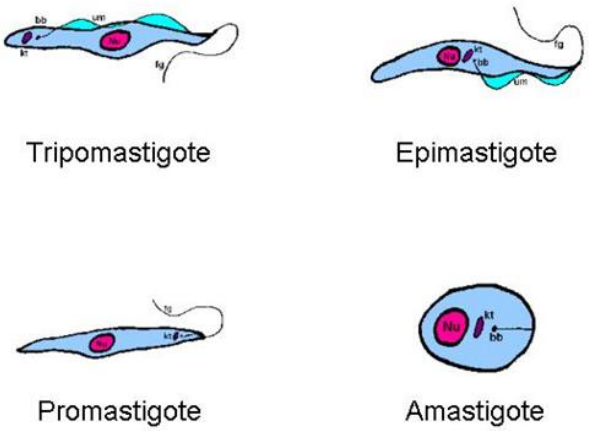

Fig. 17: Morphological stages of trypanosomatids from Cevallos and Hernández (2020).

be feasible only if considerable international cooperation is achieved (Odeniran et al. 2018). Eradication by means of insecticides of chemical origin has an important disadvantage of affecting other arthropods and beneficial organisms. Another method is "chemotherapy"; however, resistance against different drugs has already developed (Schofield and Maudlin 2001).

The economically most important parasitic diseases transmitted to farm animals from flies were reviewed in this chapter. The present review focuses mainly on diseases transmitted biologically or mechanically by the following fly species or genera: *Musca domestica, Glossina* spp., *Stomoxys calcitrans, Haematobia irritans*, and

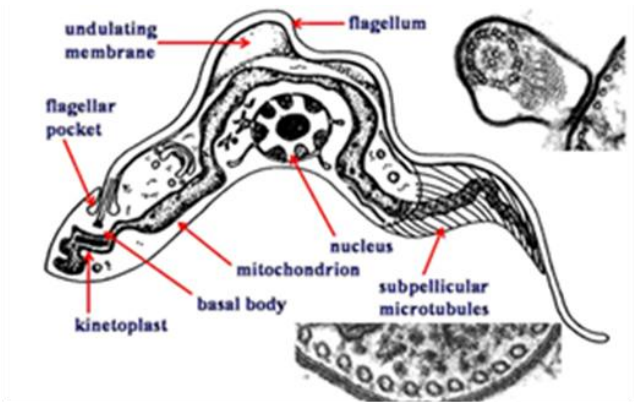

Fig. 18: The subgenus Trypanozoom is characterized by a kinetoplast, which develops in the salivary glands and midgut of the vector (taken from Mark 1999).

Tabanus spp. Similarly, some aspects of fly biology were reviewed alongside the most important parasitic diseases caused by African trypanosomes (*Trypanosoma* spp.) and the hemotropic rickettsia *Anaplasma marginale,* which was once considered as a parasite.

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INTRODUCTION TO ECHINOCOCCOSIS AND A REVIEW OF TREATMENT PANELS

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INTRODUCTION

Echinococcosis is a neglected silent cyclozoonotic parasitic disease, caused by metacestodal stages of organisms of genus *Echinococcus,* belonging to family *Taeniidae.* These parasites affect wide spectrum of animal species including livestock and wildlife, and also possess zoonotic implications (Singh et al. 2012; Sadjjadi et al. 2013; Ma et al, 2015; Rialch et al. 2018). At present, there is a huge debate over taxonomy of genus *Echinococcus* at the species level. There are considerable variations shown by this genus at the level of species in terms of morphology, developmental phases, modes of transmission, antigenicity and host specificity (Thompson and McManus 2001; Lymbery and Thompson 2012; Thompson 2008). Genus *Echinococcus* contains at least 10 valid species, each having different strains and genotypes namely *E. granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarhtus, E. canadensis, E. equines, E. felidis, E. shiquicus* and *E. ortleppi* (Nakao et al. 2013).

Echinococcus species require two different hosts, the intermediate and the definitive. The definitive hosts are the carnivores, especially the dogs that carry this parasite in their small intestines. Both wild and domesticated ruminants, camels and human beings serve as intermediate hosts of different *Echinococcus* species (Eckert et al. 2001; Jenkins et al. 2005; Elham et al. 2014). The size of adult worm varies from 2-11 mm, having 2-7 proglottid segments. Each of the proglottid has single genital opening and mature proglottid is called as penultimate segment. Scolex has two rows of rosttelar hooks.

After entering the small intestine of the definitive host, the scolex with its suckers and rostellar hooklets, becomes exvaginated and develops into adult worm. After fertilization, eggs are fully developed in the uterus and gravid proglottids release eggs that are passed into the environment along with dog feces (Figure 1). Ingestion of contaminated water or vegetation by the intermediate host leads to release of oncosphere from embyronated eggs that penetrate the intestinal wall, spreading to various other tissues of the body through circulation. Cyst formation primarily occurs in the lungs and liver and such infected tissue when eaten by the canids leads to release of protoscoleces, which become mature worm resulting in the completion of the life-cycle of parasite (Thompson and McManus 2002; Brehm 2010; Pourseif et al. 2017; Conceição et al. 2017;).

Geographical distribution

Echinococcosis is included in the World Health Organization (WHO) list of neglected tropical diseases (NTDs) (Butt and Khan, 2020). This disease is widespread in its distribution and persists in a variety of environmental conditions, varying from temperate to circumpolar, tropical and sub-tropical regions. The parasite has ability to survive very well in climatic conditions varying from arid to subpolar oceanic environment. Eurasia, Australia, Africa and South America have very high prevalence of the disease and around 50 milllion people are infected with the disease worldwide (Eckert et al. 2001; Hammad et al. 2018). This ailment encompasses wide geographical area, extending from Eastern parts of Asia to the Northern America and from upper northern hemisphere to the southern countries of African continent (Schneider et al. 2010; Sadjjadi et al. 2006) (Figure 2).

Pathology of echinococcosis

Echinococcosis can appear in four different forms namely Cystic Echinococcosis (CE), Alveolar Echinococcosis (AE), Poly-cystic Echinococcosis (PE) and Uni-cystic Echinococcosis (UE), caused by *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus vogeli and Echinococcus oligarthrus*, respectively (Table 1). All of these species share the common definitive hosts which are the canids, except *E. oligarthrus* which have members of the family Felidae as the definitive host. Definitive hosts harbor the adult stage of worms in their small intestine and shed the embryonated eggs of the parasite in the environment with the feces. *Echinococcus granulosus* and *E. multilocularis* enjoy wide range of intermediate hosts, including bovines which harbor the larval stages of the parasite in their visceral organs after ingestion of the embryonated eggs (Pleydell et al. 2008; Santa et al. 2018).

For all of the species of *Echinococcus*, human and monkeys serve as aberrant hosts and the disease may take its course from asymptomatic to severe clinical infection, which may lead to death (Eckert and Thompson 2017). Disease pattern of Echinococcosis is usually asymptomatic in livestock and diagnosis is generally made on necropsy findings in the abattoir and it has serious implications in terms of condemnation of the carcasses. In human Cystic Echinococcosis (CE), clinical

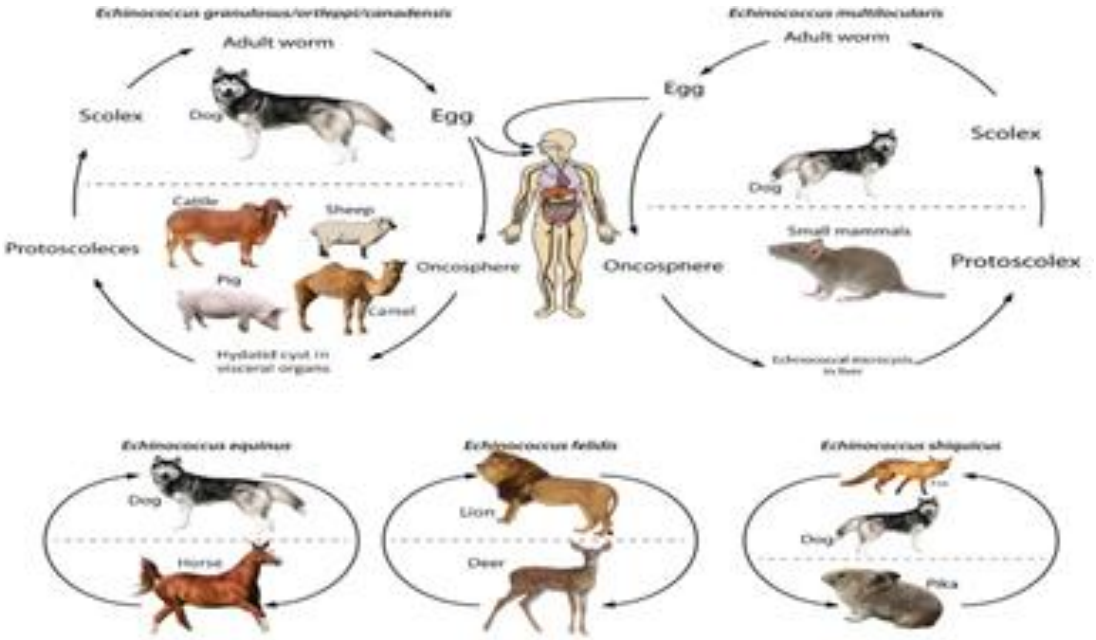

Fig. 1: Overview of life cycle of *Echinococcus* species.

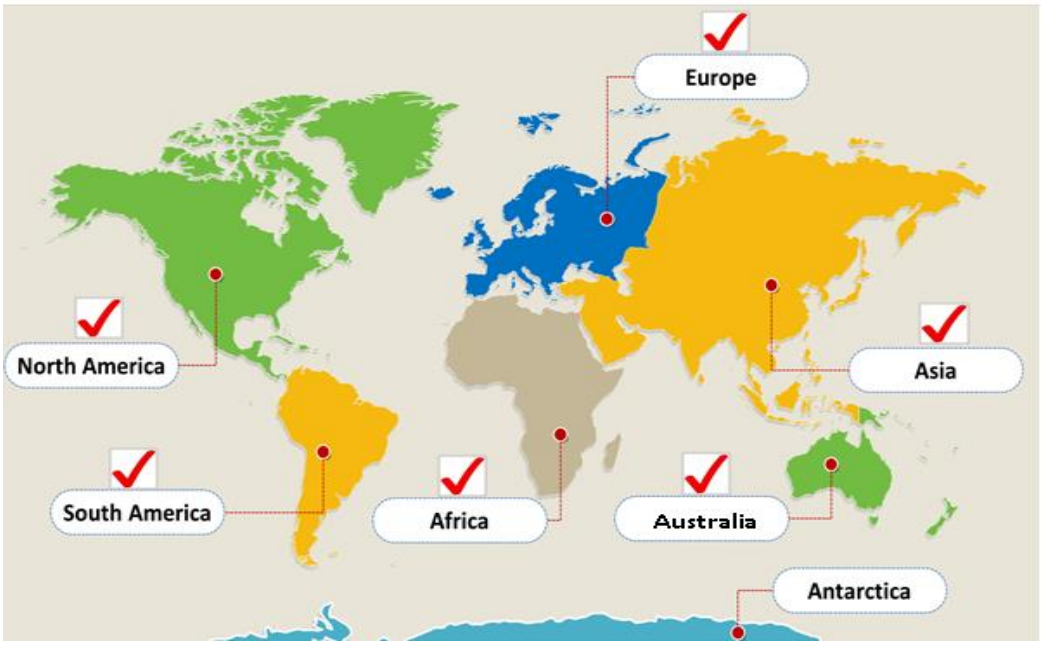

Fig. 2: Cosmopolitan distribution of *Echinococcus* species.

Table 1: Forms of Echinococcosis*

Forms of Echinococcosis	Causative agent	Typical definitive host	Typical intermediate host	Aberrant host
Cystic Echinococcosis	*E. granulosus*	Dog, wolf, jackal and other canids	Sheep, goat, cattle, pig, horse	Human, monkey, and many other mammals
Alveolar Echinococcosis	*E. multilocularis*	Fox, wolf, raccon dog, domestic dog, cat	Small herbivorous mammals and rodents	Human, monkey, dog, pig, horse
Polycystic Echinococcosis	*E. vogeli*	Bush dog, domestic dog	Paca, agouti	Human, monkey
Unicystic Echinococcosis	*E. oligarthrus*	Wild cats	Agouti, Paca, Opossums	Human

*Table derived from Thompson et al. (2017).

signs include asthenia, weight loss, epigastric pain, hepatomegaly and cholestatic jaundice (Brunetti et al. 2010). It may become a fatal disease when the cysts rupture and their fluid contents and protoscoleces are drained into peritoneal cavity, leading to anaphylactic shock (Yang et al. 2015).

Economic thrashes and public health significance

Echinococcosis is a neglected tropical disease of public health concern and has serious economic setbacks. Out of total ten known species of *Echinococcus*, *E. granulosus* and *E. multolocularis* pose significant threats to human

and animal health in addition to substantial economic losses (Torgerson and Macpherson 2011). This infection leads to economic and social thrashed in terms of treatment cost, production loss and mortality in the infected animals and aberrant human host infections. Cosmopolitan distribution of this ailment has led to losses of US dollar (USD) 3.0 billion annually (WHO Echinococcosis Fact Sheet 2017).

According to Ehsan et al. (2017), Echinococcosis has caused annual economic losses of USD 212.35 million in India, USD 232.3 million in Iran and USD 7.708 million in Turkey. It has been estimated that in Chile Cystic Echinococcosis leads to economic losses amounting to USD 14.35 million per year (Rojas et al. 2017). Followed by *Fasciola hepatica*, Cystic Echinococcosis is the most important cause of condemnation of livestock viscera.

Echinococcosis also causes substantial economic losses in Pakistan. It has been estimated that the malady causes losses accounting to USD 276.20 per 100 infected goats and sheep and USD 165.72 per 100 infected large ruminants and camels. Losses occur in terms of poor quantity and quality milk, poor quality wool and meat, retarded growth, reduced fertility and condemnation of carcasses (Latif et al. 2010).

The burden of human Echinococcosis is expressed in terms of disability adjusted life years (DALYs). For Alveolar Echinococcosis, overall global burden of disease was approximated to be 18,200 cases per annum that account up to about 666,000 DALYs (37 DALYs per case) (Torgerson et al. 2010). About 91% of cases and 95% of the DALYs were reported from China and 1600 cases per annum from Europe, Russia and central Asian countries, resulting in 21 DALYs per case. Survival analyses investigations of French and Swiss Alveolar Echinococcosis patients have indicated that modern treatment techniques like resection of liver lesions and extended therapy with benzimidazoles can lead to survival of Alveolar Echinococcosis patients comparable to those of healthy human populations (Torgerson et al. 2008; Piarroux et al. 2011). The regions where treatment choices are available, DALYs burden is moderate because of the better prognosis. For example, in Switzerland, there is a total burden of 3.7 DALYs per case which is 10 times less than that of global estimates (Torgerson et al. 2008). This accounts for one of the most important factors for the dominance of the global burden in China, where the greater part of cases were estimated to come from resource poor communities like the Tibetan plateau. In the central Asian countries, the situation seems to be quite comparable (Torgerson 2017). The most recent global estimate for the Cystic Echinococcosis burden is 188,000 cases per annum, resulting in 0.98 DALYs per case (Torgerson et al. 2015), which is quiet lower than Alveolar Echinococcosis burden, and is due to the lower death rate of Cystic Echinococcosis patients as compared to Alveolar Echinococcosis ones.

Treatment

Treatment of Cystic Echinococcosis cases involves destruction of the metacestode which can be achieved by a number of ways like sterilization of the parasite contents, aspiration of cystic fluid or by surgical removal of the entire cyst (Sayek and Onat 2001; Buttenschoen et al. 2003; Junghanss et al. 2008; Dziri et al. 2009). Surgical procedures are often complicated due to spillage of the cystic fluid into the body cavity, leading to immunological reactions (Brunetti et al. 2010).

To overcome such situations, use of anthelmintics and herbal medications is among other resorts without any risk of side-effects. Some of the medicinal based (chemical, herbal and allopatheic) options to treat Cystic Echinococcosis cases are summarized below.

Treatment with anthelmintics

Mebendazole (MBZ)

In humans, mebendazole (MBZ), an anthelmintic, is only effective against small cysts having size <5 cm (Kern 1983). Long term treatment with MBZ is not well-tolerated by all the patients equally because investigations have shown that 5-40% of patients exhibited adverse effects related to MBZ like hair loss, gastro-intestinal tract (GIT) distress, anaphylactic reaction, headache, hematotoxic effects and altered level of transaminases. Moreover, MBZ can lead to congenital anomalies in the fetus when administered during the first trimester of pregnancy (De Silva et al. 1999). When an epoxy group is introduced to MBZ, two isoforms (M-C1 and M-C2) are obtained, with M-C2 having higher parasiticidal effect than M-C1, as well as simple MBZ (Xu et al. 2019).

Albendazole (ABZ) and their combinations

Initially, MBZ was considered as the first line of treatment of Echinococcosis. But with time, this drug was proved to be less efficacious than albendazole (10 mg/kg/day, maximum 800 mg in 2 doses orally) due to its low solubility in water and poor bioavailability (Davis et al. 1989). Also, the results of a study conducted in murine models have shown that the therapeutic activity of albendazole increases when co-administered with *Zataria multiflora* (herbal product) (Moazeni et al. 2019). In humans, absorption of ABZ shows wide fluctuations and co-administration of ABZ with a fatty meal can enhance its absorption (Anand et al. 2012).

The synergistic effect of ABZ has been reported in recent studies when it is administered in combination with other drugs. The combined treatment of ABZ with metformin appears to be a good option, having admirable results than the therapy done by using ABZ alone (Gorgas et al. 2017). The co-administration of metformin with ABZ has proved effective *in vivo* and *in vitro* (Loos et al. 2015; Loos et al. 2017). Similarly, the combination of ABZ with atovaquone killed protoscoleces of *Echinococcus* within 24 hours, while atovaquone alone took a long time for showing the therapeutic effects against protoscoleces (Kouguchi et al. 2021). The combined treatment of ABZ plus thymol also showed higher efficacy as compared to

that when these drugs were used singly against *E. multilocularis* (Albani et al. 2015).

Recent studies have also shown that there is increased sensitivity of *Echinococcus* protoscoleces to ABZ when given in combination with sodium arsenite. A 100% protoscolicidal effect was seen when 80 µM albendazole and 20 µM sodium arsenite were given in combination (Xing et al. 2019). The nano-crystal formulation of ABZ demonstrated inhibition of cyst 3.7 times higher than that of the albendazole commercial oral product (Albenda) (Hu et al. 2019). When ABZ was loaded into mesoporous material (SBA-15 and SBA-16), the highest drug loading, as well as dissolution, rate was showed by SBA-15 carriers. It enhanced ABZ bioavailability and could be formulated as capsules (Adrover et al. 2020). Lv et al. (2013) conducted an experiment on protoscoleces by using albendazole liposome (L-ABZ), Huaier aqueous extract, and their combination. The results revealed that there was maximum treatment efficacy when L-ABZ and Huaier aqueous extract were given in combination *in vitro,* as well as *in vivo.*

According to Spincher et al. (2008), 2-methoxyestradiol destroyed metacestodes of *E. multilocularis* alone or in combination with ABZ but neither of the drugs had a true killing effect. Both of these drugs showed good results when they were given in combination *in vitro* and also resulted in the reduction of parasite load after 6 weeks *in vivo*. Albandazole 100 µg/ml and povidone iodine (1/10) killed all protoscoleces *in vitro* after 15 minutes (Polat et al. 2009). Tibetan medicine *Elsholtzia eriostachya* in combination with ABZ damaged germinal membrane cells of metacestodes and exhibited good efficacy against secondary *Echinococcus multilocularis* infection in rats (Ji-hai et al. 2020).

The efficacy of ABZ is reported to be higher in the case of the pulmonary or hepatic cysts as compared to that of other anthelmintics. Patients having effective immune status show better response to ABZ than the other patients with CD4+ less than 500 cells/mm^3 (Magdalena et al. 2015). Another study on HIV patients indicated that the continuous treatment with ABZ for more than six months was ineffective and CD4+ count might have been the important factor for poor response to the treatment (Dumitru et al. 2015).

Albendazole is considered to be a safe drug and is well tolerated by the patients; therefore, it is preferred to use ABZ continuously rather than discontinuous therapy. Side effects of ABZ include GIT disturbances, liver toxicity, hair loss, jaundice (less common), cough, itching, and vertigo (Karabulut et al. 2014). In pregnant women, ABZ can lead to teratogenic and embryotoxic effects (Horton, 1989). Age is an important factor that influences outcome of the treatment because drug effectiveness is better in young patients as compared to that in old ones. The site of the cyst also affects the response of infected animal to the treatment; susceptibility of the bone cyst to ABZ is much less as compared to that of alveolar or hepatic cysts (Hemphill et al. 2010; Dumitru et al. 2015).

In the last few years, the development of resistance against ABZ has been widely discussed which considerably restricted cystic echinococcosis treatment. Both miRNA and genetic profile of helminths influence their response to albendazole sulfoxide (Mortezaei et al. 2019). Now-a-days, researchers are making efforts to assemble new ABZ preparations with enhanced organ and tissue penetration to improve the therapeutic effects of the drug (Panwar et al. 2010).

Praziquantel (PZQ) and its combinations

Antiparasitic efficacy of **praziquantel** (PZQ) for cystic echinococcosis is less than that of ABZ. However, it has shown promising results in diffused and incurable cases when it was administered in combination with ABZ (Jamshidi et al. 2008; Bygott et al. 2009). It has also been proved that PZQ becomes more effective when co-administered with ABZ. In combination, the PZQ is given once a week with a dose rate of 40 mg/kg with 800 mg/day albendazole, both before PAIR (puncture, aspiration, injection and reaspiration)/ surgical procedure and in inoperable cases (Jamshidi et al. 2008; Jiang et al. 2017). Jelowdar et al. (2017) evaluated the effect of ABZ and PZQ loaded solid lipid nanoparticles on Cystic Echinococcosis and found that their efficacy was more than that of the pure ABZ and PZQ.

Other anthelmintics

Richter et al. (2013) investigated the efficacy of triclabendazole and clorsulon on *E. multilocularis* larvae and found that maximum damage was caused by triclabendazole at a concentration of 20 µg/ml within 12 days and at 25 µg/ml within 13 days; triclabendazole sulfoxide showed the highest efficacy at concentrations of 20 and 25 µg/ml within 20 and 14 days, respectively. On the other hand, clorsulon showed no effects on vesicles at 5, 10, and 15 µg/ml concentrations in the *in vitro* culture.

According to Hokelek et al. (2002), ivermectin, a nematocidal drug, was effective against scoleces when it was injected into cysts and cyst size was significantly decreased. Ahmadpour et al. (2019) observed that nano lipid carrier combined with ivermectin brought about 100% death of cestodes after 1 and 2 hrs of administration at 800 µg/ml and 400 µg/ml concentration, respectively and was also responsible for the augmented expression of mRNA caspase 3 that showed its strong apoptotic activity against the parasite.

Insecticides

Lufenuron

Lufenuron (benzylphenylurea) is an inhibitor of insect growth and is commonly used to control fleas on companion animals. When it was used against *E. granulosus* hydatid cyst in mice, a 20-30% reduction in cyst size was observed which indicates that it can be used to augment the scolicidal activity of ABZ (Breijo et al. 2011).

Fluralaner

The drug fluralaner is used to control canine ectoparasites like mites, fleas and ticks. L-glutamate gated channels and GABA-gated chloride channels are potently inhibited by this drug. Studies have revealed that the activation of caspase 3, which is an indicator of apoptosis, was prominent after treatment with fluralaner. Treatment with this drug also causes damage to metacestode layers and protoscoleces, which shows that fluralaner can be effective for the treatment of Echinococcosis (Zahran et al. 2020).

Antiprotozoals

Nitazoxanide

Nitazoxanide kills all vesicles *in vitro* culture. It causes disintegration of the vesicles at a high dose of 10 µg/ml after 7 days and its effects are rapid than those of ABZ. Nitazoxanide, itraconazole and methiazole possess parasitostatic effects, while combination of ABZ with nitazoxanide shows parasitocidal effects *in vitro* (Reuter et al. 2006).

Buparvaquone

Buparvaquone is an anti-theilerial drug, with cytotoxic effects (Xing et al. 2019). This drug damages mitochondria of *in vitro* cultured *E. multilocularis* metacestodes and might be an effective choice for the treatment of patients with Cystic and Alveolar Echinococcosis (Rufener et al. 2018).

Anti-malarial drugs

Mefloquine is another drug with antiparasitic efficacy and is used in the treatment of malaria caused by *Plasmodium* species and has proven to have efficacy against metacestodes of *E. multilocularis* (Stadelmann et al. 2011; Küster et al. 2011; Küster et al. 2015; Rufener et al. 2018; Lundström-Stadelmann et al. 2020). Similarly, atovaquone (ATV) is another antimalarial drug and enzymatic analysis has shown its inhibitory action on mitochondrial complex III. Culture experiments have revealed the activity of ATV under aerobic condition, but not under anaerobic condition, against protoscoleces. The combination of ATV with atpenin completely destroys protoscoleces in culture, whereas *in vivo* experiments also demonstrated a significant reduction in cyst formation in mouse liver after oral drug administration (Enkai et al. 2020). In a recent study, Enkai et al. (2021) treated protoscoleces of *E. multilocularis* with atovaquone, praziquantel, rotenone, artemisinin and pyrvinium pamoate in different media culture at a concentration of 50 µM, and observed that the co-administration of artemisinin or praziquantel with ATV completely eliminated the protoscoleces. Li et al. (2020) used pyronaridine, an anti-plasmodium drug, against Cystic Echinococcosis and suggested that intraperitoneal injection with pyronaridine at the dose rate of 57 mg/kg, once a day for 3 days induced 100% cyst inhibition. When it was given orally with the same dose rate q.d. for 30 days, there was a significant reduction in number of parasites in experimentally infected mice as compared with the ABZ treated group.

The use of dihydroartemisinin, another anti-malarial drug, resulted in the morphological changes and loss of viability of protoscoleces. It induced apoptosis by endoplasmic stress-caspase3 pathway *in vitro* (Ma et al. 2020). Artesunate is responsible for damaging the DNA of metacestodes and protoscoleces of *E. granulosus* and this damage is provoked by oxidative stress (Wen et al. 2020). Li et al. (2020) reported that the combined effect of veliparib and artesunate at a higher dose (325 µM) on cysts of *E. granulosus* showed reasonably high scolicidal activity *in vitro* and *in vivo*.

Plant Extracts

Eskandarian (2012) evaluated the efficacy of hydroalcoholic and chloroformic extracts of squash seeds, hazelnuts, and garlic and observed that among these plant extracts the chloroformic extract of garlic had the highest potency (98%) against protoscoleces at a concentration of 50 mg/ml after 20 min of exposure. For *Allium sativum* (garlic), ultrasonic flower extract showed a potent scolicidal activity at concentration of 100 mg/ml at different time intervals (Rahimi-Esboei et al. 2016). In another study, Barzin et al. (2019) found that garlic chloroformic extract possessed a high protoscolicidal effect. The effectiveness of garlic extract was also compared with silver nitrate and sodium chloride at one and two minutes of exposure and it was found that the anti-protoscoleces effect of garlic was higher than that of other chemicals, whereas no difference was noticed between the garlic and sodium chloride effects when the exposure time was increased to 5 minutes.

According to No et al. (2016), ethanolic extract of *Zingiber officinale* (ginger) exhibited a strong activity against hydatid disease after 20 and 10 minutes at concentrations of 30 mg/ml and 50 mg/ml, respectively. Significant scolicidal effect of ginger and eucalyptus (Blue gum) was reported by Faizei et al. (2015); the methanolic extract of ginger and eucalyptus at the concentration of 100 mg/ml killed 100% protoscolices after 40 min of exposure. Ginger extract exhibits high scolicidal activity *in vitro;* the efficacy was 100% at a concentration of 200 mg/ml after half an hour of exposure (Houshmand et al. 2019).

El-Bahy et al. (2019) conducted a study on protoscoleces of camel hydatid cysts by using *Nigella sativa* (black cumin) and *Punica granatum* (pomegranate) extracts. The highest scolicidal activity of *Nigella sativa* was noted at concentrations of 100 mg/ml and 10 mg/ml after 30 and 60 minutes exposure, respectively, while *P. granatum* showed its highest efficacy at a concentration of 100 mg/ml after 120 minutes of exposure. Essential oil of *Curcuma zadoaria* (white turmeric) showed remarkably high scolicidal activity against protoscoleces at 300 and 150 µl/ml concentration and could eliminate parasites

after exposure of 5 and 10 minutes, respectively *in vitro* (Mahmoudvand et al. 2020). Similarly, essential oil of *Cinnamomum zeylanicum* (true cinnamon) and cinnamaldehyde exhibited dose and time-dependent efficacy against protoscoleces, with the maximum efficacy was found at 50 µg/ml of cinnamaldehyde. The viability of protoscoleces decreased after 4 days of treatment and reached 0% on the 8th day (Fabbri et al. 2020).

The methanolic extract of *Myrtus communis* (myrtle) and *Tripleurospermum disciforme* (mayweed) have been found to exhibit scolicidal effects (Barzin et al. 2019). Both of these plant extracts can be given during cyst surgery, as they also inhibit secondary bacterial infections. The maximum scolicidal effects could be achieved at concentrations of 100 mg/ml and 50 mg/ml of *Myrtus communis*. The type and nature of active ingredients present in these extracts is not yet clear (Amiri et al. 2019). Moreover, the combination of pomegranate extract and albendazole possesses anti-inflammatory and anti-hydating effects which help in preventing relapse (Labsi et al. 2019).

Yuan et al. (2019) have reported that anacardic acid, a product obtained from Brazilian cashew nut shell liquid, has higher efficacy than albendazole and dihydroartemisinin against *E. multilocularis* and *E. granulosus* sensu stricto metacestodes *in vivo* and *in vitro*. Ampelopsin, which is extracted from *Ampelopsis grossedentata* (moyeam), has been used for the treatment of different types of diseases like cancer. It also indicated profound efficacy against protoscoleces of *E. granulosus* and metacestodes of *E. multilocularis in vitro* (Xin et al. 2019). *Ferula gummosa* (galbanum) and *Pelargonium roseum* (geranium) oils have activity against scolexes with no side effects (Tabari et al. 2019).

Moudgil et al. (2020) compared the protoscolicidal effect of methanolic extract of three herbs *Ferula asafetida* (dried latex), *Trachyspermum ammi* (fruits), and *Hippophae salicifolia* (leaves) for 20, 40 and 60 minutes interval at different concentrations with ABZ and the extracts of two herbs (*F. asafetida* and *T. ammi*) showed comparable or better results than those of *H. salicifolia* extract. In a recent study, Ranjbar et al. (2020) investigated the scolicidal activity of extract of *Mentha aquatic* (water mint), *M. spicata* (spear mint), *M. longifolia* (Asian mint) and *M. piperita* (peppermint). The results revealed that the activity of methanol extract of *Mentha aquatic* was the highest (99.54%) half an hour after application.

Pestalotiopsis sp. (plant pathogen), which is an endophytic fungus sp. from the Neem plant, showed 97% scolicidal activity due to its bioactive compound responsible for damaging protoscoleces of *E. granulosus* (Verma et al. 2013). Another study performed by Verma et al. (2014) revealed changes in ultrastructures of protoscoleces following their expoxure to an endophytic extract of *Eupencillium* and *Chaetomium* fungi.

Metabolite of *Piper longum* (Pippali) disrupted the ultrastructure of hydatid cysts and its effectiveness was highest in 50 mg/ml within one hour of exposure to the extract (Cheraghipour et al. 2020). Trans retinoic acid was used against protoscoleces at different concentrations including 1.67 and 0.167 µM, and 16.7 nM/L and it was observed that the death of protoscoleces was dose-dependent and occurred within a few minutes to seven days of exposure. The ultrastructural changes observed were disorganization of rosteller, changes in hook shape, and loss of hook (Yones et al. 2014). When protoscoleces were exposed to essential oils of *Pinus nigra* subsp. *pallasiana* (lamb), 100% deaths of protoscoleces were recorded after 60 min of exposure at a concentration of 50 mg/ml (Kozan et al. 2019). Moazeni et al. (2019) carried out a study on live protoscoleces of sheep liver and reported that essential oil (1%) of *Eucalyptus globulus* (blue gum), povidone-iodine (10%), and silver nitrate (0.5%) showed 100% protoscolicidal activity.

Vakili et al. (2019) exposed *E. granulosus* protoscoleces to three concentrations of extract of *Artemisia sieberi* (white wormwood) and observed that the mortality at different time intervals (2, 5 and 10 minutes) with concentration of 75 mg/ml was 80.0, 78.0, and 86.4%, respectively. *Ziziphora tenuior* (kohi purchink) extract killed protoscoleces in 20 minutes at a concentration of 10 mg/ml. By increasing the concentration of the extract, time of exposure was decreased to achieve the desired effects (Shahnazi et al. 2016).

According to Larki et al. (2017), gallic acid at 35 mg/ml concentration resulted in 92.08 and 100% mortality of protoscoleces after 1 and 3 minutes of exposure, respectively. Berberine, which is an active compound obtained from the root extract of *Berberis vulgaris* (barberry), showed 100% inhibition of protoscoleces at 2 mg/ml concentration after 10 minutes exposure (Mahmoudvand et al. 2014). Scolicidal effect of aqueous extract of *B. vulgaris* was observed at a low concentration of 4 mg/ml after 5 minutes (Rouhani et al. 2013). Similarly, carvacrol, which is the main chemical constituent obtained from *Satureja khuzistanica* (Marzeh Khuzestani), has shown 100% scolicidal effect at 10 mg/ml concentration after 10 minutes exposure *in vitro* (Moazeni et al. 2012). When protoscoleces and cysts of *Echinococcus* were treated with carvacrol, maximum scolicidal activity was observed at a concentration of 10 µg/ml and reduction in cyst weight was also observed at a concentration of 40 mg/kg 20 days after treatment in mice (Fabbri et al. 2016).

Thymoquinone is a principal active scolicidal agent found in *Nigella sativa* extract and found to have potent activity against protoscoleces at 1 mg/ml concentration after 10 minutes exposure *in vitro* (Mahmoudvand et al. 2014). Similarly, thymol, gamma-terpinene and p-cymene constitute 50.07, 23.92, and 22.9% of ajowan *(Trachyspermum ammi*) essential oil, and showed the highest scolicidal activity at a concentration of 10 mg/ml after 10 minutes exposure (Moazeni et al. 2012). An experiment conducted by Bahrami et al. (2016) showed that essential oil of *Lepidium sativum* (gardencress) possessed a high level of activity against scoleces.

According to Haleem et al. (2019), trials on experimental animals revealed that plants like *B. wallichiana* (zeresk),

B. vulgaris (barberry), and *E. heliscopia* (umbrella milkweed) are effective against protoscoleces of *E. granulosus* and could be used as a treatment. Similarly, Norouzi et al. (2021) indicated that hydroalcoholic extract of *Taxus baccata* L. (common yew) possessed scolicidal properties and killed 66.6% protoscoleces after 60 minutes exposure at a concentration of 150 mg/ml *in vitro*. Yazdi et al. (2020) found solicidal activity of *Zataria multiflora* (shirazi Thyme) essential oil nanoemulsion and emulsion at different concentrations (1, 2, 5, 10, 15 and 20 µl/ml). Emulsion at a concentration of 20 µl/ml for 15 minutes and nanoemulsion at the same concentration for 10 minutes resulted in 100% mortality of protoscoleces. Protoscoleces were killed by 100% when *Thymus capitatus* (zaatar) essential oil was used at concentrations of 2 and 3 mg/ml after 5- and 1-min exposure, respectively (Hizem et al. 2020).

Methanolic extract of *Rhus coriaria* (sumac) has 98.89 and 100% efficacy against scoleces at a concentration of 30 mg/ml after 10 and 20 minutes, respectively (Moazeni et al. 2012). Youssefi et al. (2020) studied the effect of important phytoconstituents including isofuranodiene, a-bisabolol and farnisol on scoleces and concluded that isofuranodiene possessed the highest activity against protoscoleces, followed by a-bisabolol and farnisol. Osthole, a coumarin derivative from medicinal plant, eliminated protoscoleces by 100% with 120 µM concentration within 3 days *in vitro*. When three infected groups of mice were treated with osthole (100 mg/kg), ABZ (100 mg/kg), and honey/PBS (100 mg/kg) daily for 6 weeks, there was a significant reduction in metacestodes in osthole and ABZ treated groups compared to the control group (Yuan et al. 2016).

Gholami et al. (2013) assessed the effect of methanolic extract of *Sambucus ebulus* (danewort). They used four concentrations of extract 1%, 10%, 50%, and 100% with 5, 10, 30, and 60 minutes of exposure and found highest scolicidal activity *in vitro* after 60 minutes of exposure. The extract of *Satureja khuzestanica* (Marzeh Khuzestani) and *Olea europaea* (olive) leaves showed scolicidal effects on protoscoleces of hydatid cysts, however, the extract of *S. khuzestanica* showed better effect than that of the *O. europaea* (Zibaei et al. 2012). Protoscoleces of *E. granulosus* can also be killed by using propolis (resinous material) at a concentration of 1 µg/ml for 3 minutes *in vitro*; it has no side effects when used intraperitonially (Kismet et al. 2006).

In another study, Jasim et al. (2020) evaluated the bioactivity of leaf extract of *Lepidium sativum* (gardencress) on scoleces of sheep origin and showed that it caused 100% mortality at a concentration of 100 mg/ml after 15 minutes of exposure. Methanolic extract of *Sideritis perfoliata* (cyprus) also showed scolicidal activity (57.9, 71.8, and 79.1%) on protoscoleces of *E. granulosus* at a concentration of 0.4 mg/ml after 10, 20, and 30 minutes, respectively which indicates that scolicidal activity is increased by increasing the exposure time (Çelik et al. 2021). Derakhshan et al. (2017) evaluated the scolicidal activity of *Bunium persicum* (boiss). The rate of dead protoscoleces was 100% at 15 mg/ml concentration after 10-60 minutes exposure *in vitro*. *Tordylium persicum* and fruit of *Citrullus colocynthis* (bitter apple) possessed scolicidal activity in a dose-dependent manner (Sharifi-Rad et al. 2016; Hussein et al. 2019). Similarly, the methanolic extract of *Hymenocarter longiflorus* (Lamiaceae) showed larvicidal effects against *E. granulosus* (Taran et al. 2013).

Anti-fungal drugs

Amphotericin B, an antifungal agent, has been proved to have effective activity *in vitro* against *E. multilocularis* at 2.7 µM concentration (Reuter et al. 2003a; Reuter et al. 2003b; Reuter et al. 2010). As salvage therapy in human patients, amphotericin B has activity against Alveolar Echinococcosis (Reuter et al. 2010) but it is not commonly used due to its nephrotoxic effects (Reuter et al. 2003a; Reuter et al. 2003b).

Anti-cancerous drugs

BI2536, originally designed to inhibit the human ortholog of EmPlk1, can effectively inactivate germinative cells of *E multilocularis* larvae *in vitro* by inactivation of *E. multilocularis* EmPlk1 protein and can be considered a promising compound in the treatment of Alveolar Echinococcosis cases. Direct inhibition of EmPlk1 provokes mitotic arrest and killing of germinative cells (Schubert et al. 2014).

Hemer et al. (2012) revealed the scolicidal effect of anti-cancerous imatinib and it was found to be highly active in killing stem cells, protoscoleces, and metacestode vesicle of *Echinococcus* at a concentration of 25 µM *in vitro*. Stadelmann et al. (2014) suggested that bortezomib, another anti-cancerous drug, had anti-metacestode activity *in vitro* due to inhibition of proteasome of cestodes. Tamoxifen, a non-steroidal antioestrogen, represents a significant advance in treatment of female breast cancer; it may also be used in human *Echinococcus* treatment. The reduction in cyst weight of *E. granulosus* was observed after 3 or 6 months of treatment with 20 mg/kg concentration in mice (Nicolao et al. 2014). Similarly, pyrvinium pamoate completely destroyed the protoscoleces after 5 days under aerobic and 7 days under anaerobic conditions (Enkai et al. 2020).

According to Xin et al. (2020), lonidamine and 6-aminonicotinamide showed remarkable effects against both adult and larval stage of *E. granulosus* and *E. multilocularis in vitro*, and combined drug treatment revealed significantly higher efficacy than the single drug treatment. *E multilocularis* MPK2 activity can be inhibited by ML3403 and SB202190, which are inhibitors of p38MAPKs, cause EmMPK2 dephosphorylation and effectively damage vesicles of parasite at concentrations that do not harm mammalian cells in tissue culture (Gelmedin *et al.* 2008). In another study, the anti-echinococcosis effect of SB202190 was evaluated by using different concentrations (10, 20, 40, and 80 µM) and results indicated dose-dependent death of scoleces *in vitro* (Lv et al. 2013). Egp38, a 368 amino acid MAPK

protein was identified in *E. granulosus* and when protoscoleces were treated with p38- MAPK inhibitor ML3403 *in vitro*, it suppressed Egp38 activity, leading to protoscoleces mortality within 5 days, hence, MAPK Epg38 is believed as a target site for anti-cystic echinococcosis drugs (Lü et al. 2016).

Nano Particles

Mahmoudvand et al. (2014) evaluated the scolicidal efficacy of selenium nanoparticles (SeNPs) prepared from *Bacillus* species Msh-1 and revealed that SeNPs showed scolicidal activity at different concentrations, particularly at 250 and 500 µg/ml, after 20 and 10 minutes exposure, respectively. According to Rahimi et al. (2015), different silver nanoparticle have been used in different concentrations (0.025, 0.05, 0.1 and 0.15 mg/ml) and concentration of 0.15 mg/ml after 2 hours exposure has shown 90% activity against protoscoleces. Another study on the same drug found that the drug was less effective against protoscoleces and eliminated only 71.6% protoscoleces in 1 hour at a concentration of 4 mg/ml, whereas hypertonic saline (20%) eliminated 100% protoscoleces in 10 minutes (Lashkarizadeh et al. 2015).
Nassaf et al. (2019) reported that ABZ-loaded silver nanoparticles showed the highest activity when compared with silver nanoparticles and ABZ alone. There was a marked decrease in the weight of the cyst and size of granuloma with albendazole-loaded silver nanoparticles. In another study conducted by Norouzi et al. (2020), the scolicidal activity of silver (Ag), silicon (Si), copper (Cu), iron (Fe), and zinc (Zn) nanoparticles was compared. Results revealed that Ag-NPs had the strongest protoscolicidal effect (80%) at 1 mg/ml after 1 hour of treatment. Furthermore, Si-NPs at concentration of 1 mg/ml, Cu-NPs at concentration of 0.5mg/ml, Fe-NPs at concentration of 1 mg/ml and Zn-NPs at concentration of 1 mg/ml exhibited 52.33, 41, 28 and 15.67% scolicidal effects, respectively, after 1 hour exposure *in vitro*.
Cerium dioxide and *Holothuria leucospilota* are also found to show effective scolicidal activity when used separately or in combination. Studies have shown that *H. leucospilota* extract showed 70% scolocidal effect at a concentration of 20 mg/ml after 1 hour of exposure, followed by the combination (63%) at a concentration of 15 mg/ml after the same time (Aryamand et al. 2019). Ibrahim et al. (2020) evaluated zirconium dioxide nanoparticles at 1000, 2000, and 4000 µg/ml concentration. After 1 hour, it damaged 49.6, 52.7, and 53.1% of scoleces. Napooni et al. (2019) found that gold nanoparticles possessed the strongest scolicidal effect at a concentration of 4000 µg/ml after 1 hour of treatment. According to Barabadi et al. (2017), gold nanoparticles, formed by a green process through utilizing *Penicillium aculeatum,* showed potent scolicidal activity. Different concentrations (0.05, 0.10, 0.20 and 0.30 mg/ml) were used for 10, 30, 60 and 120 minutes and variation between scolicidal effects was significant statistically at different concentrations and different time intervals. Gold nanoparticles effectively killed protoscoleces at a concentration of 1 mg/ml after 60 minutes (Malekifard, 2017).
The highest scolicidal activity of copper nanoparticles is reported at a concentration of 750 mg/ml *in vitro* and copper nanoparticles killed 73.3% protoscoleces after 60 minutes of treatment. The maximum mortality is reported when copper nanoparticles are given along with ABZ at a concentration of 750 mg/ml and 200 mg/ml, respectively for 10 minutes exposure time (Ezzatkhah et al. 2021). Silver-copper nanoparticles (core-shell) have a dramatic adverse effect on the vitality of protoscoleces compared with ABZ. Even the lowest concentration of 50 mg/ml showed a significant effect. The killing rate was 100% at the concentration of 500 mg/ml of nanoparticles (Aljanabi et al. 2021).
In a recent study, Navvabi et al. (2019) indicated that the scolicidal effect was 84% when gonad extract of sea urchin was used in combination with titanium dioxide at 15 µg/ml concentration for 60 minutes exposure time *in vitro*. When gonad extract and titanium dioxide nanoparticles were given orally in infected mice for three months, significant reductions in size, volume and weight of hydatid cysts were observed. Napooni et al. (2019) suggested that nano-particles of chitosan having curcumin (Ch-Cu NPs) could be regarded as an anti-protoscolex drug that showed good efficacy against *E granulosus*.

Chemical solutions

Hypertonic saline was evaluated by Adas et al. (2009) for its scolicidal activity. A comparison of effectiveness of hypertonic saline with ABZ sulfone and ABZ sulfoxide combinations was also made. The results showed that hypertonic saline possessed similar efficacy as shown by ABZ combinations used in the comparative study. Hosseini et al. (2006) also conducted a comparative study to evaluate the activity of hypertonic glucose with silver nitrate (0.5%), cetrimide (0.5%), and hypertonic saline (20%) against protoscolices of *Echinococcus*. It was observed that 50% hypertonic glucose had more scolicidal activity than 0.5% silver nitrate but fewer efficacies were found when compared with cetrimide (0.5%).
Honey, which has antibacterial activity as well, was used by Kilicoglu et al. (2006) in order to determine its effects on protoscoleces. Different concentrations (1, 5, 10, 25, and 50%) of honey were used for the treatment of protoscolices *in vitro*. All protoscoleces were killed at 10% or higher concentrations, indicating anthelmintic potential of honey.
Topcu et al. (2009) studied the effect of chlorhexidine gluconate on hydatid cyst during surgery of a patient suffering from Cystic Echinococcosis. It was found that all protoscoleces were killed with 0.04% chlorhexidine-gluconate solution after 5 minutes of intra-cystic injection. In another study, commercial chitosan was found to show high level of deacetylation and was proven to have anti-scoleces activity *in vitro*. It was also noted that chitosan from fungus could also be used as effective as commercial chitosan for the treatment of hydatidosis (Rahimi-Esboei et al. 2013).

Zeghir-Bouteldja et al. (2009) studied the effect of peroxynitrite, nitrite, and nitrate on the viability of protoscoleces. Peroxynitrite and nitrite damaged the germinal layers after 24hr and 3hr when used at the concentrations of 320 and 80 µM, respectively. Kuster et al. (2013) suggested that dicationic diguanidino
drugs having thiophene core groups were effective against metacestodes of *E. multilocularis*.
Auranofin (MMV688978) is a thioredoxin-glutathione reductase inhibitor, and shows anti-protoscoleces activity against metacestodes of *E. multilocularis*, and *E. granulosus* (Bonilla et al. 2008; Ross et al. 2012). Good efficacy was reported after 48 hrs of administration *in-vitro* (Saiz et al. 2014; Salinas et al. 2017).
In another study, Aydin et al. (2012) exposed Echinococcocus protoscoleces to taurolidine in petri plates. All protoscoleces were killed in 90 minutes. Moreover, administration of taurolidine *in vivo* also showed significant results. Ekçi et al. (2010) reported that when mice were treated with polyvinylprolidone iodine (1%) and taurolidine (2%) for 2 and 5 minutes, polyvinylprolidone seemed to have strong activity as compared with taurolidine. According to Abdulkareem et al. (2020), ozonated saline solution has the potential to kill scoleces *in vitro* (100%) and can be used during hydatid cyst surgery *in vivo* without any fear of toxicity because ozone is converted into oxygen afterwards.

Other options

The venom of *Androctonus crassicauda* (scorpion) was used against protoscoleces and the efficacy was found to be 100% at a concentration of 100 µg/ml after 240 minutes of exposure (Al-Malki et al. 2020). Likewise, octenidine dihydrochloride (used for skin, mucous membrane and wound antisepsis) has been found to have strong efficacy against scoleces at a concentration of 0.1% *in vitro* after 15 minutes (Ciftci et al. 2007).
Treatment with interleukin IL-17A at various concentrations including 100, 125 and 150 pg/mL *in vivo* stopped metacestode growth by 72.3, 93.8, and 96.9%, respectively (Labsi et al. 2018). Metformin, which is an antihyperglycemic and antiproliferative agent, induced dose-dependent killing of stem cells and protoscoleces *in vitro*. When it was given orally at a concentration of 50 mg/kg per day for 8 weeks, it caused a significant reduction in parasite weight and suppression of Em-TOR in Alveolar Echinococcosis models (Loos et al. 2020). Shi et al. (2016) treated protoscoleces with chenodeoxycholic acid at different concentrations (500, 1000, 2000, and 3000

Compound	Disease	Experiment Setting	Dosage	Treatment Duration	Efficiency Assessment	References
Albendazole + Sodium Arsenate	CE	In vitro	80µM +20µM	48 hr	100%	Xing et al., 2019
Albendazole + povidone Iodine	CE	In vitro	100 µg/ml + 1/10	15 min	100%	Polat et al., 2009
Praziquantel + Albendazole	CE	In vivo	40mg/kg + 800mg/day	Once a week	Significant	Jamshidi et al., 2008
Triclabendazole	CE	In vitro	25 µg/ml	13 days	Significant	Richter et al., 2013.
Ivermectin Nano-lipid carrier	CE	In vitro	800 µg/ml	1 hr	100%	Ahmadpour et al., 2019
Nitazoxanide	CE	In vitro	10 µg/ml	7 days	Significant	Reuter et al., 2006
Artemisinin or Praziquantel + Atovaquone	CE	In vitro	50 µM	7 days	Significant	Enkai et al., 2021
Pyronaridine	CE	In vivo	57 mg/kg (IP)	3 days	100%	Li et al., 2020
Imatinib	CE	In vitro	25 µM	7 days	Significant	Hemer et al., 2012
Tamoxifen	CE	In vivo	20 mg/kg	3 months	Significant	Nicolao et al., 2014
Pyrvinium pamoate	CE	In vitro	50 µM	5 days (Aerobic)	100%	Enkai et al., 2021
SB202190	CE	In vitro	80 µM	4 days	95%	Lv et al., 2013
Selenium nanoparticles	CE	In vitro	500 µg/ml	10 min	Significant	Mahmoudvand et al., 2014
Silver nanoparticle	CE	In vitro	0.15mg/ml	2 hr	90%	Rahimi et al., 2015
Hypertonic Saline	CE	In vitro	20%	10 min	100%	Lashkarizadeh et al., 2015
Gold nanoparticles	CE	In vitro	4000 µg/ml	1 hr	Significant	Napooni et al., 2019
Copper nanoparticles + Albendazole	CE	In vitro	750mg/ml + 200mg/ml	10 min	Significant	Ezzatkhah et al., 2021
Gonad extract of Sea Urchin + Titanium dioxide	CE	In vitro	15 µg/ml	60 min	84%	Navvabi et al., 2019
Garlic chloroformic extract	CE	In vitro	50 mg/ml	20 min	98%	Eskandarian 2012
Piper longum extract	CE	In vitro	50 mg/ml	1 hr	Significant	Cheraghipour et al., 2020
Eucalyptus extract	CE	In vitro	100 mg/ml	40 min	100%	Faizei et al., 2015
Carvacrol	CE	In vitro	10 mg/ml	10 min	Significant	Moazeni et al., 2012
Thymoquinone	CE	In vitro	1 mg/ml	10 min	Significant	Mahmoudvand et al., 2014
Curcuma zadoaria essential oil	CE	In vitro	300 mg/ml	5 min	Significant	Mahmoudvand et al., 2020
Thymus capitatus essential oil	CE	In vitro	3 mg/ml	1 min	100%	Hizem et al., 2020
Androctonus crassicauda (Scorpion)	CE	In vitro	100 µg/ml	240 min	100%	Al-Malki et al., 2020
Metformin	AE	In vitro	50 mg/kg	8 weeks	Significant	Loos et al. 2020
FBG	CE	In vitro In vivo	20%	8 hr	98%	Rostami et al., 2015

µmol/L) and recorded mortality of protoscoleces and changes in morphology like loss of hooks, microtriches destruction, stroma region contraction, blebs, and lipid droplets formation. Fluoride containing bioactive glass (FBG) is a novel scolicidal agent used to prevent post-surgical infections particularly of hydatidosis. FBG damaged 98% protoscoleces with 20% fluoride after 8 hours of treatment. By increasing the concentration of bioactive glass and fluoride ratio, scolicidal activity was also increased (Rostami et al. 2015). A recent study (Wang et al. 2021) demonstrated the effect of galactosidases on metacestodes of *E. multilocularis in vitro* and revealed that the metacestode vesicle of the parasite was unable to infect mice after glycan digestion induced by galactosidases.

Knocking down of EgRad54 gene and treatment combined with harmine and harmine derivatives, DNA damage of *E. granulosus* was enhanced through down-regulation of Topo2a and Rad54 and up-regulation of H2A and ATM which eventually inhibited growth of *E. granulosus* (Gong et al. 2021). Signaling inhibitors, including CI-1033, U0126 and BIBW2992, showed activity against protoscoleces and metacestodes of *E. multilocularis in vitro* by changing the ultrastructure of metacestode. CI-1033 and BIBW2992 caused apoptosis of metacestodes and germinal cells. U0126 and BIBW2992 exhibited efficacy against *E. multilocularis* (Cheng et al. 2020). Among the seven mTOR inhibitors, the tacrolimus (TAC) was found to be more effective than other inhibitors against hydatid cyst *in vitro* and also there was an effective reduction in weight and number of the parasitic cyst at a concentration of 4 mg/kg/day *in vivo* (Muhedier et al. 2020). Chen et al. (2020) studied the effect of hMASP-2 DNA-nanolipoplexes against Cystic Echinococcosis and reported that they induced degradation of germinal layer cells and up-regulated T-cell immunity in mice, therefore, could be used as an alternate for Cystic Echinococcosis treatment.

Conclusion

A huge research work on treatment protocols for Cystic Echinococcosis has been done *in vitro*. As the location of the cyst, its size and type determine the response to treatment, there is a dire need of conducting investigations on standardization and drug therapy duration *in vivo*. Studies have explored that some drugs have abilities to kill the parasite *in vivo* and more research is necessary to discover new drugs/chemical agents which have high anti-*Echinococcus* activity *in vivo* for the treatment of clinically ill patients.

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SARCOCYSTOSIS IN MEAT-PRODUCING ANIMALS: AN UPDATING ON THE MOLECULAR CHARACTERIZATION

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INTRODUCTION

Sarcocystosis is a globally distributed zoonotic protozoan disease that infects a wide variety of mammals, reptiles, and birds. This disease is caused by *Sarcocystis* species, which are a coccidian intracellular protozoan parasite of the genus *Sarcocystis* that belongs to the family Sarcocystidae of the phylum Apicomplexa (Fayer et al. 2015). About 200 valid species of *Sarcocystis* have been identified, which vary in their pathogenicity to the host, ranging from avirulent to highly virulent; some species are zoonotic; however, complete life cycles are known only for 26 species (Dubey 2015; Dubey et al. 2016).
This parasite has an obligatory two-host life cycle (prey and predator) known as intermediate and definite hosts. The asexual part of its life cycle is completed in the intermediate host, which is usually a herbivore animal. At the same time, sexual stages of its development occur in the definite or final host, a carnivore or omnivore animal (Lindsay and Dubey 2020). *Sarcocystis* is non-pathogenic in the definite host, and several species are also non-pathogenic in the intermediate hosts. In general, species transmitted by the canid's definite host are pathogenic, while those transmitted by the felid's definite host are non-pathogenic (Lindsay and Dubey 2020).

Human infection with Sarcocystis spp. is relatively rare. Still, humans serve as definite or intermediate hosts for this parasite. They become the final host by consuming under-cooked beef or pork meat, infected with *S. homonis* and *S. suihominis*, respectively. Symptoms of human intestinal *Sarcocystis* infection include nausea, abdominal discomfort, stomach ache, and diarrhea (Fayer et al. 2015). Humans can become an intermediate or aberrant host for seven or more *Sarcocystis* species, including *S. nesbitti*, which was identified in humans through18SrDNA sequence analysis. *S. nesbitti* infection is considered as a new zoonotic disease caused by the ingestion of food or water contaminated with this *Sarcocystis* species. Moreover, *S. heydorni,* which mostly infects cattle, is also considered as a zoonotic protozoan (Dubey 2015; Dubey et al. 2016). Infection with *Sarcocystis* protozoan in the intermediate host can lead to morbidity, mortality, abortions, decreased meat production, and increased meat condemnation due to presence of macrosarcocysts. Up to now, the importance of human sarcocystosis has failed to gain necessary attention regardless of the potential for muscular or intestinal Sarcocystis infection to infect a considerable number of people (Fayer 2004; Tappe et al. 2013).
Sarcocysts caused by sarcocystosis are common in many domestic and wild animals and can cause mortality in these infected animals. There are two types of *Sarcocystis* cysts, the microscopic and the macroscopic sarcocysts (Dubey 2015).
Sarcocystosis is also considered to be one of the most prevalent intracellular protozoan diseases of livestock (Fayer 2004). Animals including cattle, goats, and sheep are susceptible to sarcocystosis (Dubey and Lindsay 2006) and are considered as intermediate hosts. Cysts are only formed within the muscles of the intermediate hosts. Therefore, they are called sarcocysts, which commonly indicate a wide-ranging hosts and worldwide distribution (Abdel-Ghaffar *et al*., 2009). Several species of *Sarcocystis* develop macroscopic sarcocysts in the tissue of domesticated animals. Amongthese, S. *caprafelis* (syn. *S. moulei*) is found in goats, while *S. gigantea* (syn. *S. ovifelis*) and *S. medusiformis* in sheep (Dubey and Lindsay 2006).Similarly, *S. hirsuta* and *S. hominis* form macroscopic sarcocysts in cattle, while *S. buffalonis and S. fusiformis* form these cysts in the water buffalo (Lindsay and Dubey 2020).

History of Sarcocystosis

In 1843, milky white threads in the muscles of a deer mouse, *Mus muscula,* were observed by Friedrich Miescher in Switzerland (Miescher 1843). These threads were known as Miescher's tubules for many years. Later, the causative organism of the problem was named as *Sarcocystis* after Lankester (1882), a Greek word derived from “sarkos” that means muscle or flesh, and “kystis” meaning bladder, describing the parasite's form that encysted in the tissue of the intermediate hosts (Fayer 2004). Thereafter, *S. muris* was the first species named in the genus *Sarcocystis* by the Swiss scientist Friedrich Miescher. The house mouse was the only known intermediate host for this organism (Ruiz and Frenkel 1976). In 1967, crescent-shaped structures typically found in cultures of sarcocystiswere investigated and the organism was identified as a protozoan, a close relative of *Toxoplasma* spp. and *Eimeria* spp. (Fayer 2004).
In 1969, Mandour identified a new *Sarcocystis* species in rhesus macaques named *S. nesbitti*, after Mr. Nesbitt, who

observed the trophozoites in stained smears. The definite hosts of *S. nesbitti* are now-a-days known to be snakes, whereas several primates, including human beings, can be intermediate hosts (Mandour 1969).

Scientists debated whether the *Sarcocystis* spp. were protozoa until 1967, after the first Sarcocystis report when the spindle or crescent-shaped bodies (bradyzoites) in the sarcocystis were studied by electron microscopy. Its organelles resembled to other apicomplexan protozoa, such as *Toxoplasma* and *Eimeria* (Fayer 2004).

The terminology for *Sarcocystis* spp. was suggested by combining the Latin names of both intermediate and definite hosts, such as *Sarcocystis ovicanis (S. tenella)*, for which sheep is an intermediate host, while the dog acts as a final host.Similarly, *S. ovifelis (S. gigantea)* in which sheep serves as an intermediate host, while the cat is the final host. *S. bovicanis* comes from two Latin words: *bos* for cattle and *canis* for dog (Mehlhorn 2016). Some historical landmarks concerning *Sarcocystis* are listed in Table 1.

Classification of *Sarcocystis* species

According to the classification system proposed by Levine (1986), *Sarcocystis* parasite is classified as:

Phylum Apicomplexa	Levine (1970)
Class Sporozoasida	Leuckart (1879)
Subclass Coccidiasina	Leuckart (1879)
Order Eucoccidiorida	Leger and Duboscq (1910)
Suborder Eimeriorina	Leger (1911)
Family Sarcocystidae	Poche (1913)
Subfamily Sarcocystinae	Poche (1913)
Genus *Sarcocystis*	Lankester (1882)

Life cycle of *Sarcocystis* species

The life cycle of *Sarcocystis* species was unknown until 1972, when some investigators ultrastructurally studied the gametogonic and oocyst formation properties of *S. falctula* in poultry during *in vitro* experiments (Fayer 1972; Rommel et al. 1972). In 1973, Wallance (1973) fed experimental mice with coccidia collected from the feces of a naturally infected cat and thus, he induced sarcocysts formation in mice.

The life cycle of *Sarcocystis* species needs two obligatory prey-predator hosts for its completion, intermediate host and definite host, followed by one another sequentially and designated as a di- heterogeneous parasite (Odening 1998) (Fig. 1). The stages occurring during the life cycle of *Sarcocystis* species are as follows:

Asexual stages

The asexual stages of *Sacocystis* parasite development occur only in the intermediate host, which is generally a prey animal. The infection starts when these animals ingest *Sacocystis* oocysts or sporocysts from the food or water contaminated with feces of the final host. Sporozoites are released from the ingested sporocysts by the action of trypsin and bile. The free sporozoites invade the gut wall and lodge themselvesin the endothelial cells of the small arteries. Four cycles of asexual reproduction are distinguished. Several nuclear divisions occur in the sporozoites, followed by segmentation to generate merozoites, which are motile and crescent-shaped. Following these schizonts' cycles, the *Sarcocystis* encysts itself in the muscles and form metrocytes, which are changed to bradyzoites. Sarcocyst having bradyzoites indicates the last encysted stage in the skeletal, cardiac, and smooth muscles of infected herbivores, infectious for carnivorous animals as definite hosts (Dubey 2015; Fayer et al. 2015; Dubey et al. 2016).

Two types of tissue sarcocysts can occur in sheep, goats, and cattle; either as microscopically or macroscopically visible structures of different *Sarcocystis spp*, as shown in Fig. 2 and Fig. 3. Fig. 4 illustrates the Banana-shaped bradyzoites prepared by muscle mincing and squash methods of fresh esophageal tissue from infected sheep and goats and stained with Giemsa stain.

Sexual stages

A definite host acquires the infection by ingesting mature sarcocysts from muscles of an infected animal (Lindsay and Dubey 2020). These sarcocysts are digested in the digestive system of the definite host and release the bradyzoites, which invade mucosa of the small intestine. Then, they are transformed into male and female gametes, called microgametes and macrogametes, respectively. After fertilization, these gametes form a zygote, which develops into the non-motile oocysts. The oocysts sporulate in the small intestine. The sporulated oocysts are thin-walled, each having two sporocysts: Each sporocyst contains four sporozoites. Then, it ruptures, liberating the sporocysts into the lumen of intestine; these sporocysts are defecated with feces (Lindsay and Dubey 2020), as has been shown in Fig. 1.

Ultrastructure of *Sarcocystis* spp

Sarcocystis spp. are single-cell eukaryotic organisms that contain a nucleus, nucleolus, endoplasmic reticulum, ribosomes, Golgi apparatus, and mitochondria. They also have the characteristic apicomplexan organelles, such as the apical rings (conoidal rings), polar ring, a conoid, pellicle, subpellicular microtubules, micropores, rhoptries, and micronomes, as shown in Fig. 5 (Ghaffar et al. 1989; Al-Quraishy et al. 2014; Dubey et al. 2016).

The intermediate host becomes infected by ingesting the sporocysts, the latter release sporozoites in the small intestine. These sporozoites appear in the mesenteric arteries and lymph nodes, where they eventually liberate merozoites into the blood, initiating the development of the sarcocysts in muscle. Resultantly, bradyzoites areformed, which is known as the infective stage for the consumer (Markus et al. 2004).

A sarcocyst plays a significant role in the transmission, as well as in the taxonomy, of *Sarcocystis* spp. It results from the invasion of merozoite into a myocyte or neural cell. Then the merozoite becomes rounded to change into a

metrocyte, with several organelles of the apical complex, like the micronemes, conoid and apical rings disappear. In contrast, ribosomes, endoplasmic reticulum and mitochondria become more abundant, and the nucleus becomes larger (Dubey et al. 2016). Within the cysts, the parasite multiplies by endodyogony or endopolygony, leading to the formation of metrocytes and later thousands of infectious cystozoites (bradyzoites), as shown in Fig. 6.

Sarcocystis species that are infecting ruminants

Sheep, goats and cattle are ungulates, 'hooved' animals that are members of the Order Artiodactyla (animals with cloven hooves), suborder Ruminantia (ruminants or cud-chewing animals), and Family Bovidae. These animals are herbivores, and they meet all their glucose requirements from gluconeogenesis. The subfamily Capra includes sheep and goats (Underwood et al. 2015).
Numerous spp. of *Sarcocystis* infect sheep, some of them are transmitted via canids and others by feline (cat). The species transmitted by dogs are mostly pathogenic and produce microsarcocyst in the skeletal and cardiac muscles of the animal. These species, like *S. tenella,* can lead to pathological effects in sheep, like anorexia, anemia, weight loss, abortion, neural symptoms, and even death (Dubey et al. 1988; Abdel-Baki et al. 2009). However, the species transmitted by cats, for instance *S. gigantea* and *S. medusiformis,* form macrosarcocyst in the esophagus, tongue, and larynx; the pathological effects of macrosarcocyst are more severe than those of the microsarcocysts (Collins et al. 1979; Dubey et al. 2016).

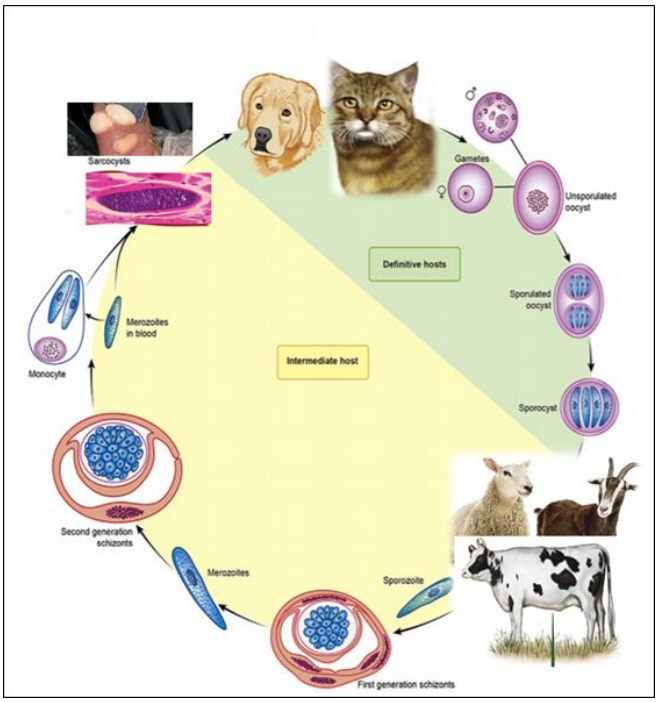

Fig. 1: The life cycle of *Sarcocystis* spp. from ruminants (Modified from Lindsay and Dubey 2020; Swar and Shnawa 2021).

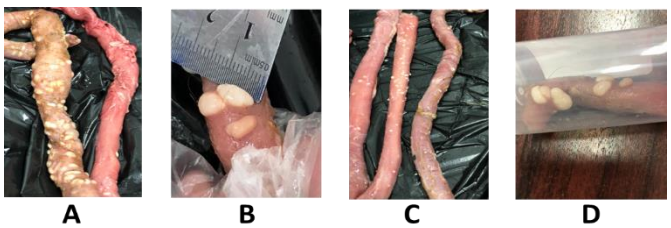

Fig. 2: Appearance of macroscopic sarcocysts in the esophagi of goats (A&B) and sheep (C&D) (Swar and Shnawa 2021). Note several cysts appear with different sizes and shapes on the surface of infected esophagus.

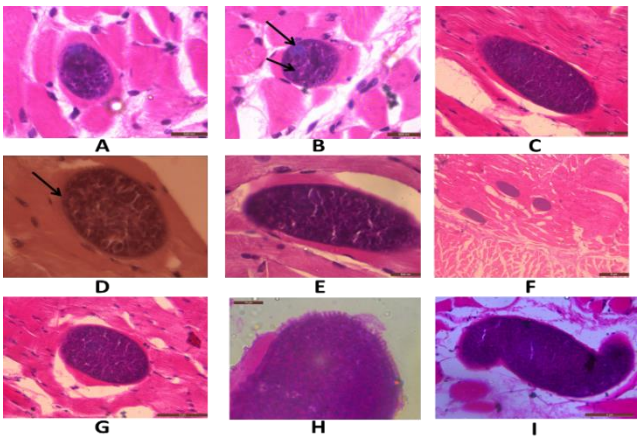

Fig. 3: Microscopic sarcocysts within the esophagi of infected sheep and goats (Swar 2021).

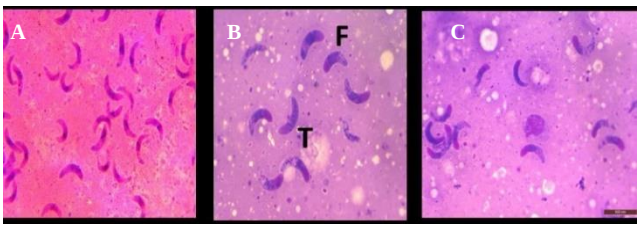

Fig. 4: Banana-shaped bradyzoites by muscle mincing and squash technique of fresh esophageal tissue from sheep and goats stained with Giemsa stain (A, B, and C). Bradyzoites from macroscopic sarcocyst of goats and sheep. B also shows fat (F) and thin (T) types of bradyzoites. Scale bar =500nm. (Swar 2021).

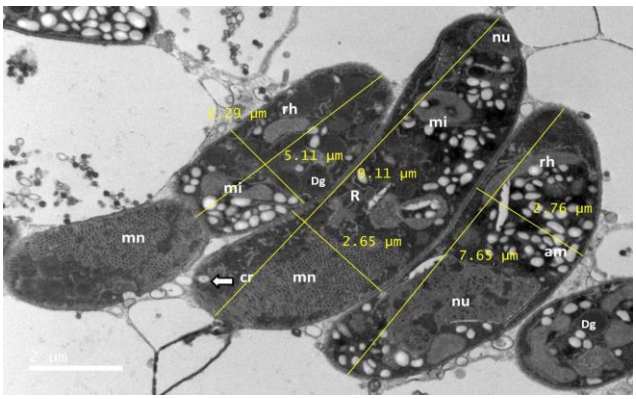

Fig. 5: Transmission electron micrograph of a longitudinal section of macroscopic sarcocyst of *Sarcocystis* spp. of sheep shows bradyzoites. Note conoidal ring (cr), amylopectin (am), numerous micronemes (mn), subterminal nucleus (nu), rhoptries (rh), dense granules as black structure (white arrow), amylopectin as white granules (am), ribosome(R) and mitochondrion (mi). Scale bar= 2 µm. (Swar and Shnawa 2020).

Three *Sarcocystis* species have been identified in domestic goats; these include *S. caprafelis* (synonym *S. moulei*), *S. hiricanis*, and *S. capracanis*. The *S. hiricanis* and *S. capracanis* are usually associated with microscopic sarcocysts, whereas *S. moulei* produces macroscopic cysts (Dubey et al. 2016). Medically, *S. capracanis* shows more severe pathological effects than those of the other two species (Collins and Charleston 1979). The infected goats may show fever, anorexia, weight loss, tremors, abortion, and death in severe cases (Dubey et al. 1981). However, some investigations documented the infection of sheep and goats with Sarcocystis species that are unusual in these hosts, such as the infection of sheep with *S. moulei* that commonly infects goats in Saudi Arabia (Al-Hoot et al. 2005) and Iran (Kalantari et al. 2016). Similarly, *S. gigantean*, which usually infects sheep, can also cause infection in goats (Ghaffar et al. 1989) and it was suggested that goats can be a host for three species of Sarcocystis described as *S. moulei*, including *S. ovifelis (S. gigantea)*. Moreover, using molecular and ultrastructural techniques, Hong et al. (2016) evidenced the infection of Korean goats with *S. tenella*, which is commonly known as sheep specific.

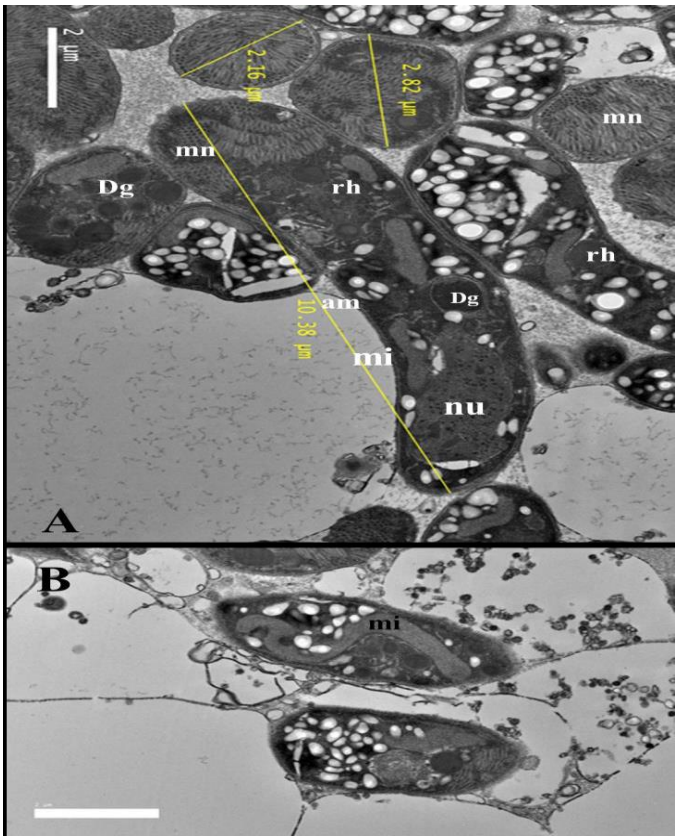

Fig. 6: Transmission electron micrographs of macroscopic sarcocyst of *Sarcocystis spp* from sheep: (A). Longitudinal and cross-sections of bradyzoites showing conoidal ring (cr), amylopectin (am), micronemes (mn), nucleus (nu), and dense granules (dg), and mitochondrion. Scale bar= 2 µm. (B). Cross-section of bradyzoite with one long twisted mitochondrion (mi), nucleus (nu), dense granules (dg), and amylopectin as white granules (am). The parasite is surrounding by a membrane of parasitophorous vacuole within the host cell cytoplasm-scale bar= 2 µm for both micrographs (Swar 2021).

Five species of Sarcocystis have been identified in cattle: these include *Sarcocystis cruzi*, *Sarcocystis heydorni*, *Sarcocystis hirsuta*, *Sarcocystis hominis*, and *Sarcocystis rommeli*; the definite hosts for these species are canines (*S. cruzi*), felines (*S. hirsuta*, *S. rommeli*), and primates (*S. heydorni*, *S. hominis*).
Generally, four *Sarcocystis* species can infect water buffalo, including *S. buffalonis*, *S. dubeyi*, *S. fusiformis*, and *S. levinei*.

Molecular Characterization of *Sarcocystis spp*

In recent years, researchers gained outstanding achievements in several molecular procedures to identify various *Sarcocystis* spp that can infect different animals and are recognized as host-specific. These molecular procedures include 18S rRNA, 28S rRNA, 18S rDNA, mitochondrial cytochrome C oxidase subunit 1 gene (COX1) and ITS-1 region (Dubey et al. 2014; Blazejewski et al. 2015; Ng et al. 2015; Hu et al. 2017; El-Morsey et al. 2019).
The first genetically identified species of the genus *Sarcocystis* through genome sequence was the *S. neurona*. Its genome contains 127-Mbp, and its size is twice that of the size of other coccidian genomes (Blazejewski et al. 2015). Tenter et al. (1992) recognized two monophyletic groups of *Sarcocystis* spp. One group represents the species for which cats arethe definite hosts. In contrast, the second group has the species that need dogs as definite hosts in their life cycles.
Previously, *Sarcocystis* spp. were known to be primarily host-specific, but, during the last few years, a large number of *Sarcocystis* spp which use different animals as intermediate hosts were identified. Consequently, host specificity for various *Sarcocystis* spp. is questionable. In this regard, Al-Hoot et al. (2005) characterized ultrastructurally the *S. moulei* in sheep infection, the species which usually infects goats in Saudi Arabia. Moreover, in Iran, *S. moulei* was documented genetically in sheep (Kalantari et al. 2016). Similarly, *S. capracanis* was identified from the cerebrospinal fluid of sheep suffering from meningoencephalitis in the United Kingdom (Formisano et al. 2013). Based on these findings it can be suggested that sheep can act as an alternative intermediate host for these species in addition to goats. In a study regarding *Sarcocystis* infection from Egypt, Elmishmishy et al. (2018) documented the resemblance of *S. gigantea* from sheep with *S. moulei* from goats. They proposed the cross-transmission of *S. moulei* amongst sheep and goats, and suggested them to be phylogenetically related. Other researchers confirmed the genetic similarity between *S. tenella* of sheep and *S. capracanis* from goats and considered them as sister species (El-Morsey et al. 2019).
Moreover, Saudi Arabian researchers also revealed the presence of phylogenetic association among *Sarcocystis* spp from both hosts, sheep and goats (Metwally et al. 2019). According to Yang et al. (2001), morphologically identical species from two different intermediate hosts should be classified as similar species. However, many *Sarcocystis* spp. seem to have a more comprehensive intermediate host opportunity than previously recognized.

Table 1: Historical landmarks regarding *Sarcocystis,* modified from Dubey et al. *(*2016)

Scientist's name	Year	The outcomes
Miescher	1843	*Sarcocystis* reported from the muscle of house mouse
Lankester	1882	The genus *Sarcocystis* named
Fayer	1972	Sexual stages cultured in vitro
Rommel et al.	1972	Two obligatory hosts required for completion life-cycle
Fayer & Johnson	1973	The pathogenicity proved with recognition of vascular phase
Heydorn	1975	Several *Sarcocystis* species are recognized within a given host.
Fayer & Johnson	1975	Chemotherapy (Amprolium) is documented
Fayer et al.	1976	Abortion results from *Sarcocystis* infection in cows
Dubey et al.	1981	Protective immunity against sarcocystosis is proved in goats
Dubey et al.	1989	Classification of *Sarcocystis* species depending on cyst wall morphology is recommended.
Dubey et al.	1991	First detection of the causative agent of equine protozoan myeloencephalitis and classified *as Sarcocystis neurona.*
Blazejewski et al.	2015	First characterization of *Sarcocystis* genome that related to *S.neurona.*

Table 2: Several recent articles concerning the molecular characterization of *Sarcocystis* spp. of meat-producing animals.

Sarcocystis spp.	Molecular technique	Host	Country	Reference
S. tenella	RFLP-PCR for the 18S rRNA	Sheep	Brazil	(da Silva et al. 2009)
S. fusiformis,S.cruzi, S. homonis, S. hirsuta	RFLP-PCR for 18S rDNA	Cattle & Water buffalo	Vietnam	(Jehle et al. 2009)
S. gigantea and *S. tenella.*	Mitochondrial cytochrome c oxidase subunit I gene (cox1) and the nuclear ssr RNA	Norwegian Sheep Argentinean cattle	Norway	(Gjerde 2013a)
S. hirsuta and *S. sinensis*				
S. tenella	18S r RNA gene sequence with PCR-RFLP	Sheep	Iran	(Shahbazi et al. 2013)
S. cafferi	18S Rrna& COX1	Buffalo	South Africa	(Dubey et al. 2014)
S. gigantea and S. tenella	18S rRNA	Sheep	Iran	(Bahari et al. 2014)
S. gigantea and *S. medusiformis*	18S rRNA	Sheep	Iran	(Farhang-Pajuh et al. 2014)
S. cruzi	18S rDNA	Cattle	Malaysian	(Ng et al. 2015)
S. capracanis	18S rRNA	Goats	Malaysia	(Kutty et al. 2015)
S. tenella	18S rRNA	Sheep	Italy	(Bacci et al. 2016)
S. arieticanis & *S. capracanis*	18S rDNA	Sheep	Brazil	(Bittencourt et al. 2016)
S. tenella	18S rDNA	Goats	Korea	(Hong et al. 2016)
S. tenella	18S rRNA	Sheep	Iraq	(Whaeeb and Faraj 2016)
S. gigantea & *S. moulei*	18S rRNA	Sheep	Iran	(Kalantari et al. 2016)
S. gigantea	18S rRNA	Sheep	Argentina	(Gual et al. 2017)
S. fusiformis & S. moulei	18S rRNA	Water buffaloes	Iraq	(Dakhil et al. 2017)
S. tenella & S. arieticanis	18S r RNA gene,28S r RNA, cox1, and ITS-1 region	Sheep	China	(Hu et al. 2017)
S. tenella & S. capracanis	18S rRNA	Sheep &goats	Tunisia	(Amairia et al. 2018)
S. tenella	18S rRNA	Sheep	Egypt	(Hussein et al. 2018)
S. tenella and *S. arieticanis*	cox1	Sheep	China	(Dong et al. 2018)
S. gigantea& S. tenella	18S rRNA	Sheep	Egypt	(Elmishmishy et al. 2018)
S. fusiformis	18S rRNA&cox1	Buffaloes	China	(Mei Ren et al. 2019)
S. cruzi & S. hjorti	18S rRNA	Cattle	Egypt	(El-kady et al. 2018)
S. cruzi	18S rRNA	Cattle	Korea	(Choi et al. 2018)
S. tenella & S. capracanis	Cox1	Sheep & goats	Saudi Arabia	(Metwally et al. 2019)
S. bovifelis	Cytochrome C Oxidase subunit I mitochondrial (mtDNA COI) gene	Cattle	Italy	(Rubiola et al. 2019)
S. tenella and *S. arieticanis*.	18S rRNA, 28S rRNA, cox1 and ITS-1	Sheep	Egypt	(El-Morsey et al.2019)
S. arieticanis, S. tenella, S. gigantea, S. medusiformis, & S. mihoensis	Cox1,18S and 28S rRNA	Sheep	Spain	(Gjerde et al. 2020)
S. arieticanis	18S rRNA	Sheep	Egypt	(Hussein et al.2020)
Sarcocystis spp.	18SrRNA	Sheep	Iraq	(Al-Saadi et al. 2020)
S. gigantea, S. moulei, & S. medusiformis	18S rRNA	Sheep & goats	Iraq	(Swar and Shnawa, 2020)

More recent genetic findings propose that *S. medusiformis*, *S. gigantea* and *S. moulei* have identical sister genome sequences. These species are documented to form macrosrcocysts in sheep, while cats serve asthe final host for them (Gjerde et al. 2020). This fact has also been detected in the cattle and water buffalo with uncommon *Sarcocystis* spp infection., Results of several other studies also suggest that *Sarcocystis* spp. are non-specific for the intermediate host (Jehle et al. 2009; Xiang et al. 2011; Gjerde et al. 2016; Dakhil et al. 2017; El-kady et al. 2018).

Gjerde (2013a; 2013b) proved that the sequences of COX1 are better than the ssRNA gene for identifying the closely associated spp of *Sarcocystis* and recommended it as a

novel bio- genetic marker for further studies. Gjerde et al. (2016) recommended COX1 as a superior gene to 18S and 28S rRNA genes as a bio-genetic marker. In the same aspect, El-Morsey et al. (2019) proved the priority of COX1 and ITS-1 genes as the best genetic markers compared to other genes to differentiate the closely related *Sarcocystis* spp owing to the high divergence among them. Another study evaluated the sequences of 4 genetic markers (18S rRNA, 28S rRNA, mitochondrial *COX1* and *ITS-1)* specific for *S. tenella* and *S. arieticanis*.

It has been confirmed that the ITS-1 region is more helpful for distinguishing the closely related *Sarcocystis* spp. because of their high divergence (Hu et al. 2017). The same was true for the genetic resemblance between sheep and goats infection with *Eimeria* spp., which was phylogenetically confirmed by the ITS-1 sequences technique in Egypt. The sequence of ITS-1 gene of *E. ahsata* was 100% identical with *E. ahsata* and clustered in one clade with *E. cardinalis* and *E. faurei*. Alternatively, the similarity was 100% between *E. arloingi* and *E. arloingi* of goats. It is clustered with *E. ellipsoidalis* of bovine sources (Hassanen et al. 2020). Conventionally, the Sarcocystis' ultrastructural features are considered as fundamentals for the identification of numerous *Sarcocystis* spp. within the same intermediate host (Hu et al. 2017; Huang et al. 2019). Currently, genomic sequence analysis is an essential technique to clarify whether morphologically similar *Sarcocystis* spp. from different intermediate hosts are the same species or not. Findings of several recent studies concerning the molecular characterization of *Sarcocystis* spp isolated from meat-producing animals have been summarized in Table 2.

Conclusion

Recently, researchers achieved outstanding success in molecular aspects to identify various *Sarcocystis* spp that infect livestock. Various molecular techniques evaluated for this purpose are 18S rRNA, 28S rRNA, 18S rDNA, mitochondrial cytochrome C oxidase subunit 1 gene (COX1), and ITS-1 region. Usually, the Sarcocystis' ultrastructural features are considered as fundamentals for the identification of many *Sarcocystis* spp. within the same intermediate host. At present, genomic sequence analysis is necessary to clarify whether morphologically similar *Sarcocystis* from different intermediate hosts are the same species or not. *Sarcocystis* spp. are known as primarily host-specific, but a large number of Sarcocystis spp using different animals as intermediate hosts were identified during the last few years. As a result, the issue of host specificity of *Sarcocystis* species remains questionable.

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THE EPIDEMIOLOGY OF ZOONOTIC PARASITES OF FARM ANIMALS AND BIRDS IN CHINA IN THE PAST TEN YEARS

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INTRODUCTION

Toxoplasmosis

In 1908, Nicolle and Manceaux first discovered *Toxoplasma gondii* in Africa (Ferguson and Henriquez 2005). Since then, people have recognized Toxoplasmosis. Toxoplasmosis is a worldwide zoonotic parasitic disease, caused by *Toxoplasma gondii* of the genus *Toxoplasma* (Hernández-de-Los-Ríos et al. 2019). This intracellular parasite can infect humans and almost all warm-blooded animals, including mammals and birds. According to an estimate, about one-third of the world's population is infected with *Toxoplasma gondii* (Robert-Gangneux and Dardé 2012).

Morphology

Toxoplasma gondii is an intracellular parasite, divided into five forms according to its developmental stages: (1) Tachyzoites, which rapidly divide into nucleated cells and occupy the entire cytoplasm of cells of the host, and called as pseudocysts (Fig. 1). (2) Cysts which slowly proliferate in the cyst wall secreted by the worm, called a cyst, which contains hundreds of bradyzoites (Dubey et al. 1998). (3) Schizont comprises bradyzoites or sporozoites, etc. Schistosomes proliferate in cat small intestinal epithelial cells to form a collection of merozoites. (4) Gametophytes are large gametes (female) and small gametes (male), which form zygotes after fertilization and finally develop into oocysts. (5) Oocysts refer to the inside of oocysts.

A sporophyte develops and reproduces, forming 2 sporangia, and then each sporangium differentiates into 4 sporozoites. *Toxoplasma gondii* develops in two stages, namely the extra-intestinal mucosa stage and the intra-intestinal mucosa stage. The former develops in various intermediate hosts and main tissue cells of end-staying infectious diseases. The latter only develops in the epithelial cells of the small intestinal mucosa of the last host (Aguirre et al. 2019).

Life style

The life history of *Toxoplasma gondii* comprises two stages, divided into sexual reproduction and asexual reproduction. Sexual reproduction only occurs in cats (Robert-Gangneux et al. 2012). When cats eat meat containing cysts, the cyst wall is destroyed by gastric acid, and the bradyzoites in the cyst are released and settle in the intestinal epithelial cells. Then it undergoes sexual development, forming male and female gametes (gamete reproduction) (Ferguson 2002). After fertilization, the oocysts enter the intestine and are excreted with feces (Fig. 2).

When the intermediate host eats food or drinks water contaminated by oocysts, the oocysts will enter the digestive tract and rupture. Resultantly, it spreads through the intestinal wall with the blood to the tissue of the entire body and proliferates in the cells through budding. The host's immunity can slow down the reproduction of the protozoa and form a cyst wall, which becomes a cyst. Cysts are most commonly associated with the brain and skeletal muscles and can survive for a long time (Ferguson and Hutchison 1987).

Epidemiology

Toxoplasmosis is one of the most common infectious diseases in mammals, with a worldwide distribution (Mohammed et al. 2019). According to the survey, the incidence rate in developing countries is higher than that in developed countries, and the incidence is higher in some backward regions, such as Latin America, the Middle East, and Africa (Montoya and Liesenfeld 2004). Diseased animals and insect-carrying animals are the sources of infection of *Toxoplasma gondii*. Cats are the last host of this parasite. Oral infection is the fundamental way of transmission of the disease. Humans and different carnivores are infected by eating raw or undercooked meat contaminated by encapsulated parasites. At the same time, *Toxoplasma gondii* can be transmitted to offspring through the placenta. Table 1 shows the factors that trigger the Toxoplasma outbreak.

Pathogenesis

The pathogenic effects of *Toxoplasma gondii* depend upon virulence of the parasite and immune status of the host (Zhang et al. 2018). The tachyzoite stage is the primary treatment stage of *Toxoplasma gondii*. After the worms invade cells of the body, they proliferate in an enormous

Table 1: As of 2018, a summary of factors that triggered the outbreak of *Toxoplasma gondii*. Data referenced from a previous research (Pinto-Ferreira et al. 2019).

Related factors	Incidence (%)
Contaminated meat	47.1
Oocysts	44.1
Intake of oocysts in water	20.6
Contact with sand and soil	17.6
Consumption of vegetables	5.9
Tachyzoites in raw milk	8.8

amount, destroying cells of the body. At the same time, *Toxoplasma gondii* produces toxins, causing inflammation in the body (Burger et al. 2018). Chronozoites in the cyst can cause chronic infection. The continuous proliferation of the chronic zoites leads to a larger size, which can squeeze the organs and impair their functions.

Symptoms

The common clinical symptom is head and neck lymph node disease, but inguinal lymph node, retroperitoneal lymph node, and mesenteric lymph node disease may also occur. Usually, swollen nodules of 0.5-3.0 cm diameter appear in the lymph nodes (Montoya and Liesenfeld, 2004). It is also accompanied by symptoms such as fever, malaise, sore throat, and rash. In a host with strong immunity, chronic infection is usually asymptomatic. In contrast, in chronically infected people with immunodeficiency, the brachyzoites in the body transform into actively replicating tachyzoites, leading to necrotizing encephalitis and even death.

Diagnosis

According to the patient's immune background, disease status and clinical signs, the diagnosis can be made through pathogenic examination and serological examination. Pathogen inspection includes examination of body fluids or tissues for worms through a microscope. For direct necropsy of animals, tissues such as lungs and liver are stained for microscopic examination. For serological examination, IHA and ELISA methods are often used at present. The analysis should be carried out 2-4 weeks after the first examination because there is possibility of false positive results. If the IgA antibody titer increases by over 4 times, it is taken as positive.

Trichinosis

Trichinosis is a zoonotic disease caused by *Trichinella spiralis,* a parasite of the genus *Trichinellaceae.* Besides humans, animals including pigs, dogs, cats, rodents and wild boars can be infected with this parasite (Arefkhah et al. 2020). *Trichinella spiralis* is a common pathogen that causes human diseases and can cause infection after eating raw or undercooked meat containing *Trichinella spiralis* larvae (Dimzas et al. 2019). There are many clinical symptoms, including general fever, abdominal pain, diarrhoea, nausea, vomiting or muscle pain, and symptoms of myocarditis and encephalitis (Rawla and Sharma 2021).

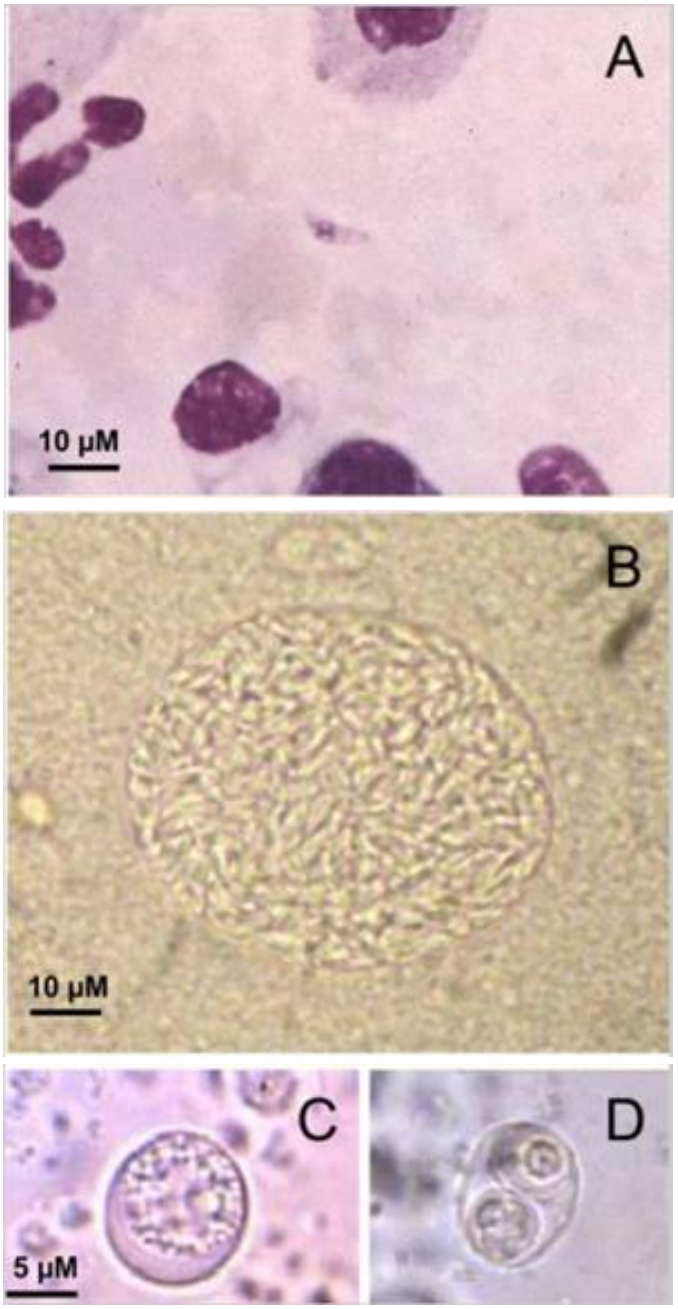

Fig. 1: Biological stages of *Toxoplasma gondii*: Microscopic examination of tachyzoites in a bronchoalveolar lavage fluid sample stained with Giemsa (A) (magnification, ×500); a cyst in the brain of an infected mouse (B) (magnification, ×500); unsporulated (C) and sporulated (D) oocysts (magnification, ×1,000). (Robert-Gangneux et al. 2012).

Morphology

Adult *Trichinella spiralis* are small and dioecious. The average length of adult males is 1.2 mm, and that of females is 2.2 mm (Peters and Pasvol 2007). The front part of the worm is thinner, the oesophagus; the back part is thicker, containing the intestines and reproductive organs. The larvae are located in the cyst and are coiled in a spiral shape. The fully developed larva usually has 2.5 discs. The cyst is fusiform, with its long axis parallel to the muscle fibres, and has two walls. Generally, there is only one larva, but some may have 6-7 (Fig. 3).

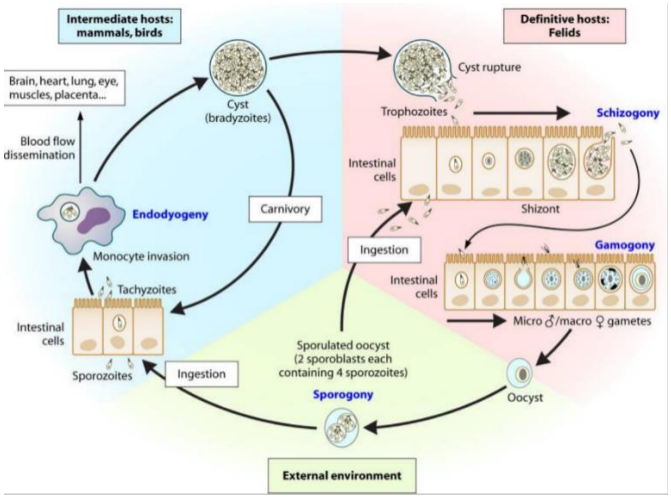

Fig. 2: Life cycle of *Toxoplasma gondii*. Figure shows the biology, infection, and replication of three infective stages of parasites in their respective hosts (Robert-Gangneux et al. 2012).

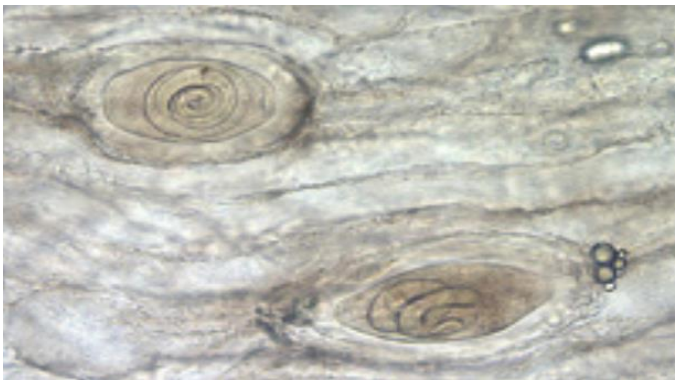

Fig. 3: Bear muscle showing the encysted larvae at 200x magnification. United States Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/dpdx/trichinellosis. Public domain.

Life style

Adults and larvae parasitize themselves on the same host. When the host is infected, it becomes the terminal host first and then becomes the terminal host. The life cycle of *Trichinella spiralis* in humans and other host animals consists of three consecutive stages: the intestinal stage, the extraintestinal stage, and the encapsulation stage (Fig. 4). Life cycle of the parasite begins with the intestinal stage in the stomach and small intestine, where gastric acids and pepsin release the larvae encased in the striated muscles (Peters and Pasvol 2007). The released larvae invade the mucosa of the small intestine and grow into adults within one week and begin to mate and reproduce. The pregnant female releases the larvae into the lymphatic circulation and shifted to the striated muscles for subsequent development. After the larva enters the striated muscle fibres through the capillary, the release of vascular endothelial growth factor-stimulated by the cytokine causes the larva to form a cyst (Capo et al. 1998). The cysts begin to calcify after 6-9 months, but the larvae can remain viable for up to 11 years. The host is infected by eating cysts containing infectious larvae. The cysts are

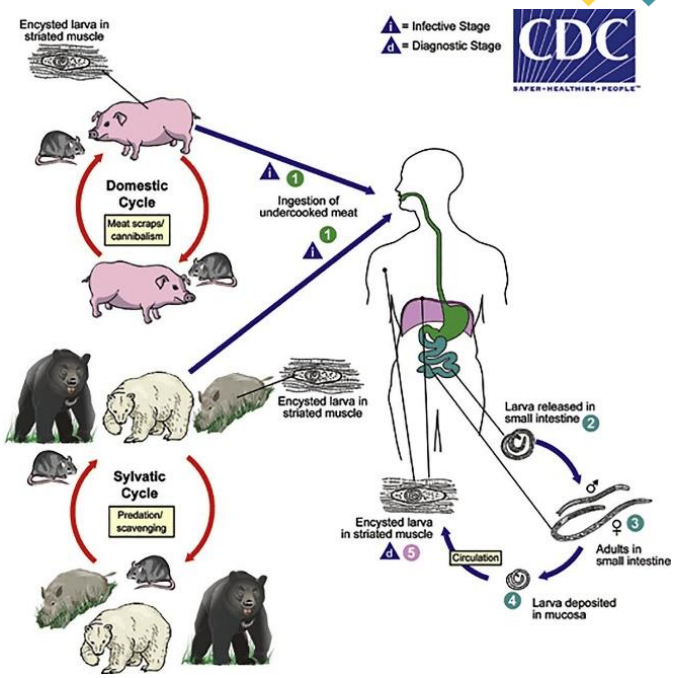

Fig. 4: The domestic and sylvatic life cycles of Trichinella species. United States Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/dpdx/trichinellosis. Public domain.

dissolved under the action of gastric acid and pepsin in the host, releasing the larvae into the intestines, where they can develop into adults in a short time.

Epidemiology

Trichinosis occurs worldwide, and it is estimated that about 10,000 cases occur every year. The cases are usually concentrated in people, who eat common animal infected meat. The host range and infection range of *Trichinella spiralis* are very wide. It is popular among various wild and domestic animals, and many marine animals worldwide. There are nine species of *Trichinella* disease. So far, 12 genotypes of *Trichinella* have been reported. The most common *Trichinella spiralis* that can cause human diseases is *Trichinella* swine. According to the Centers for Disease Control and Prevention, in the 1940s, about 400 cases of Trichinosis were reported every year, but the number of reported cases has dropped significantly since 2010. In the past 40 years, few Trichinosis cases have been reported, and the risk of the disease in commercially raised and properly prepared pork is very low. However, eating uncooked game, especially bear meat, can cause people to suffer from this disease. Table 2 shows the Trichinella species, their geographic distributions, and hosts.

Pathogenesis

The symptoms of animal infections are like those of humans. The severity of the pathogenic effects of *Trichinella spiralis* on the human body is related to factors such as the number of ingested larvae cysts and their vitality, as well as the immune function status of the host.

Table 2: Trichinella species, their geographic distributions, and hosts. Data referenced from a previous report (Diaz et al. 2020).

Species	Geographic distributions	Host
Trichinella britovi	Europe, Asia, Africa	Wild and domestic swine, wild carnivores (bears, foxes, hyenas, lynxes, wolves)
T. murrelli	North America	Black bears, grizzly bears, raccoons, red foxes, bobcats, coyotes, cougars, moose, wild boar
T. nativa	Arctic and subarctic regions worldwide	Arctic foxes, polar bears, black bears, brown bears, grizzly bears, walruses, sea ice-associated seals, wolves, other wild carnivores
T. nelsoni	Africa	Wild carnivores (wild dogs, hyenas, lions, tigers)
T. papuae	Papua New Guinea, Taiwan, Thailand	Wild and domestic swine, saltwater crocodiles, soft-shelled turtles
T. patagoniensis	Argentina	Cougars
T. pseudospiralis	Worldwide	Birds, mammals, reptiles
T. spiralis	Worldwide	Wild and domestic swine, horses
T. zimbabwensis	Africa	Nile crocodiles, monitor lizards
Trichinella T8 (unnamed genotype)	Africa, South Africa, Namibia	Lions, spotted hyenas (not detected in humans at present)
Trichinella T9 (unnamed genotype)	Japan	Bears, wild carnivores

The larvae penetrate the intestinal wall and mature, causing extensive duodenal inflammation, mucosal congestion and oedema, bleeding, and even superficial ulcers. Vascular inflammation can occur where larvae migrate, causing a significant heterogeneous protein reaction (Shimoni and Froom 2015). The acute inflammation subsides with intramuscular cyst formation, and the systemic symptoms are eased, but the myalgia may last longer.

Symptoms

Initial symptoms of Trichinosis are gastrointestinal diseases, which usually occur 1-2 days after eating raw or undercooked meat from animals infected with *Trichinella*. These symptoms include nausea, diarrhoea, vomiting, and abdominal pain. The following typical Trichinosis symptoms usually occur within two weeks after eating contaminated meat and can last up to 8 weeks: muscle pain, fever, facial swelling, weakness or fatigue, headache, etc. Symptoms can range from very mild to severe and are related to the number of *Trichinella* eaten in the meat (Gottstein et al. 2009). Furthermore, many patients with Trichinosis do not show any symptoms.

Diagnosis

Diagnosis of animals before death is very difficult, and pig Trichinosis is often detected after slaughter. For this purpse, the diaphragm is examined with naked eyes, and when small white spots are seen between the muscle fibres, a microscope is used to observe whether there are cysts. In humans, acute Trichinosis usually occurs after eating raw or undercooked meat or having contact with the patient. Currently, commonly used laboratory testing method is ELISA. The positive coincidence rate of serum antibody detection with ELISA method can reach up to 93-96% (Dupouy-Camet et al. 2002).

Fasciolopsis buski

Fasciolopsis buski (Manning and Ratanarat 1970) is a member of the family *Fasciolopsis* and is parasitic in the small intestine of humans and pigs, most often in the duodenum. Occasionally, it can infect dogs and rabbits, where it is attached to the mucosa by powerful suckers or implanted in the mucosa, and is a large intestinal parasitic trematode (Malviya 1985). *F. buski* is known as a giant intestinal trematode (Lee et al. 2011) and is one of the largest digenean infecting humans globally, mainly confined to Asian nations, including China (Murugesh et al. 2007). The infection with *F. buski* develops through the consumption of raw or undercooked fishery foods contaminated with cysts containing the parasite (Chen et al. 2016). *F. buski* can also co-infect with other parasites, especially trematodes (Gupta et al. 1999). It can occur in the form of moderate and severe infections of the digestive tract, with the severe cases can adversely affect the immune system of the host, induce acute nephritis and even death, leading to major public health problems and losses in endemic areas (Karthikeyan et al. 2013).

Morphology

The adult worm body is flat, fat, and flesh-red when alive, grayish-white when fixed; body shape resembles ginger, so it is also called ginger worm (Fig. 5). The body of parasite is 20~75 mm long, 8~20 mm wide and 0.5~3.0 mm thick, and it is the largest parasitic trematode in the human body. The oral sucker is located on the ventral surface of the front of the body, about 0.5 mm in diameter, and the ventral sucker is 2~3 mm in diameter, located near the back of the oral sucker, funnel-shaped, with well-developed muscles, and visible with the naked eye (Wiwanitkit et al. 2002).

Life style

Life style peculiarities of the worm are as follows: Immature eggs are released in the intestine and excreted via stool. After that, in water, eggs are embryonated, releasing miracidia that invade the appropriate intermediate host snail. In snails, parasites go through various stages of development (sporocysts, rediae and cercariae). The cercariae are discharged from snails and cysts and become metacercariae on aquatic plants.

Fig. 5: Left: *F. buski* egg in an unstained wet mount. Center: Adult fluke of *F. buski* next to a scale. Right: Snail of the genus *Hippeutis*, an intermediate host for *F. buski*. (Credit: DPDx; Conchology, Inc., Mactan Island, Philippines).

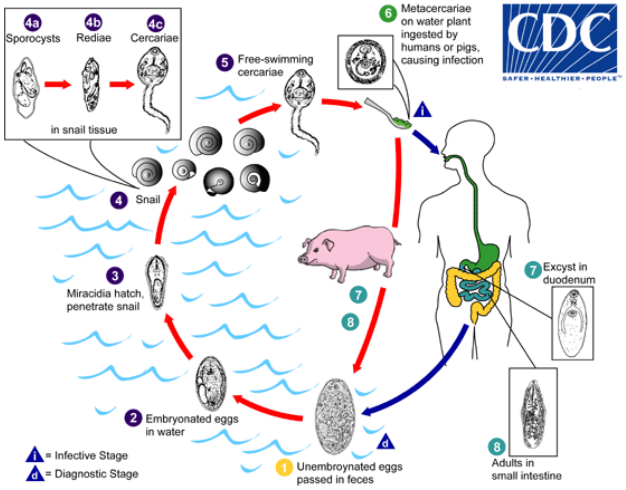

Fig. 6: Life cycle image and information of *F. buski*. United States Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/parasites/fasciolopsis/biology.html.

Mammalian hosts get infected by eating the metacercariae present on the aquatic plants. After ingestion, metacercariae enter the duodenum, leave the cyst and ascribe to the intestinal wall (Weng et al. 1989). They are evolved into adult flukes (20-75 mm x 8-20 mm) over a period of about 3 months and adhere to the intestinal wall of mammalian hosts (humans and pigs). Adults have a lifespan of approximately one year (Fig. 6).

Epidemiology

The disease is endemic and mainly occurs in temperate and subtropical regions of Asian countries, such as Vietnam, Laos, Cambodia, Thailand, Myanmar, Malaysia, Bangladesh, India, Indonesia and the Philippines (Chai et al. 2009). In China, it is mainly distributed in the Yangtze River basin and southern China, such as Jiangsu, Zhejiang, Fujian, Anhui, Jiangxi, Yunnan, Shanghai, Hubei, Hunan, Guangxi, Guangdong, Guizhou, Sichuan, Chongqing, Hainan, and Taiwan. It also occurs in north of the Yangtze River in Shandong, Henan, Hebei, Shaanxi and Gansu (Yeh and Mitchell 2016).

Host vectors

The intermediate hosts, the flatworm snails, are widely distributed in ponds, marshes, ditches and paddy fields, often inhabiting under leaves of plants. Most of the aquatic plants can be used as the vector for the cysts of the ginger borer. Some aquatic plants commonly used for green fodder are essential vectors of swine infection (Achra et al. 2015).

Pathogenesis

Mechanical damage: The ginger sucker only sucks the intestinal mucosa with its strong oral and ventral suction discs, causing mechanical damage to the suction site, resulting in enteritis, intestinal mucosal detachment, bleeding and even abscess formation.
Mechanical blockage: When the intensity of infection is high, the intestine can be mechanically blocked, adversely affecting digestion and absorption and even causing intestinal rupture or intestinal entrapment and death.
Nutrient seizure: Due to its large size, the worm absorbs a vast amount of nutrients, which retards the growth of the sick animal and causes anaemia, wasting and malnutrition. The animal absorbs toxic effects of the worm, which can cause anaemia and oedema, inflammation, haemorrhage, ulceration and necrosis of the intestinal mucosa (Graczyk et al. 2000).

Symptoms

Ginger suckers mostly attack young pigs, resulting in stunted growth, sparse and lusterless coat, depressed spirit, low head, salivation, pale eye mucosa and dullness. Appetite of the affected animal is reduced, and digestion is poor, but sometimes animal feels hungery. There are signs of dysentery, thin faeces mixed with mucus. Affected animal shows signs of abdominal pain, diarrhoea, swelling and ascites (Wu et al. 2020). The lactation yield and length of the affected sow is reduced, which affects the growth of piglets.

Comprehensive preventive measures

Manure disposal: In endemic areas, manure of diseased pigs is the primary source of transmission and should be composted and fermented as much as possible before being used as fertilizer (Chung et al. 1996). Also, human manure and pig manure should be equally avoided by mutual transmission between humans and animals.
Regular deworming: In endemic areas, regular deworming should be carried out every year in spring and autumn (Manning and Ratanarat 1970). The intermediate hosts of flatworm should be eliminated. In the drier season of late autumn and early winter each year, digging pond mud to accumulate fertilizer and dry pond mud to kill snails is advised. In low-lying areas, where the pond water is not easily drained, snails can be exterminated by chemicals, such as copper sulfate with a concentration of $10\sim50\times10^{-5}$, 0.1% lime, 0.01% tea cakes and ammonia sulfate, lime nitrogen, etc (Roy et al. 2009).

Cryptosporidiosis

Cryptosporidiosis is caused by infection with protozoan parasite *cryptosporidium* in a wide range of host species,

such as humans, livestock, companion animals, wildlife, birds, reptiles, and fish (Chalmers et al. 2013). In 1907, it was first described by Tyzzer in the small intestine of mice (Tyzzer 1912). The disease causes severe diarrhoea in mammals and distressing respiratory symptoms in birds, posing a serious threat to the health and production of the affected animals. It is also a common-established cause of sporadic gastroenteritis in immunocompromised humans, and symptoms include watery diarrhoea, nausea, abdominal pain, vomiting and low-grade fever (Lal et al. 2019).

Morphology

Cryptosporidium belongs to the *Eucoccidiorida* and *Cryptosporidiidae* in taxonomy. The Cryptosporidium species that can cause human infections are *C. cuniculus, C. parvum, C. ubiquitum, C. viatorum, C. meleagridis, Cryptosporidium* mink genotype, *C. felis, C. hominis, C. canis*, Chipmunk genotype I and *C. muris*. Several species related to ruminant infection include *C. muris, C. andersoni, C. parvum, C. hominis, C. felis,* and *C. bovis*.

Biological characteristics: Invasive forms of Cryptosporidium have an apical complex with polar rings, rhoptries, micronemes, conoid, and subpellicular microtubules. Locomotion in invasive forms occur by body flexion, gliding, or undulation. Male and female gametes develop independently. Homoxenous (one host life cycle) shows developmental stages just under the membrane of the host cell. Oocyst are without sporocysts and with four sporozoites, while microgametes are without flagella (Current and Garcia 1991). The oocysts of Cryptosporidium are rounded in shape and measure 4.2 µ-5.4 µm in diameter (Fig. 7).

Life style

The oocysts of Cryptosporidium are transmitted among many hosts via the faecal-oral route. Furthermore, direct contact with faeces of infected animals or indirect transmission of contaminated food or water mediated by environmental contamination can also occur. Akin to coccidia, the developmental history of *Cryptosporidium parvum* includes excystation, merogony, gametogony, fertilization, oocyst formation, and sporogony. After ingesting infective *Cryptosporidium* oocysts, the environment of the gastrointestinal tract promotes oocysts to decyst and release four sporozoites. Sporozoites penetrate host cells and develop into trophozoites (uninucleate meronts), which form merozoites through asexual division. Invasive merozoites enter adjacent host cells to form type I (recycling) or type II meronts. Type II meronts enter host cells to form microgametes and macrogametes. Approximately 80% of the zygotes formed after fertilization of the microgametes, develop into thick-walled oocysts (environmentally resistant), released in faeces and transmit the infection from one host to another. Moreover, approximately 20% of the zygotes are transformed into thin-walled oocysts. These oocysts are released in the host and cause autoinfection (Current and Garcia 1991).

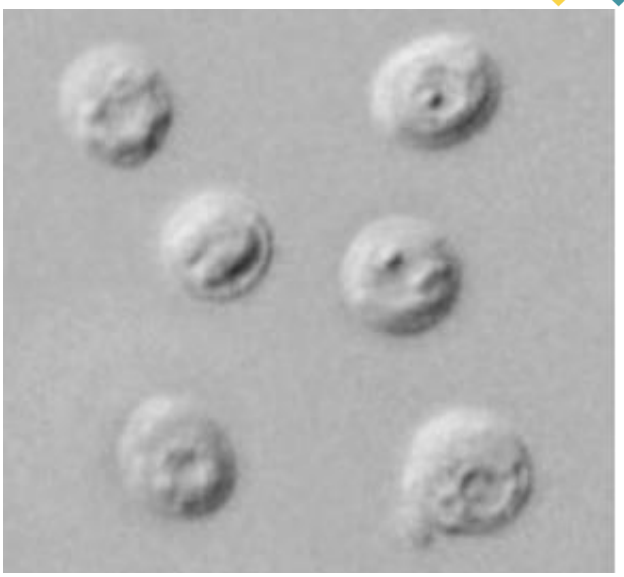

Fig. 7: Oocysts of *Cryptosporidium parvum* (Current and Garcia 1991).

Epidemiology

Cryptosporidium has a global distribution, and most countries have reported *Cryptosporidium* infections in humans and livestock. Cryptosporidiosis outbreaks have been recorded many times in the European Union countries, and their outbreaks are mainly associated with the spread of drinking or recreational waters, outdoor activities, animal contact, food consumption and person-to-person spread (Rochelle and Giovanni 2014; Robertson and Chalmers 2013). Table 4 shows valid named species of Cryptosporidium.

Infection source: The infection source of *Cryptosporidium* is diseased animals or animals that discharge oocysts to the environment. These oocysts are highly resistant to the external environment.

Route of transmission: Oocysts are transmitted between infected animals and susceptible hosts orally through contaminated feed, drinking water or faeces and can even spread through the air.

Susceptible hosts: *Cryptosporidium* has a wide host range, which can parasitize more than 150 species of mammals, more than 30 species of birds, fish, and 57 species of reptiles. Additionally, *Cryptosporidium* does not have any apparent host specificity. For example, *Cryptosporidium* from quail can cause infection in chickens, ducks, mice, and other animals.

Pathogenesis

Cryptosporidium damages intestines of the host and induces a series of clinical symptoms, which may be caused by *Cryptosporidium* through altering the activity of host cells or absorbing nutrients from the gut.

Symptoms

Cryptosporidium infection usually causes watery diarrhoea, and the duration of symptoms is prolonged in

Table 3: Related factors of Fascioliasis outbreaks described in the previous review (Siles-Lucas et al. 2021).

Country	Year	Infected number (species)	Attributed source
HUMAN OUTBREAKS			
China	2011	29	Wild vegetables
Turkey	2011	24	Wild watercress
Romania	2010	4	NS
ANIMAL OUTBREAKS			
Brazil[b]	2016	19	Water
Italy	2014	NS (sheep)	Pasture
Croatia	2007	20 (sheep)	NS

NS, not stated.

Table 4: Valid named species of Cryptosporidium (Sunnotel et al. 2006).

Cryptosporidium species	Size (μm)	Host	Location
C. andersoni	5.5×7.4	Bovines	Abomasum
C. baileyi	4.6×6.2	Birds	Cloaca, bursa,respiratory tract
C. canis	5.0×4.7	Canids, human	Small intestine
C. felis	4.5×5.0	Felids, human	Small intestine
C. galli	8.0–8.5×6.2–6.4	Birds	Proventriculus
C. hominis	4.5×5.5	Human	Small intestine
C. meleagridis	4.5–5.0×4.6–5.2	Birds, human	Intestine
C. molnari	4.7×4.5	Fish	Stomach
C. muris	5.6 ×7.4	Rodents, human	Stomach
C. parvum	4.5×5.5	Ruminants, human	Intestine
C. saurophilum	4.2–5.2×4.4–5.6	Lizards, snake	Intestinal and cloacal mucosa
C. serpentis	4.8–5.6×5.6–6.6	Snakes, lizards	Stomach
C. suis	5.1×4.4	Pigs, human	Small intestine
C. wrairi	4.0–5.0×4.8–5.6	Guinea pigs	Small intestine
C. bovis	4.2–4.8×4.8–5.4	Ruminants	Small intestine
C. scophithalmi	3.0–4.7×3.7–5.0	Fish	Intestine

people with weakened immunity. Other common symptoms include nausea, low grade fever, abdominal pain, and vomiting. Occasionally, symptoms of weakness, myalgia, headache, malaise and anorexia can be seen.
Cryptosporidium commonly infects young animals, such as calves and lambs. In these animals, symptoms include diarrhoea with lethargy, poor body condition, inappetence, fever and dehydration.

Lesions

In *Cryptosporidium* infection, the intestinal villi shrank and become merged. The epithelial lining of the intestinal mucosa turns into low columnar or cuboidal cells, degenerates or falls off. Moreover, the cecum, colon and duodenum can also be infected. These lesions cause the host to reduce the absorption of vitamin A and carbohydrates.

Diagnosis

Many infective oocysts excreted with faeces, and the low infectious dose indicates that Cryptosporidiosis is highly contagious. However, in immunocompetent animals, this disease is self-limiting. Therefore, the exact diagnosis can only rely on laboratory methods to observe the various stages of *Cryptosporidium* or use immunological techniques to detect antigens or antibodies.
Diagnosis before death: Saturated sucrose solution is used to collect *Cryptosporidium* oocysts in the faecal samples; these samples are examined under microscope. It is essential to recognize that oocysts are too small; therefore, an oil lens with 1000x magnification is recommended.
Postmortem diagnosis: Giemsa dye solution is used to stain the digestive tract mucosa smears scraped off. In positive samples, the cytoplasm of the parasites can be observed to be blue, containing several dense red particles.
Immunologic and molecular biologic techniques: Specific tests, including acid-fast or fluorescent stains to stain faecal smears, Enzyme-linked immunosorbent assay (ELISA), Polymerase chain reaction (PCR), and immunochromatographic lateral flow assays are recommended to confirm diagnosis (Chalmers and Katzer 2013).

Clonorchiasis

Clonorchis sinensis, also known as the Chinese liver fluke, belongs to the family *Opisthorchiidae*. This parasite causesa series of hepatobiliary diseases in the hepatobiliary duct of the host. Clonorchiasis is a freshwater fish-derived zoonotic parasitic disease, and the parasite is one of the most important foodborne parasites, endemic in Eastern Asian countries, including China, Korea, Vietnam, Thailand and the Far East regions of Russia (Lun et al. 2005; Traub et al. 2009; Hong and Fang 2012; Qian et al. 2012). Based on the national survey in China in 2003, it is estimated that over 12 million people are infected with *C. sinensis*, and the prevalence of this parasite is expanding in this country.

Morphology

The adult *C. sinensis* is a leaf-shaped hermaphroditic trematode, 10-25 mm in length and 3-5 mm in width with

an anterior oral sucker, a centrally located ventral sucker and genital pore.

The egg of *C. sinensis* is small, with an average of 29 microns × 17 microns, shaped like a light bulb, brown, with an operculum cover at the upper end and a small protuberance at the back end, which contains miracidium.

Life style

With a relatively wide host range, *C. sinensis* can infect to several snail species and more than 100 freshwater fish species (intermediate hosts). Many of such fish species belong to the *Cyprinidae* family, such as minnows and carps. In addition to humans, *C. sinensis* can affect canines, felines, mustelids, pigs, piscivorous mammals as definitive hosts. The life cycle of the *C. sinensis* involves three types of hosts: freshwater snails, freshwater fish, and piscivorous mammals, including humans.

Many freshwater snails (belonging to order *Mesogastropoda*) can be used as the primary intermediate host. Miracidium enters the sporocyst stage, during which it reproduces asexually. The asexual reproduction only occurs during summer, when the eggs develop to mature cercariae and are released into the water (Liang et al. 2009). Once the cercariae encounter the second intermediate host, they penetrate the skin mucosa of the fish, encyst as metacercariae and develop into mature metacercariae. At this time, when mammals ingest infected fish or fish meat, the metacercariae excyst in the duodenum and rapidly migrate into the intrahepatic bile ducts within 10–20 mins, develop into adults and begin to spawn. It is reported that *C. sinensis* lays about 4000, 2400 and 1600 eggs a day in humans, cats and guinea pigs, respectively (Kim et al,2011). The eggs flow to the intestines with bile and discharge water through faeces to continue the life cycle. Adults may survive in humans for over 26 years (Attwood and Chou 1978), as shown in Fig. 8.

Epidemiology

Clonorchis sinensis is a widespread parasite of humans, dogs, and cats in the southeast of Asia. It is extraordinarily common in China and is also found in Korea and Japan.

In the survey conducted in Guangdong, China, from 2004 to 2012, 15,435 out of 179756 people were infected, with an infection rate of 8.6%. In the survey of Guangxi from 2005 to 2014, 14,234 of 144,073 people were infected, with an infection rate of 9.9%. In addition, the population infection rates in Hunan, Jilin, Heilongjiang, Liaoning and Zhejiang were 34.3 (3195/9313), 18.5 (1979/10694), 12.4 (518/4177), 3.1 (123/3972) and 2.3% (19/821), respectively (Tang et al, 2016). It is speculated that the high infection rate in endemic areas is related to local eating habits, including eating raw or half-raw freshwater fish and shrimp, which may also be an important reason for the widespread prevalence of the disease.

The epidemic level of Clonorchiasis in China is on the rise. Two national surveys show that the population's

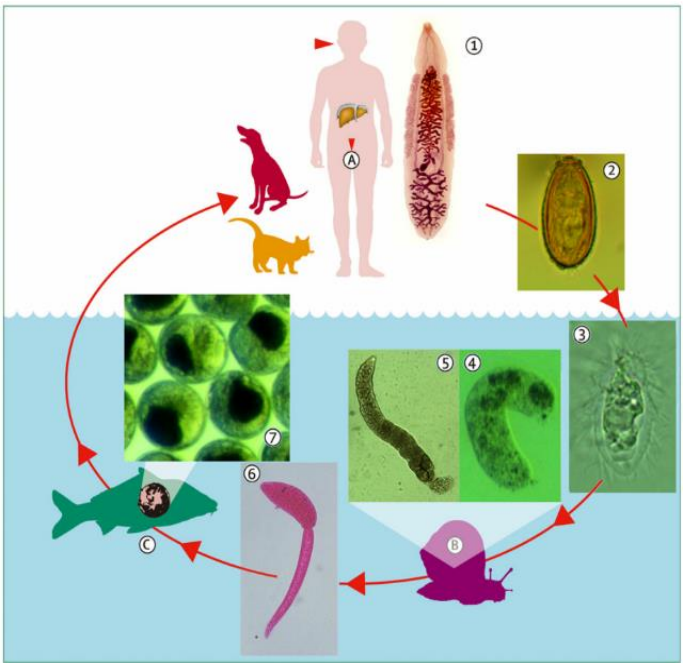

Fig. 8: Life cycle of *Clonorchis sinensis*. (1) lives mainly in the intrahepatic bile duct and gall bladder. Fully mature egg with miracidium (2) excreted in the fecal environment (water) and enters the body of the (B) first intermediate host, the freshwater snail. The miracidium (3) is discharged from the egg and enters snail's gastrointestinal tract, where it develops into sporocysts (4) and then into rediae (5). A large number of motile cercariae (6) are released into the water. Free-swimming cercariae invade the skin, fins, or muscles of freshwater fish as intermediate host (C) and develop into metacercariae (7). Consumption of raw or undercooked metacercariae infected freshwater fish can transmit the infection in the definitive host. Modified from Lun et al. (2005).

infection level is rising significantly, from 0.31% in the first survey to 0.58% in the second survey. Guangdong, in particular, showed the rise from 1.82% to 5.35% (Chen et al. 2012). It shows that the prevention and treatment of the disease have not been paid enough attention. Moreover, the level of prevention and control of Clonorchiasis in China lags behind other major foodborne Clonorchiasis endemic countries. For example, the main foodborne parasitic disease in South Korea is *Clonorchiasis sinensis*, but its epidemic level has dropped from 4.6% in 1971 to 2.4% in 2004 (Qian et al. 2014). In Thailand, the main endemic country of Retroclonorchiasis in muskcats, it fell from 14% in the early 1980s to 8.7% in 2009 (Sithithaworn et al. 2012). The situation of prevention and control of Clonorchiasis in China is grim, which should be paid more attention (Chen et al. 2012).

Pathogenesis

After infecting the host, *Clonorchis sinensis* moves in the bile duct through the suckers of the head and abdomen, causing mechanical damage to the bile duct epithelium. When the larvae mature, the injury is more serious, which can cause bile duct epithelial ulcers (Liu et al. 2010). With the advancement in the course of the disease, many adults and eggs block the bile duct, resulting in increased bile

duct pressure. Consequently, eggs enter the surrounding liver tissue through ulcers, inducing numerous inflammatory cell infiltration to cause granuloma reaction; the bile duct obstruction will also cause bacterial infection, further aggravating bile duct injury. Repeated mechanical injury and inflammatory stimulations are important factors leading to bile duct epithelial hyperplasia and cholangiocarcinoma (Pan et al. 2017; Liu and Sun 2016). *Clonorchis sinensis* moves in the bile duct and continuously produces excretory/secretory products (ESPs). The researchers isolated 39 known functional proteins from ESPs, including glucose metabolic enzymes, detoxifying enzymes, structural proteins, etc., which play an important role in the interaction between host and parasites (Tang et al. 2016).

Symptoms

Symptoms of Clonorchiasis are associated with worm load. Most infections are mild and asymptomatic. The clinical manifestations are associated with inflammation, periodic obstruction of the bile ducts, mechanical damage caused by the mucosal tissue nutrition by worms, toxic effects of the worm's metabolic products, or byproducts, and secondary bacterial infections. In mild cases, symptoms include mild abdominal manifestations. Patients with a high burden of worms often present with other nonspecific symptoms such as abdominal pain (especially in the upper right abdomen) and various physical symptoms (such as headache, dizziness, etc.). Prolonged infection can exacerbate symptoms and cause hepatomegaly and malnutrition. It can sometimes increase the incidence of biliary complications (e.g. cholelithiasis, cholangitis, cholangiocarcinoma, and cholecystitis etc.), liver abscesses, and pancreatitis.

Diagnosis

The etiological diagnosis of *Clonorchiasis sinensis* is mainly based on the detection of eggs in faeces. The eggs in faeces are collected by precipitation method, floating method, washing method, aldehyde ether concentration method, modified Kato method, and the morphological characteristics of eggs observed under a microscope. This method is also the standard method for detecting *Clonorchiasis sinensis*. The immunological diagnostic methods of Clonorchiasis include intradermal test, indirect hemagglutination (IHA) test, ELISA, immunofluorescent antibody technique, (immunofluorescence technique), immune colloidal gold technique and serum circulating antigen detection. The widely used methods are ELISA and immuno-colloidal gold technique. ELISA mainly includes dot-ELISA, biotin-avidin-ELISA, monoclonal antibody-ELISA, FAST-ELISA and cysteine protease-ELISA. Immunogold techniques include immunocolloidal gold chromatography (immunochromatography), dot immunogold silver staining (Dot-IGSS) and dot immunogold filtration assay (DIGFA).

Cysticercus

The causative agent of Cysticercosis cellulosae is the larva of *Taenia solium*, which is parasitic in the human body (Héctor et al. 2003). Pigs and wild boars are the main intermediate hosts, followed by dogs, camels, cats, and humans (Assana et al. 2019). Humans are the final host of *Taenia solium*. These organisms parasitize the striated muscles, heart, brain, eyes and other human and pig organs (Clinton et al. 2017). It not only affects the pig industry but also poses a huge threat to the human health.

Morphology

Cysticercus cellulosae is commonly known as cysticercosis. Mature *Cysticercus cellulosae* has an oval shape and is of a soybean size (6-10 mm×6 mm). It is characterized by a translucent cyst filled with liquid. The wall of the cyst is a thin film. There is a milky white nodule of the size of a round millet grain on the wall. There are 4 circular suction cups on the head section in the turned head section, and there are many small endoplasmic hooks on the top protrusion at the front end, arranged in two circles. The adult Cysticercus measures 2-5 m in length and some can grow up to 8 m. There is 700-1000 nodal plate in the bug. The scolex is a spherical shape with a diameter of 1 mm, and there are 20-25 horny little hooks on the rostellum. There are 4 sucking discs in the rear of the rostellum. The eggs are round or slightly elliptical with a diameter of 35 μm.

Life cycle

The adult parasitizes in the anterior part of small intestine and its cephalic segment is buried deep in the mucosa. Worm eggs or gravid proglottid are discharged with faeces and contaminate the ground and food. The intermediate host (mainly pig) swallows the eggs, or gravid proglottid can enter the intestinal wall under the action of the secretions of oncosphere. It enters the lymphatic and blood vessels and is carried to the muscles, heart, brain and throughout the body with blood circulation. After 2 months, it develops into infectious mature Cysticercus.

Epidemiology

Cysticercus disease is mainly prevalent in 18 countries and regions, where pork is the main food. This disease has been recorded in most provinces and autonomous regions of China, and it is more severe in the north. According to statistics, the national economic loss caused by cysticercosis can reach more than 80 million yuan every year. The epidemic of this disease has the following characteristics:

Pigs can be infected by ingestion of eggs in feed contaminated with human faeces. Human may become infected with Cysticercus by eating raw or under-cooked infected pork. The infection of this disease can occur in all

Table 5: The prevalence data of Cysticercosis in some Asian countries (Rajshekhar et al. 2003).

Country	Human Cysticercosis (%)	Taeniasis (%)	Porcine Cysticercosis (%)
China	3 –/4	0.112 (0.06 –/19)	5.4 (0.8 –/40)
Indonesia	1.7 –/13	0.8 –/23	0.02 –/2.63
Vietnam	5 –/7	0.5 –/6	0.04 –/0.9
India	NA	2	9.3
Nepal	NA	10 –/50	32.5
Korea	3	NA	NA

NA, not available

Table 6: Types of Echinococcosis currently prevalent and their approximate geographic range (Romig 2003).

Causative agent	Term for disease	Geographical range
Echinococcus granulosus (species group)	Cystic echinococcosis (hydatidosis, hydatid disease)	Cosmopolitan
Echinococcus multilocularis	Alveolar echinococcosis	Northern hemisphere
Echinococcus vogeli	Polycystic echinococcosis	Central and South America
Echinococcus oligarthrus	Polycystic echinococcosis	Central and South America

seasons, but it tends to increase in the warm season when the environment is suitable for survival and development of eggs. The disease is generally sporadic, but endemic in some places, and its severity is positively correlated with the number of local tapeworm patients. Pigs are susceptible animals under natural conditions, and Cysticercus can survive in pigs for 3-5 years. Wild boars, dogs, and cats can also be infected. Although humans can serve as intermediate hosts, the disease is often fatal. Table 5 shows the prevalence data of Cysticercosis in some Asian countries.

Pathogenesis

Pathogenesis of Cysticercus disease varies remarkably with the number and location of Cysticercus parasitism. The migration of hyacercaria *in vivo* at the early stage of the disease can cause tissue damage. The pathogenicity of mature Cysticercus often depends on the location of the parasite, followed by their number. When parasite is in the brain, it can cause neurological symptoms and reduce the defence capability of the body by damaging integrity of the brain.

Moreover, it can cause death if brain lesions become severe. When it is parasitic in the eyes, it can cause visual disturbance and even blindness. When parasitic in the muscles and subcutaneous tissue, there are generally no obvious pathogenic effects.

Clinical symptoms

Generally, no obvious clinical symptoms are seen in pigs in the early stage of the disease. However, pigs infected with Cysticercus can show obvious clinical signs such as malnutrition, anemia, slow growth, and edema in the later period. Moreover, Cysticercus can parasitize in the lungs and throat, and can cause dyspnea, hoarseness and dysphagia. Cysticercus parasitization in the tongue can cause feeding difficulties. Cysticercus parasitized in the myocardium can cause blood circulation disorders. Cysticercus parasitized in the brain can cause epilepsy, acute encephalitis and even death. If Cysticercus is parasitized in the small intestine of humans, it can causenutrients depriviation and the secreted toxins can result in weight loss, abdominal pain, indigestion, and diarrhea. When Cysticercus parasitizes in the human brain, it can cause headache, mobility impairment, paralysis and other neurological symptoms.

Diagnosis

Blood and cerebrospinal fluid examination: Routine blood examination showed normal haematological values of most patients, but eosinophil counts were slightly elevated in a few patients. Moreover, cerebrospinal fluid pressure, lymphocyte and protein contents in patients with cerebral Cysticercosis were significantly increased, while sugar and chloride levels were within normal range.

Etiological examination: Fecal examination is carried out to check for eggs or nodules in the stool. Patients with subcutaneous and muscle Cysticercosis can undergo a subcutaneous nodule biopsy.

Immunologic tests: The purified Cysticercus fluid or serum can be used as an antigen to perform the intradermal test (ID), indirect hemagglutination test (IHA), enzyme-linked immunosorbent test (ELISA), enzyme immunoassay (EIA) to detect specific antibodies of Cysticercus in serum and cerebrospinal fluid of patients. **Imageological examination:** This examination includes CT scan and MRI of the skull, ophthalmoscopy, slit lamp, or B-mode ultrasonography.

Pathological examination: Subcutaneous nodules can be examined through biopsy, and *Cysticercus cephalon* in the lumen can be confirmed by pathological section.

Schistosomosis Japonicum

Schistosomiasis is a parasitic disease that seriously endangers the health of humans and livestock (Ayé et al. 2020). It has a high incidence in the Middle East, South America, and Southeast Asia, especially sub-Saharan Africa. Schistosomiasis is endemic in 76 countries around the world and approximately two hundred million people were infected. Infectious larvae grow in freshwater snails and then penetrate the skin of the final host. Adults colonize the mesentery or pelvic vein, where females lay eggs, which are secreted in feces or urine. Eggs trapped in surrounding tissues and organs, such as the liver and

bladder, can cause an inflammatory immune response, leading to diseases of the intestine, liver and spleen or genitourinary system (Mcmanus et al. 2018).

Morphology

Adult organisms are dioecious and long cylindrical. The male is stubby, milky white, with a smooth body surface, 10-20 mm long and 0.5-0.55 mm wide. The female is slender and darker than the male, with a small front end and a thick rear end.

Life style

Oncomelania snails are the only intermediate host of *Schistosoma japonicum*. Humans or mammals are parasitized by adults and are called terminal hosts. *Oncomelania* snails are parasitized by vegetative larvae, and are called intermediate hosts. These two methods of reproduction alternate with each other, called generational alternation (Mcmanus et al. 2018). The approximate course of its life history is that patients or livestock infected with Schistosomiasis excrete worm eggs with their faeces or urine, the excrement contaminates the water, the worm eggs are taken into the water, and when they meet the right temperature, they will decompose, releasing the mircaria in the water (Fig. 9).
The miracidia can penetrate the snails, continue to develop into mother spores, and then reproduce asexually to produce daughter spores and cercariae. Cercariae can escape from the spiral body and swim freely in the water under suitable temperature, humidity, light, and other external environmental conditions. The water body containing cercariae is called diseased water. If people or other susceptible animals come in contact with the diseased water, cercariae penetrate the skin and turn into young worms after entering the skin. After migration and a period of growth and development, they finally settle in the portal vein system or the bladder, pelvic venous plexus, and develop into adult worms. The male and female adults mate together. Eggs and worms can be excreted with feces or deposited in the liver to form nodules (McCreesh et al. 2015). In general, the life history of Schistosomiasis includes six stages: adult, egg, miracidia, larvae, cercariae, and juvenile worms. Among these, from egg to juvenile is asexual reproduction; mirabilia and cercariae can live freely in the natural water for a short time.

Epidemiology

Schistosomiasis is considered a neglected tropical disease that occurs mainly in tropical and subtropical regions (Fig. 10). About 779 million people worldwide are at risk of infection, and more than 250 million people are infected with Schistosomiasis. *Schistosomiasis japonicum* was endemic in Japan until it was eliminated in the late 1970s with effective control measures (Rollinson et al. 2013). The remaining species have a low global prevalence and are currently distributed mainly in South Africa, West and Central Africa and the Mekong River in southern

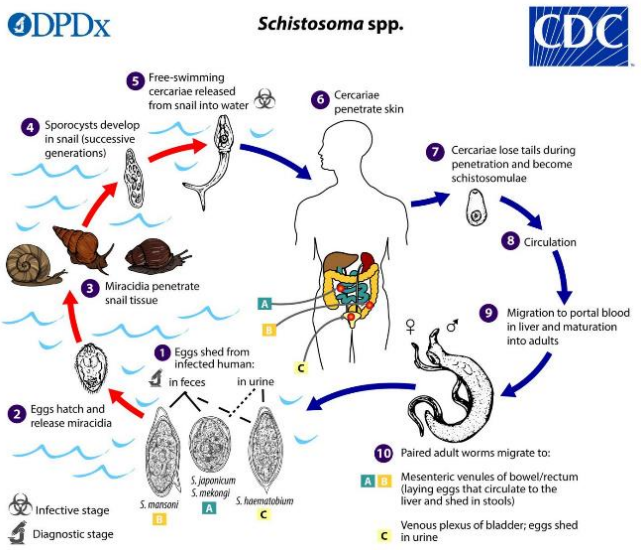

Fig. 9: The lifestyle of *Schistosomosis japonicum* (CDC 2018)

Fig. 10: Global distribution of Schistosomiasis in 2012 (Mcmanus et al. 2018)

Cambodia and the Lao People's Democratic Republic. The main intermediate hosts appear to be dogs and domestic pigs, but other animals, particularly cattle, may also be involved in transmission of the infection (Latif et al. 2013).

Pathogenic mechanism

After the cercariae penetrate the intact skin, part of the infected larvae will die in the skin, and the rest will directly enter the venous circulation through small blood vessels or lymphatic vessels. The larvae flow through the blood to the mature part of the liver. In the skin, the innate immune response to dying or dead larvae can cause an allergic reaction, and when exposed to water containing cercariae, a popular itching reaction on the body part, called cercariae dermatitis, is noted (Mcmanus et al. 2018). Symptomatic acute Schistosomiasis is also known as Katayama fever or Katayama syndrome. It usually occurs when a person comes in contact with a Schistosoma species for the first time, between 2 weeks and 3 months after exposure. These symptoms are caused by a systemic allergic reaction and the formation of immune complexes, which are responses to antigens released when the young Schistosomiasis worms migrate or deposit their eggs. Symptoms are usually accompanied by eosinophilia and transient lung infiltration (Chen et al. 2021).

Symptoms

Many people show no symptoms when they are first infected. However, within a few days after infection, they may develop a rash or itchy skin. Symptoms that may occur within 1-2 months after infection include fever, chills, cough, and muscle pain. If left untreated, Schistosomiasis can last for many years. Symptoms and signs of chronic Schistosomiasis include abdominal pain, enlarged liver, blood in the stool or urine, and urination problems. Chronic infection can also lead to an increased risk of liver fibrosis or bladder cancer.

Diagnosis

There are two main diagnostic methods for *Schistosoma japonicum* in animals based on serology and parasitology. The parasitology-based diagnostic tool is the gold standard, but it takes a long time, and the false-negative result rate is high at low infection intensity, due to which it is gradually replaced by serological diagnosis. Still, the serological diagnosis has its shortcomings like cross-reaction of other parasitic diseases, false-positive results, and problem of distinguishing the current infection from the previous infection. Therefore, there is an urgent need for more accurate and sensitive diagnostic methods to control Schistosomiasis (Mcmanus et al. 2018).

Parasitological diagnosis

The diagnosis based on parasitology refers to examining Schistosome eggs in the faeces or tissues of terminal host to be tested and examining the eggs after slaughter. The detection methods of eggs in faeces include the direct smear method, sediment collection method and nylon sieve egg method. KKM is usually used to detect faecal eggs. Pathogen detection is the most accurate diagnostic method for Schistosomiasis. The detection of eggs or miracidia hatched from faeces of the final host is the gold standard for Schistosomiasis diagnosis (Chen et al. 2021).

Serological diagnosis

Serological diagnosis is the use of the immune response of the specific combination of antigens and antibodies to detect diseases. It is also an important means for diagnosing and detecting Schistosomiasis. To improve the diagnosis efficiency, a variety of serological methods for the diagnosis of Schistosomiases such as IHA, ELISA, colloidal gold test strips, and nucleic acid detection have been developed (Chen et al. 2021).

IHA

The IHA applies antigens (or antibodies) to the surface of red blood cells, converts them into sensitizing carriers, and then binds to the corresponding antibodies (or antigens). Then, the red blood cells gather together, and a visible agglutination reaction occurs. IHA is suitable for on-site diagnosis because it is low-cost, easy to operate, and does not require any expensive equipment (Chen et al. 2021).

ELISA

The basic principle of enzyme-linked immunosorbent assay is to specifically bind the analyte in the sample with the enzyme through the specific immune reaction between the antigen and the antibody. The colour reaction between the enzyme and the substrate is then used to determine the analyte content. Enzyme-linked immunosorbent assay has become a commonly used qualitative and quantitative analysis method. Many Schistosome antigens are used for diagnoses, such as soluble egg antigens, soluble worm antigen preparations and recombinant antigens. However, SEA may be more effective in terms of the sensitivity of the diagnosis of Schistosomiasis (Cai et al. 2019).

Colloidal gold test strips

Because of its simple operation, low cost, long-term storage potential, and unlimited experimental conditions, the test strip method is gradually becoming popular for on-site rapid screening and diagnosis of Schistosomiasis. Types of test paper methods include test paper dye immunoassay (DDIA), latex microsphere labeling method, and colloidal gold immunochromatography.

Nucleic acid testing for diagnosis

Nucleic acids are present in all developmental stages of Schistosomes. The life cycle of Schistosome and the peculiarity of its parasitic site determine Schistosome nucleic acid detection technology (Cai et al. 2019).

Echinococcosis

Echinococcus disease, also known as hydatid disease, is caused by the larval stage of E*chinococcus granulosus*, a tapeworm that is about 2-7 mm long. It is found in dogs (ultimate hosts) and sheep, cattle, goats and pigs, which are intermediate hosts (Grech-Angelini et al. 2019; Tamarozzi et al. 2020). Although most human infections are asymptomatic, chronic pancreatitis can cause harmful, slowly expanding cysts in the liver, lungs, and other organs that often remain unnoticed and ignored for many years (Matsumoto, 2020). The World Health Organization (WHO) has listed Echinococcosis as one of the 17 neglected diseases.

Morphology

The shape of hydatid cysts varies widely due to their parasitic parts. They are generally approximately spherical, with a diameter of 5-10cm. Cyst wall can be divided into two layers, the inner layer is the germinal layer or germ layer, and the outer layer is the milky white stratum corneum. Adult parasites are very small, with a total length of 2-6mm, consisting of a head and three to four segments. There are suckers, apical processes and small hooks on the top of the head. There are several apical glands on the apical processes, which are covered by a radial embryonic membrane (Fig. 11).

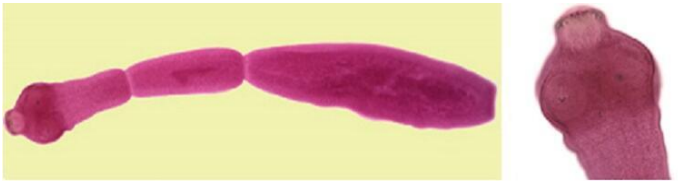

Fig. 11: From left to right are; an adult *Echinococcus granulosus* and the close-up of scolex section. United States Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/parasites/echinococcosis/.

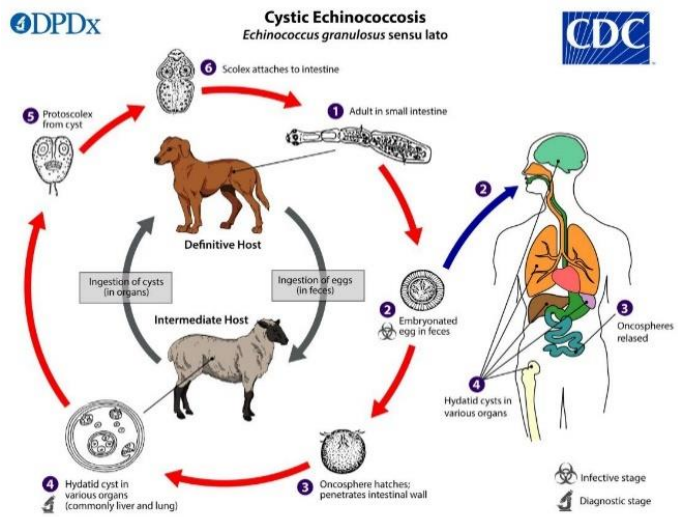

Fig. 12: Life cycles of *Echinococcus spp* (Hao et al. 2019).

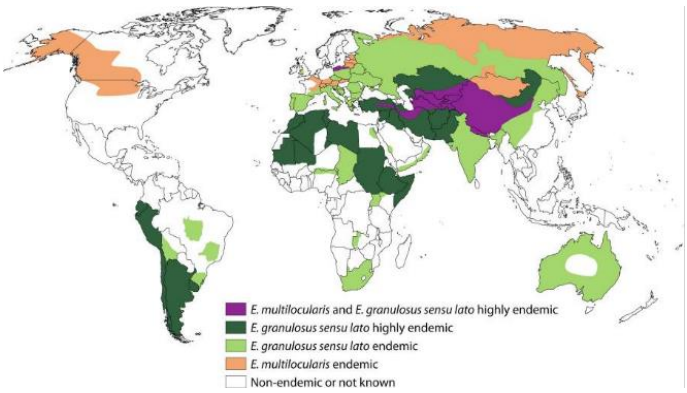

Fig. 13: Global distribution of *Echinococcus granulosus sensu lato*, responsible for Cystic Echinococcosis (CE), and *Echinococcus multilocularis*, responsible for Alveolar Echinococcosis (AE) (Woolsey and Miller 2020).

Life style

Carnivores (canines and cats) are the ultimate hosts of adult tapeworms, and herbivorous prey (ungulates, rodents and lagomorphs) are the intermediate hosts of tapeworms. However, in some unique and unusual circumstances, such as those reported in Turkana, Kenya, humans can act as intermediate hosts, but humans are usually not directly involved in transmission (Woolsey and Miller 2020). Adult worms develop in the intestine of their final host; the last segment (or anterior segment) of each worm matures to produce eggs, which are released into the external environment through faeces of carnivores. In turn, humans or intermediate hosts ingest eggs, which hatch in the intestines and release floats that pass through the portal vein and lymphatic vessels to reach the liver, where they usually settle and develop as larvae (metacysts or hydatid cysts). Occasionally, they may also reach the lungs, brain, bones or other organs of humans or intermediate hosts. The protoschi, the fertile form of the parasite, is asexually produced by the cysts and is released into the hydatid fluid; when it is finally ingested, the protoschi, with the help of bile salts, makes the scrotum everted. After the intestinal wall, they develop into mature egg-laying adults (Hao et al. 2019) (Fig. 12).

Epidemiology

Cystic Echinococcosis is distributed all over the world and is a major public health problem in some regions (Fig. 13). It is recorded in Peru, Chile, Argentina, Uruguay, southern Brazil, Mediterranean region, Central Asia, western China, and East Africa. It is an endemic problem. Cystic Echinococcosis has not been found in Antarctica. It has been eliminated through comprehensive control plans in Iceland, New Zealand, Tasmania, Falkland Islands and Cyprus; in addition, it has been previously recognized as endemic in France, Switzerland, Germany and Austria. The incidence of adverse events has doubled. As far as North America is concerned, the central and northern United States, the northwestern part of Alaska, and the northwestern part of Canada have long been endemic to *E. multilocularis*. It is expanding, at least in part due to increased and improved sampling efforts and targeting end hosts other than foxes (such as coyotes). Until recently, Cystic Echinococcosis was considered a mainstream human health problem in North America, except for Alaska, and Echinococcus has not been reported from Mexico or the southern United States.

Pathogenic mechanism

The pathogenic effects of Echinococcus on humans and animals include mechanical compression, toxic effects, and allergic reactions. The severity of symptoms depends on the size, location and number of hydatids. Echinococcus parasitizes mostly in the liver of animals, followed by the lungs. Mechanical compression can cause atrophy of the surrounding solid tissues and severe functional disturbances in the affected parts. After the metabolites are absorbed, the surrounding tissues will cause inflammation and systemic allergic reactions. The disease can be fatal, and the harm to humans is particularly obvious. *Echinococcus multilocularis* is more harmful to humans than *Echinococcus granulosus*.

Symptoms

Cystic Echinococcosis is usually asymptomatic, unless there are complications. Rupture of cysts can lead to infection or allergic reactions and fistula formation in adjacent structures (such as biliary tract, intestine, and

bronchus). Mass effect on adjacent structures is the main mechanism for the usual symptoms of cysts. Incidental discovery of cysts during imaging studies conducted for other reasons are not uncommon.

Diagnosis

Imaging technology is essential for diagnosis of Echinococcosis. Ultrasound, which is relatively cheap and portable, is widely used to diagnose CE or AE induced liver lesions; X-rays are used for lung cysts. Both techniques are also used for diagnosis and population screening, and follow-up. Serology, which involves detection of specific antigens, is also used for Echinococcosis diagnosis (Mcmanus et al. 2003).

Magnetic resonance cholangiopancreatography (MCRP) is also useful in preoperative assessment of complications such as bladder-biliary fistula. This technique is also used in identifying bile duct obstruction. Like MCRP, endoscopic retrograde cholangio-pancreatography (ERCP) is also non-invasive and used for diagnostic purposes. Bladder-biliary fistulas become obvious only after the internal capsule ruptures (mainly CE2 and CE3b). The sensitivity and specificity of MRCP in detecting bladder-biliary fistulas at these stages are 75 and 95%, respectively (Kahlfu et al. 2004).

Clinical laboratory analyss, including chemical and hematological tests, is non-specific in patients with Cystic Echinococcosis. For patients with biliary obstruction, increased levels of bilirubin, transaminase and gamma-glutamyltransferase can be observed. In the case where the cyst leaks into the bile duct tree or the cyst ruptures, a significant increase in γ-glutamyltransferase, alkaline phosphatase and eosinophil counts can also be observed. However, such findings are usually not observed in cases of intact cysts.

Conclusion

Parasitic diseases mainly occur in areas dominated by animal husbandry, and the prevalence is usually higher in rural areas than that in urban areas. Attention needs to be paid to hygiene and deworming during the breeding process to avoid the widespread transmission of parasitic diseases among populations. In this chapter, description regarding the main zoonotic parasites that are prevalent in China, including *Toxoplasmosis*, *Trichinosis*, *Fasciolopsis buski*, *Cryptosporidiosis*, *Clonorchiasis*, *Cysticercus*, *Schistosomosis japonicum* and *Echinococcosis* has been given. It is hoped that more knowledge of parasites will be widely known to everyone, which will help reduce the incidence of parasitic diseases in livestock and humans worldwide.

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SEXUALLY TRANSMITTED PARASITIC INFECTIONS BOTH IN HUMANS AND ANIMALS

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INTRODUCTION

The increasing numbers of infectious diseases are believed to be sexually transmitted, having great human and veterinary health concerns. Trichomoniasis is one of the most common parasitic, non-viral, and sexually transmitted disease worldwide. Different symptoms are reported in the patients such as dysuria and vaginal discharge in women, whereas dysuria and urethral discharge is seen in men (McClelland et al. 2007). Many of the infected people may be asymptomatic. It is also a major public health concern, as it increases the incidence and risk of HIV (Van Gerwen and Muzny 2019). Giardiasis may lead to marked economic losses and severe public health problems (Minetti et al. 2016). Amoebiasis is another protozoan infection and mostly asymptomatic in nature (Haque et al. 2003). Worldwide, it has been assessed that in developing countries ≥30 million peoples are infected by *E. histolytica,* which causes ≥100000 deaths a year (Bercu et al. 2007). Giardiasis is an important enteric zoonotic disease of humans and animals worldwide. It affects ≥300 million individuals per year (Minetti et al. 2016). Scabies is a mite infestation; it prompts the predominantly nocturnal itching with some papules in particular sites including sex organs of the host (Singhal et al. 2017). Scabies is a health-related disorder from the centuries, initiated by digging the mites, but its significance is often underestimated. Despite their problems and risks, parasitic infections, including sexually transmitted diseases, are vastly understudied and have received considerably less public attention (Chen et al. 2020; Lin et al. 2020; Mohsin et al. 2021a; 2021b). This chapter aims to elaborate the importance of sexually transmitted parasitic infections and possible protocols for controlling these diseases.

Trichomoniasis

Trichomonas vaginalis is one of the most common parasites and sexually transmitted organisms worldwide. Various symptoms are reported in the patients having clinical *T. vaginalis,* such as dysuria and vaginal discharge in women whereas dysuria and urethral discharge in men (Wendel et al. 2003). Many of the infected people may be asymptomatic. Untreated and prolonged infections in females are frequently linked with birth-related issues and infertility (Cotch et al. 1997). Although relatively less common in men than in women, *T. vaginalis* is a frequent cause of epididymitis, prostatitis, and nongonococcal urethritis (NGU). It is also a major public health concern, as it increases the incidence and risk of HIV (McClelland et al. 2007). Various treatment regimens are employed, which are mainly based on the oral administration of metronidazole. But the treatment is often unsuccessful, has many adverse effects, and the infection relapses after cessation of therapy. Intra-vaginal drug delivery is also a lucrative option available for physicians. Despite its problems and risks, *T. vaginalis* is understudied problem and has received considerably less public attention (Van Gerwen and Muzny 2019).

Epidemiology

According to WHO data, more than half (about 276.4 million) of the total treatable venereal diseases (498.9 million) are caused by *T. vaginalis.* In particular, it is higher in the Americas and Africa as compared to the other regions. Various studies conducted in Western Europe have shown that the incidence of Trichomoniasis is decreasing in industrialized and developed nations. Studies in Iran have given the prevalence rates of 2-8% however, it can be an underestimation due to socio-economic factors and the real figure can be as high as 30% (Matini et al. 2012; Bouchemal et al. 2017; Van Gerwen and Muzny 2019). Although no association between HIV and *T. vaginalis* infection was reported in the US, many studies in the African region have confirmed that the incidence of *T. vaginalis* infections is higher in the HIV patients and vice versa (Bouchemal et al. 2017; Van Gerwen and Muzny 2019). The exact mechanism about

how *T. vaginalis* increases HIV incidence is not understood but *T. vaginalis* is responsible for the severe inflammatory response, thus facilitating the HIV establishment in the vagina. Another study conducted in the United States has revealed that *T. vaginalis* contributes to HIV and its contribution exceeds the contributions of other sexually transmitted diseases (STDs) (Sorvillo et al. 2001).

T. vaginalis is a sexually transmitted infection, affecting both of the sexes equally. A study on women conducted in Norway has elaborated that Trichomoniasis enhanced the likelihood of cervical neoplasia (CN) due to human papillomavirus (HPV) (Gram et al. 1992). A similar correlation between Trichomoniasis and HPV-induced cervical cancer was reported in India (Ghosh et al. 2017) and Finland (Viikki et al. 2000). Another study showed that Trichomoniasis facilitated HIV establishment by a factor of 6.5, thus resulting in cervical cancer (Lazenby et al. 2014).

Some strains of the *T. vaginalis* can themselves act as carriers of various viruses which further increase the inflammatory response. The Trichomonas virus emitted by the *T. vaginalis* infected patients causes further inflammation on medication with metronidazole (Fichorova et al. 2012). It is also a well-established fact that *T. vaginalis* can be transmitted by a pregnant female to her fetus. *T. vaginalis* does not mainly infect young women of non-productive age (15-25 years), which is in contrast to other STDs. It mainly affects women of productive age, with the incidence rates being highest among the women of 35-40 years of age (Cudmore et al. 2004). Various predisposing factors increase the incidence of the problem, for example smoking, prostitution, using oral contraceptives, marital status, low socioeconomic background and old age. The incidence, severity, prevalence and duration of the *T. vaginalis* infection depend on the individual's health status and availability of healthcare facilities, as shown in Table 1.

Pathogenesis and clinical presentation

T. vaginalis is a protozoal parasite. It is mainly pyriform in shape but changes its shape in response to its physiochemical environment. It has five flagella (four located at the anterior end and the fifth at the opposite end). These flagella help in its attachment to various surfaces and also in movement. *T. vaginalis* lacks mitochondria and possesses hydrogenosomes as alternate energy sources (Hardy et al. 1969). After initial attachment, the parasite changes its shape to an amoeboid form, which enhances its contact with epithelial cells of vagina and adhesion to various target cells. Five attachment proteins called adhesions help in the attachment process. Various other proteins and conjugated molecules, like fibronectin, and lipophosphoglycan, are also involved in the attachment process (Harp and Chowdhury 2011). The discharge from the vagina of the infected female is rich in polymorphonuclear leukocytes. The involvement of these cells and *T. vaginalis* initiates signaling pathways, leading to the production of interleukins (IL-6 and IL-8), tumor necrotic factor-alpha (TNF-α) and macrophage chemoattractant protein-1 (MCP-1). Many mitogen-activated protein kinases (MAPKs) signaling pathways, such as p38, c-jun N-terminal kinase (pJNK), and extracellular signal-regulated kinase ½ (ERK1/2) pathways, can become active. This vast array of signaling pathways increases the mRNA expression of toll-like receptors (TLRs) (Chang et al. 2006). Finally, it seems that MAPKs are responsible for the induction of apoptosis by the activation of Bcl-XL (a Bclo2 type protein) and NF-κB in the macrophages. The research is still underway to understand host-parasite interactions and it can possibly lead to the discovery of new molecular targets to design novel trichomonacidal drugs (Van Gerwen and Muzny 2019).

An estimated 25-50% of the infected females are asymptomatic. Other infected women can develop severe symptoms, which are cyclic in nature and can be more severe during the menstruation phase. Among the women who are culture positive for *T. vaginalis,* only 11-17% show pruritus, abnormal odor, dysuria, vaginal discharge, and vaginal burning (Landers et al. 2004). The "strawberry cervix" develops in only 2% of infected women (Fouts and Kraus 1980). The vaginal pH in adult and healthy women is around 4.0. During the infection of *T. vaginalis* vaginal pH can increase to more than 7.0 and facilitates the parasitic growth. The changes in blood hormones levels and vaginal pH can account for more severe symptoms in the menstruation phase. Moreover, the menstrual debris and blood create a rich media with high pH and increased concentration of iron, further stimulating the reproduction and attachment of *T. vaginalis* to the vaginal epithelium and the consequent worsening of symptoms (Harp and Chowdhury 2011). Although the *T. vaginalis* is mostly localized in the lower urogenital tract, it can occasionally cause pyosalpinx and adnexitis, leading to serious life-threatening consequences, particularly during pregnancy (Bouchemal et al. 2017).

Diagnosis

T. vaginalis was first discovered in 1836 by Donne. He noticed motile microbes in the vaginal discharge of women showing signs of infection. However, this visualization technique, called the wet mount test, is markedly less sensitive (38-82% in the symptomatic patients) (Bhesania and khedkar 2016). This wet mount technique is now-a-days mainly used for drug sensitivity evaluation. The viable organisms can only be observed if the test is performed within few minutes of sample collection. Specialized media, such as the Trichosel medium and Diamond's medium, are required for the culture of vaginal discharge. However, culture test has a number of disadvantages. It can only be performed in the laboratories having incubator and culture medium and requires at least 7 days for the identification of *T. vaginalis.* However, the inPouch device is developed to improve the culture conditions and this device is commercially available. This device comprises two-chambered plastic bags. The chambers are connected by a

Table 1: The prevalence and incidence of *T. vaginalis* in men and women of age group 15-49 years across various regions of the globe (WHO 2014; Bouchemal et al. 2017)

Regions	Prevalence				Incidence			
	Men		Women		Men		Women	
	Millions of individuals	%	Millions of individuals	%	Millions of individuals/1000		Millions of individuals/1000	
The Americas	5.2	2.2	52.7	22	43	180.6	42.5	177.7
Europe	1.3	0.6	13.0	5.8	10.9	48.4	11.6	51.7
Africa	3.9	2.0	38.9	20.2	31.6	164.8	28.1	146
Southeast Asia	3.0	0.6	25.7	5.6	24.3	50.1	18.5	40.3
Eastern Mediterranean	1.3	0.8	12.0	8.0	10.6	66.1	9.7	64
Western Pacific	2.9	0.6	27.2	5.7	23.8	47	21.9	45.6

narrow passage. The collected sample is put in the upper chamber and the lower chamber is used for culture and further observation. By the usage of Diamond's modified media, this device shows as much sensitivity as the culture method (Sood et al. 2007). The PCR-based diagnostic tests can detect very few organisms, even if they are dead. An amplification test (developed by Becton Dickinson, Sparks, MD) based on RNA, called Affirm VPIII test, can detect the *T. vaginalis* in less than an hour. The sensitivity and specificity values of this test are 90 and 99%, respectively (Harp and Chowdhury 2011). Another newly developed test is OSOM *Trich* rapid antigen test. It is an immunochromatographic capillary flow enzyme immunoassay that detects the membrane protein of *T. vaginalis*. This test is quite rapid as compared to the culture and gives results in ten minutes only. It can also be performed on frozen samples without affecting the results (Huppert et al. 2005). Many new diagnostic tests are available now-a-days. Nucleic acid amplification tests (NAATs) are also commercially available which can be used for both symptomatic, as well as asymptomatic, females and can be conducted on a variety of samples including vaginal, urethral, cervical and urinary specimens.

Treatment

Currently, five nitromidazole drugs are the most commonly available options for treating *T. vaginalis* infections and are given through either parenteral or oral routes. Among these, only tinidazole and metronidazole are FDA-approved drugs for the treatment of *T. vaginalis* and are available in US market. Metronidazole (Flagyl) was approved in the 1960s and it gives good cure rates after systemic administration. As the sexual partner(s) of an infected patient are most often also infected and many of them can be asymptomatic, the treatment of partners is also recommended due to the fear that they can re-infect their partner (Workowski and Berman 2006; Kissinger et al. 2006). Metronidazole is an effective, less costly, and easily tolerated drug and side effects (e.g. gastrointestinal problems) are most often mild. Rarely, neurotoxic and hematological effects are also reported. The resistant and recurrent infections of *T. vaginalis* can be treated with longer administration of the drug at higher doses. However, the chances of adverse side effects, treatment failure, and patient discomfort are also increased at higher doses (Howe and Kissinger 2017). But if no alternative treatment is available, the increased dose of metronidazole is the only option left. However, some cases are not treatable, even with higher doses of metronidazole and they pose a severe challenge for both the physician and the patient. The higher doses of tinidazole and metronidazole, along with vaginal drug administration, can be a useful option for the resistant cases (Sobel et al. 2001).

Tinidazole is a nitroimidazole introduced in 1969 for treating *T. vaginalis* infections. It gives good curative results at doses lower than metronidazole with even milder adverse effects (Raja et al. 2016). Its minimum lethal dose is also lesser than that of metronidazole and no resistance has been reported so far (Kirkcaldy et al. 2012).

The mechanism of *T. vaginalis* resistance to the metronidazole is not fully elaborated but it is hypothesized that it can be due to mutations in the parasite. In the case of anaerobic resistance, the activity of an important enzyme, called pyruvate ferredoxin oxidoreductase, is decreased or diminished in *T. vaginalis* (Kulda 1999). The loss of activity of the flavin reductase enzyme can cause both anaerobic and aerobic resistance (Leitsch et al. 2014). Although nitroimidazoles are effective compounds for the treatment of *T. vaginalis* infections, many other drugs such as nithiamide and disulfiram can be useful alternatives for patients who are hypersensitive to the nitroimidazole compounds (Van Gerwen and Muzny 2019). Mebendazole and albendazole have also shown *in vitro* effectiveness against *T. vaginalis*. Nitazoxanide, which is a 5-nitrothiazolyl derivative, is effective *in vitro* with IC_{90} and IC_{50} values of 2.046 and 0.034 µg/ml, respectively, although occasional treatment failures are also reported (Navarrete-Vázquez et al. 2003). So, it can be elucidated that only a few alternatives to metronidazole and its related compounds are available and there is a dire need to discover new alternatives for the patients who are resistant to the already available compounds.

Preventive measures

The use of chemotherapeutics is controversial because it results in the development of chemotherapeutic refractory strains. Vaccination for *T. vaginalis* is particularly important for high-risk individuals to protect both partners. Vaccination can be a solution to many of the problems encountered in the control measures. Vaccines against *Trichomonas fetus* are already prepared and available commercially. This is a flagellate parasite

closely related to *T. vaginalis,* and infects cattle. Various research reports confirm the usefulness of the vaccine against *Trichomonas fetus* for the protection and clearance of genital infections caused by this parasite (Chapwanya et al. 2016; Edmondson et al. 2017). As both of these parasites are quite similar to each other, it can also be possible to develop a vaccine for humans which can protect them against *T. vaginalis* infections. The huge economic losses suffered by the US dairy industry have stimulated the development of vaccines for *T. fetus,* while efforts to develop the equivalent vaccine for human infections of *T. vaginalis* are still lacking. The development of a long-lasting vaccine against *T. vaginalis* is a challenging task given that the infection does not develop long-lasting immunity after recovery. Intra-vaginal vaccination in the 1960s using heat-killed *T. vaginalis* and using abnormal heat-killed strains of lactobacillus in 1970 reported limited success, hence systemic administration was considered. Another report has elucidated that subcutaneous administration of the vaccine in the mice increased the clearance and decreased the incidence of *T. vaginalis* (Smith and Garber 2015).

Male circumcision is another major way of preventing the spread of *T. vaginalis* infections. Various randomized trials have confirmed that the sexual partner of a circumcised male has lesser chances of acquiring *T. vaginalis* infection (Bailey et al. 2007). A study conducted in South Africa has proved that female-to-male transmission of infection is also lower in the case of circumcised men. A possible explanation of this phenomenon can be the fact that the sub-preputial space in the uncircumcised males is wet, which increases the chances of *T. vaginalis* survival in such males (Asemota 2018). However, many other studies conducted in various other countries have shown no correlation between men circumcision and the transfer of infections (Turner et al. 2008; Bouchemal et al. 2017; Asemota 2018). Vaginal administration of microbicide agents presents other alternatives to the venereal acquisition of *T. vaginalis* infections. These agents are administered to the women prior to sexual intercourse. This practice can limit the *T. vaginalis* interaction with the host cells (Van Gerwen and Muzny 2019). A study has shown that intra-vaginal administration of metronidazole gel (500 µg/ml) completely prevented the infection in the mice (Lushbaugh et al. 2000).

Giardiasis

Giardiasis is an important enteric zoonotic disease of humans and animals worldwide with great economic importance. Diarrhea is the main cause of mortality in children under 5 years of age, however, it is treatable. In young children, it is a primary reason for malnutrition (poor nutrients absorption) (Kotloff et al. 2013). *Giardia intestinalis* (*G. intestinalis*), also called *G. duodenalis* and *G. lamblia*, is the unicellular parasite, belongs to phylum protozoa, infects both human and animals, and leads to intestinal problems like diarrhea (Ankarklev et al. 2010). Giardia species with their specific structure are mentioned in Table 2.

It exists in two forms, the cyst and the trophozoite. Trophozoites are teardrop-shaped, having 2 distinct nuclei, 4 pairs of flagella, and an adhesive disc on their ventral concave surface. The cysts are oval in shape, having 4 nuclei (Meyers et al. 1977). Worldwide, the common clinical sign of Giardiasis is diarrhea and every year about 180 million diarrheal cases are reported in humans (Ryan et al. 2019). The prevalence of this disease in developing countries ranges between 1.5 and 73.4%, with a large number of asymptomatic infections (Cacciò and Ryan 2008). In Giardiasis, immuno-deficient persons are mostly at high risk (Simsek et al. 2004; Prado et al. 2005; Nematian et al. 2008).

Incidence and burden of disease

G. lamblia has been documented as a common pathogen worldwide (Pires et al. 2015). In endemic areas it has great importance in both public and veterinary health (Yaoyu and Xiao 2011). According to the World Health Organization (WHO), approximately 183 million cases have been reported due to Giardiasis in Africa, Asia, and America (Torgerson et al. 2015). It has been estimated that the prevalence of Giardiasis cases is approximately 2.8 x 10^8/year over the globe. *Giardiasis* is linked with poor hygienic status, overpopulation, and low socioeconomic position (Rodríguez-Morales et al. 2016). It is a universal infection and covers a wide range of area in the world. Generally, cysts of Giardia are highly infectious, as only 10 cysts in a living organism may lead to Giardiasis. In developing countries among children, the incidence of this disease has shown a constant increasing trend.The prevalence of this disease is mostly in the summer season, with endemic or regular outbreaks occur even in the developed countries of the world (García-Cervantes et al. 2017). A study including 242 outbreaks, influencing 41000 individuals, revealed that disease transmission was due to intake of contaminated water (75.0%) and food (15.7%), individual to individual (2.4%), and animal contact (1.3%) (Adam et al. 2016). Through surveillance data, it has been observed that peak of Giardiasis infections occur at 0-9 years and 45-49 years of age groups, both in male and female individuals (Painter et al. 2015). The list of the population which is considered at high risk due to this disease includes:

1. Children who attend daycare centers (Duffy et al. 2013).
2. Adults who work in a daycare center (Thompson 1994).
3. Institutionalized individuals (Mascarini and Donalísio 2006).
4. Immunocompromised individuals (Abaza et al. 1995).
5. International travelers (Holtan 1988).

Pathogenesis and clinical features

Clinical manifestation of Giardiasis appears at 1-2 weeks after the infection. In Giardiasis, diversity of host and environmental factors can affect the disease outcomes.

Table 2: Giardia species with their host and morphology (Cacciò and Ryan 2008; Kotloff et al. 2013)

Species	Host	Morphology
G. lamblia	Dog, human including some wild species	Teardrop like appearance, having a claw-shaped median body.
G. ardeae	Herons, birds	Shaped like that of *G. lamblia.*
G. agilis	Amphibians	As compared to *G. lamblia,* slender and longer with a teardrop-shaped median body
G. psittaci	Psittacine birds	Shaped like that of *G. lamblia.*
G. muris	Rodents	As compared to *G. lamblia,* rounder and shorter with a small rounded median body
G. microti	Muskrats, Rodents and voles	Shaped like that of *G. lamblia.*

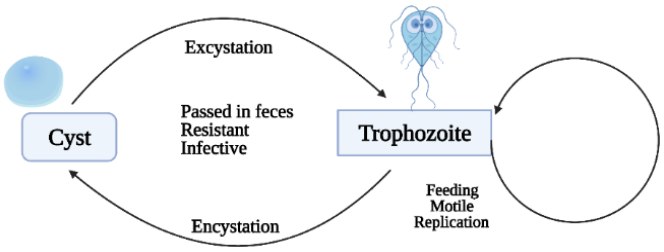

Fig. 1: The typical fecal-oral life cycle of Giardia parasite.

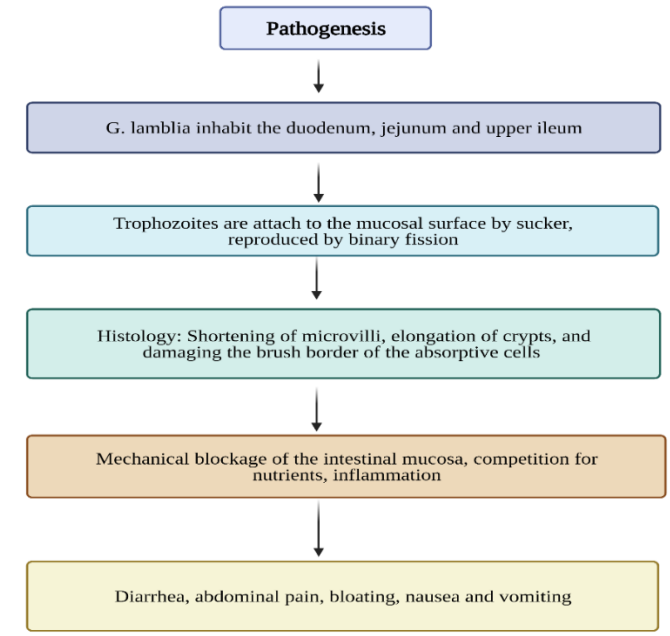

Fig. 2: Possible pathogenesis schematic diagram of Giardiasis.

These factors include age, diet, immune factor, gut microbiota, as well as concurrent infection (LJ et al. 2010; Cotton et al. 2015; Bartelt and Sartor 2015; Bartelt and Platts-Mills 2016; Beatty et al. 2017; Barash et al. 2017; Allain et al. 2017). Symptoms of acute Giardiasis include abdominal pain, watery diarrhea, weight loss, and vomiting. In case of chronic infection, malabsorption with weight loss and malaise are considered as well-known clinical signs (Ortega and Adam 1997; Gascón 2006). Moreover, stunted growth in children has also been documented (Rogawski et al. 2017). Giardia causes the non-invasive disease with mild inflammation and without toxin secretion (Buret 2007; Ankarklev et al. 2010). The trophozoites of Giardia may damage epithelial cells of the enteric system and leads to villus atrophy, loss of digestive enzymes on enteric epithelial cells of intestinal brush border and ultimately resulting in malabsorption of nutrition, water, electrolytes and maldigestion (Céu Sousa et al. 2001; Troeger et al. 2007; Ringqvist et al. 2008; Solaymani-Mohammadi and Singer 2011; Humen et al. 2011; Cotton et al. 2011; Liua et al. 2018; Dubourg et al. 2018). The typical life cycle and pathogenesis of Giardiasis are shown in Figures 1 and 2, respectively.

Preventive measures

For prevention of this parasitic disease, different treatments (Table 3) and protective measures can be adopted, as summarized below:

1. Eat and drink healthy and clean food and water.
2. Enjoy safe sex.
3. Provision of convenient purification unit for travelers.
4. Public education on the spread of infection and the method to prevent it.
5. Use of double strength iodine for killing the Giardia.

Amoebiasis

Amoebiasis or amoebic dysentery is a protozoan infection caused by *E. histolytica*. Most of the time infection is asymptomatic, but invasive intestinal infection can occur, exhibiting abdominal pain or cramping, bloody or watery diarrhea, and weight loss (Haque et al. 2003). Worldwide, it has been assessed that in developing countries ≥30 million peoples are infected by *E. histolytica,* causing ≥100000 deaths in one year (Bercu et al. 2007). The global prevalence of *E. Histolytica* is shown in Table 4. Transmission of this disease usually occurs as a result of consumption of food and water contaminated with fecal material containing cysts. Even fecal-oral spread can occur within the household and during male homosexual activities (Haque et al. 2003; Cheepsattayakorn and Cheepsattayakorn 2014).

Amoebiasis exists in both trophozoites and cysts form. The trophozoite is motile due to the presence of pseudopodia. Its multiplication occurs through binary fission within the intestinal mucosa. Before being excreted into the stool, trophozoites become rounded and develop into cysts within the intestinal lumen (Bercu et al. 2007). Trophozoites are highly brittle and may not encyst after excretion. A mature cyst is about 12.0 mm in diameter. The cyst is denatured in the small intestine when consumed by a susceptible host. The single amoeba then migrates to the large intestine and develops into a trophozoite, which then encysts and completes the life cycle (Botero 1994). The *E. histolytica* infection is mainly asymptomatic with only 10-20% patients show encyst

Table 3: Efficacy of anti-Giardia drugs with dose rates (Gardner and Hill 2001)

Drug	Dose rate	Efficacy range (%)
Bacitracin Zinc	240000 U/day x 10 days	95
Paromomycin	10-50mg/kg/day or 1500mg/day x 5-10 days	55-88
Albendazole	200-800mg/day x 1-3 days	24-81
Furazolidone	400mg/day x 7-10 days	80-85
Quinacrine	300mg/kg x 5-7 days	95-100
Secnidazole	2g single dose	86-100
Ornidazole	1-2g single dose	96-100
Tinidazole	50mg/kg single dose	80-96
	1-2g single dose	86-100
Metronidazole	500-750mg/kg x 5-10 days	60-95
	2-2.4g single dose	36-60

Table 4: The global prevalence of *E. histolytica*

Region	Prevalence	Reference
South Africa	*E. histolytica* (12.4%)	(Samie et al. 2008)
Dakar, Senegal	*E. histolytica* (5.1%)	(Gassama et al. 2001)
Ethiopia	*E. histolytica* (10.3%)	(Hailemariam et al. 2004)
Uganda	*E. histolytica* (1.4%)	(Brink et al. 2002)
Mazandaran province, Iran	*E. histolytica* (1.6%)	(Daryani et al. 2009)
Sydney, Australia	*E. histolytica/dispar* (3.2%)	(Stark et al. 2007)
India	*E. histolytica* (3.6%)	(Mukherjee et al. 2010)
Bangladesh	*E. histolytica* (2.1% vs 1.4% in diarrhea and control)	(Haque et al. 2009)
Taiwan	*E. histolytica* in HIV patients 5.8%	(Hung et al. 2008)
Northern India	*E. histolytica* (7.7%)	(Prasad et al. 2000)
Tajikistan	*E. histolytica/dispar* non -HIV (25.9%)	(Matthys et al. 2011)
Mexico	25.9%	(Moran et al. 2005)
Brazil	*E. histolytica/dispar* (3.3% and 1%)	(Bachur et al. 2008)
Venezuela	*E. histolytica* (10.8%)	(Rivero et al. 2009)
San Pedro Sula	*E. histolytica* (5.8%)	(Lindo et al. 1998)
Bogota (Colombia)	*E. histolytica* (13%)	(Missaye et al. 2013)
Cuba	*E. histolytica/dispar* (1.5%)	(Escobedo and Núñez 1999)

after excretion. A mature cyst is about 12.0 mm in diameter. The cyst is denatured in the small intestine when consumed by a susceptible host. The single amoeba then migrates to the large intestine and develops into a trophozoite, which then encysts and completes the life cycle (Botero 1994). The *E. histolytica* infection is mainly asymptomatic with only 10-20% patients show symptomatic infection. Recently, it was found that *Prevotella copri* is enriched in the gut microbiome with amoebic dysentery, representing that dysbiosis may subsidize susceptibility to the progress of colitis (Ngobeni et al. 2017).

Pathogenicity of amoebiasis

Based on the site of infection, *E. histolytica* can lead to intestinal and extraintestinal amoebiasis. Pathogenic form of the parasite stimulates the enzymes that assist its attack into the mucosa and submucosa, leading to the deep-flask shaped sores. In some cases, it can enter into the circulation and reach the inner organs such as skin, lungs, liver, kidney and spleen etc. The disease in the colon usually accounts for 90% of the clinical cases characterized by acute diarrhea and dysentery (Chadee et al. 1987; Espinosa-Cantellano and Martínez-Palomo 2000), with 1% liver involvement (Haque et al. 2003). The pathogenicity of this disease path route and virulence factors are shown in Figure 3 and Table 5, respectively.

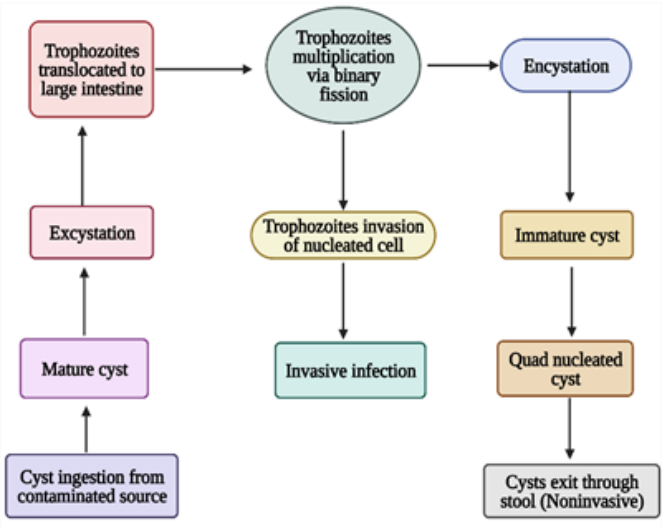

Fig. 3: Pathogenesis of *E. histolytica*; from ingestion to extra-intestinal hepatic invasion (Tharmaratnam et al. 2020).

Diagnosis

For several years, stool culture method pursued by isoenzyme analysis was regarded as a "gold" standard. The assortment of *E. histolytica* and *E. dispar* has been diagnosed by this method (Clark and Diamond 2002). Numerous assays, such as indirect hemagglutination (IHA), counterimmunoelectrophoresis (CIE), indirect immunofluorescence assay (IFA), enzyme - linked

Table 5: Virulence factors of *E. histolytica* with their outcomes

Virulence factor	Characteristics	Outcomes	References
Amoeba pore	Stored in cytoplasmic granules and released following target cell contact; form ion channels in the membranes of both eukaryotic cells and phagocytosed bacteria	Direct accountable for the cytolysis of host cells	(Leippe et al. 1991; 1992)
Cysteine proteinase	Host protein damage; delivers proteolytic cascades through degrading mucus, debris, and host cells	These are an amazing possible target for the medication of amoebiasis and their possible role in promoting invasion	(Luaces and Barrett 1988; Leippe et al. 1992; Que and Reed 2000)
Gal/GalNAc-binding lectin	Cell capping and endocytosis; cytotoxicity; resistance to the complement; polymerization of actin	This versatile virulence factor plays important role in parasite pathogenicity and a particular vaccine applicant	(Saffer and Petri 1991; McCoy et al. 1994 ; Ramakrishnan et al. 1996)

Table 6: Some commercially available antibody assay for the diagnosis of Amoebiasis

Serological Assay	Specificity (%)	Sensitivity (%)	References
IHA	97.5	93	(Robert et al. 1990)
Novagnost Entamoeba (IgG recognition)	≥ 95	≥ 95	(Knappik et al. 2005)
IHA	90.9-100, 99.8	100, 99	(Pillai et al. 1999)
RIDASCREEN Entamoeba (IgG detection)	95.6, 97.4 (100)	100, 97.7-100 (100)	(Knappik et al. 2005)
Amoebiasis serology microplate	97	95	(Hira et al. 2001)

Table 7: Laboratory diagnostic test for Amoebiasis (Alvarado-Esquivel et al. 2015; Ryan et al. 2017)

Method	Specificity (%)	Sensitivity (%)	Benefits	Consequences
Stool antigen detection	≥80%	0-88%	Increasing endemic areas There are many endoparasites to be detected, with a fast turnaround time and commercially available combination tests	Poor sensitivity to amebic liver abscess Needs fresh, non-fixative stool preserved to be analyzed
PCR	92-100%	89-100%	Gold standard; high sensitivity, colitis, and hepatic abscess specifications with increased availability. Rapid reverse, the automated system reduces engineering time and contamination risk Contaminated with multiplex panels for detection of multiple at a time.	More costly; the cost can restrict resource-limited usage Require an analytical instrument, kit, and a skilled technician --------
Serology	≥90	65-92%	High sensitivity and specificity	Serology remain positive for the year after resolution of infection so less helpful in an endemic area
Microscopy	-----	≤60%	Largely available Screen for other parasites	Poor sensitivity and specialty, Multiple stool needs to be submitted

immunofluorescent assay (ELISA), and immunoelectron-phoresis, have been developed for the recognition of *E. Histolytica* infection (Fotedar et al. 2007). For the diagnosis of this disease, some diagnostic protocols are shown in Tables 6 and 7.

Prevention

Amoebiasis can be treated by metronidazole and tinidazole (Kimura et al. 2007). No commercial vaccine is available for Amoebiasis. To control the infection, focus needs on primary prevention efforts such as water and food safety, hand hygiene, and prevention from exposure to fecal-oral through sexual activity. Before traveling to endemic areas, i.e. South America, Asia, Saharan Africa, food safety individuals should be advised to avoid enteric illness, as amebic colitis and an amoebic liver abscess may occur years after travel. Loperamide should be dodged in amoebic colitis (McGregor et al. 2007). Household associates of patients with Amoebiasis must be separated, as the infection can be transmitted among the family and household contacts (Shirley and Moonah 2016).

Scabies

Scabies is a mite infestation; it occurs due to *Sarcoptes scabies*. It prompts the predominantly nocturnal itching with some papules at a particular site i.e. flexors fold, cubital margins of hands, the anterior side of the wrists, anterior axillae, around nipples and navel, internal side of thighs, and external male genital organs. Worldwide, ≥300 million individuals are affected per year (Stadtländer 2005). Scabies is an infection initiated by digging the mites (Ectoparasite). For centuries, it has persisted in health problems, while its significance is often underestimated. *Sarcoptes scabiei var hominis* is a possible cause for human scabies. The mite develops four life stages including eggs, larva, nymph, and adult. Usually, female deposits some (2-3) eggs a day and burrows them under the skin. The eggs are converted to larvae, which migrate to the skin and are changed into nymphs. The nymphs molt into adults and then mating occurs, the impregnated female burrows back beneath the skin and persists for the rest of the life period (~ 30-60 days) (Currie and Carapetis 2000). Males are occasionally seen;

Table 8: Difference between classic and neonatal Scabies with respect to their lesions and treatment (Singhal et al. 2017; Ong and Vasanwala 2018)

	Classic Scabies	Neonatal Scabies
Site of infestation	Trunk, genitals region, soles and palms area, Interdigital webs, and skin above the neck.	Soles, face, and palm.
Lesions and burrows	Eczema, pustules, papules, excoriation, and burrow.	Ulceration, pustules, vesicles, erosions, and burrow usually absent.
Excoriation and pruritus	Present.	Absent.
Drugs	Ivermectin 200mg/kg, 5% permethrin cream, and second dose after a week.	5% permethrin, topical sulfur treatment in petrolatum daily for 3 days.

they create temporary narrow pits in the skin to feed till they find a female burrow and mate with her. The common signs of Scabies include skin rash and lesions such as papules (2-3 mm) and larger nodular lesions, mostly 5-10 mm and sometimes more than 10 mm, with itching and can affect any part of the body such as the shoulder blade, elbow, wrist and the armpit (Alvarado-Esquivel et al. 2015). The earliest symptom of the disease is extreme itching, particularly at night time. The pimple, rash and narrow pits (skin) are common symptoms of Scabies. The face, palms, head, soles and neck are often affected in children, however commonly not in adults. Occasionally, small burrows are observed on the skin; these are created by the female organisms. These burrows appear as small, raised and crooked grayish-white colored areas on the surface of the skin (Allos 2001). Scabies is spread from human to human by the infestation of the skin through the itch mite. Female Scabies mite digs the surface layer of the skin and deposits the eggs. A mite passes through infested skin to skin from one person to others. An infected individual can transmit Scabies unless the individual does not show a sign and symptom. Scabies is also sexually transmitted in adults (Stadtländer 2005). The signs normally do not occur earlier when an individual is infected with scabies (first time). If an individual has already been infested with this disease, symptoms occur much earlier (1-4 days) after exposure. The mites do not normally live longer than 2-4 days, when removed from human skin. The life period of a mite on a host body is nearly 1-2 months (Hicks and Elston 2009). This disease can be diagnosed based on historic features and characteristic signs and symptoms. The visualization methods for Scabies are optical microscopy, video dermoscopy, reflecting confocal microscopy, and handheld dermoscopy (Alvarado-Esquivel et al. 2015). It is divided into two categories (i) classical and (ii) neonatal, as shown in Table 8.

Conclusion

Sexual intercourse can be a major transmission process for major parasitic diseases, including Trichomoniasis, Amoebiasis, Giardiasis, and Scabies. The oral-genital and oral-anal exchanges predispose male homosexuals to infection with these pathogens. These parasites can cause acute and chronic disorders, including abdominal symptoms like watery diarrhea, colitis, weight loss, and vomiting. Majority of the hosts infected with these diseases are asymptomatic but proper protocols of diagnosis and treatment are necessary for the control of these diseases. Anti-parasitic drugs are used against all these parasitic diseases but due to development of resistance, attention is diverted towards vaccination and use of medicines derived from plants. Vaccination against infectious diseases, plant derivative products, and probiotics are effective approaches against these pathogens (Lin et al. 2020; Mohsin et al. 2021a). The omics studies (Transcriptomic, genomics, proteomics) are considered the best and authentic alternative approaches for controlling the parasitic diseases in the modern age (Mohsin et al. 2021a; 2021b).

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NANOPARTICLES AS A NEW APPROACH FOR TREATING HYDATID CYST DISEASE

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INTRODUCTION

Hydatidosis or Cystic Echinococcosis (CE) is a zoonotic disease resulting from *Echinococcus granulosus* metacestode infection. Several investigations described it as an emerging or re-emerging infection, with high medical and economic impacts in many countries (Moro and Schantz 2009; Eckert e al. 2000). Also, it is classified as a neglected tropical illness (WHO 2001). The disease is endemic in rural sheep-raising areas, where dogs consume the infected animal organs. In Iraq, the disease is considered hyperendemic, with high socio-economic outcomes owing to the infection of both humans and their livestock (Benyan et al. 2013). Sadjjadi (2006) reported an increasing trend in CE cases in North Africa, Middle East, and Iraq. Three techniques for curing the Echinococcosis infection of liver are known; these include operation, percutaneous aspiration, and chemotherapy (Adas et al. 2009). Surgical procedure remains the primary therapeutic method for CE, but other procedures may play an efficient role in its management (Pitt and Pitt 2013). Leakage of cystic fluid rich with protoscoleces is the leading cause of recurrence; therefore, scolicidal materials are used for inactivating the protoscoleces during surgical operation. However, these materials have many adverse effects (Shi et al. 2016).

Hypertonic saline is applied as protoscolicidal material now-a-days. Still, it causes hypernatremia, which in turn, results in convulsions, intracranial hemorrhages, necrosis and degeneration, and myelinolysis (Albi et al. 2002; Adas et al. 2009). Benzimidazole is the main therapeutic agent used for CE therapy. Unfortunately, it shows several side effects, such as leucopenia, alopecia, liver toxicity, and thrombocytopenia (Junghanss et al. 2008), as has been shown in Table 1.

Additionally, Benzimidazole is characterized by its reduced water solubility, which results in its low bioavailability. Consequently, low gastrointestinal absorption leads to insufficient systemic availability and reduced efficacy against CE (Evrard 2002). For this reason, many experimental studies were conducted to increase the effectiveness of albendazole. Among these, Shuhua et al. (2002) prepared this medication in soybean oil emulsion and tested it in murine Echinococcosis. In addition, new plans were tested to determine novel protoscolicidal green synthesized compounds from these plants. In this regard, Kohansal et al. (2017) reviewed the publications published from 1996 to 2015 and concentrated on the plant extracts that exhibited significant protoscolicidal effectiveness. Moreover, the protoscolicidal properties of *Curcuma longa, Zingiber officinale*, and Cyperus *rotundus* extracts were examined (Almalki et al. 2017; Shnawa et al. 2017). The findings revealed the possible efficacy of these plant extracts against Echinococcosis.

Nanoparticles (NPs) possess a broad choice of applications, especially in the medical aspect, which show significant signs of progress in developing different methods for improving drug ability, drug distribution, diminishing toxicity of medications, and allowing the programmed nanomaterial production (Rai et al. 2017). This chapter highlights the NPs, mainly focused on the greenish biosynthesis processes. Besides, it explains the protoscolicidal ability of NPs by reviewing the most recent published papers concerning this aspect. These studies may permit the discovery of an innovative healing alternative for hydatid cyst disease.

Classification of *Echinococcus granulosus*

Previously, *E. granulosus* was considered one species of a broad genotypic and phenotypic differences, whereas now it is recognized as a group of cryptic species that vary in shape, growth, host specificity, and ability to infect humans. Also, variation in mitochondrial and nuclear genes has created phylogenetic trees and propositions of different taxa ancestries. Recent studies have molecularly subdivided this species into *E. granulosus* sensu stricto (includes G1-3), *E. felids* (priorly 'lion strain'), *E. equinus* ('horse strain,' genotype G4), *E. ortleppi* ('cattle strain,' genotype G5) and *E. canadensis*. The last one displays the most comprehensive diversity, and it includes 'camel strain' of genotype G6, 'pig strain,' related to genotype G7, along-with double 'cervid strains,' G8 and G10 (Romig et al. 2015). In this regard, and most recently, nucleotide sequence alignments of mitochondrial cytochrome c oxidase subunit 1(cox1) gene were performed for characterizing the liver hydatid cysts of sheep and cows in Iraq. The finding showed that all samples belonged to the G1 sheep strain (Abdulla et al. 2020).

Based on the reports of Thompson (1986), Rausch (1997), and Roming et al. (2015), *E. granulosus* belongs to the following classification:

Phylum: Platyhelminthes.

Superclass: Eucestoda.
Class: Cestoidea.
Subclass: Cestoda.
Order: Cyclophyllidea Ben; Braun, 1900.
Family: Taeniidae Ludwig, 1886.
Genus: *Echinococcus* Rudolphi, 1801.
Species: *Echinococcus granulosus* Batsch, 1786

Life cycle of *Echinococcus granulosus*

Dual vertebrate hosts are needed for completing the life cycle of *Echinococcus granulosus*. Protoscoleces play an avital role in initiating the life cycle of this parasite because it is the infective stage for the carnivores (dogs), representing the final host (Galindo et al. 2008). The Echinoccocal worms attach to the mucosa of the small intestine of dogs and produce eggs. The eggs can infect humans and other susceptible herbivores, like sheep and cattle (Walker et al. 2004). Humans can be infected by accidental consumption of the embryonated eggs and are known as closed way hosts for Echinococcosis. After their consumption by the intermediate host, eggs hatch releasing the embryos that penetrate the mucosa and then find their way via blood or lymph to the liver, lungs or to other sites to produce unilocular fluid-filled hydatid cysts. When the definitive hosts consume viscera of infected animals with the metacestodes, the protoscoleces evaginate, attach to the mucosal lining of their intestine and grow into the worm stage (Fig. 1).

Hydatid Cyst Structure

The hydatid cyst structure of *E. granulosus* contains three layers: i) the external pericyst originates from host cells and produce a fibrous, protective tissue; ii) then comes the laminated membrane (exocyst layer), which is non-cellular and permits the semi permeability of nutrients; and iii) the internal germinal layer (endocyst), where the brood capsules are produced. A fully developed cyst contains brood capsules with protoscoleces and is filled with clear fluid, which is rich in daughter cysts and protoscoleces, as shown in Fig. 2 (Eriksen and Agopian 2017; Fritsche and Pritt 2017).

Treatment of Cystic Echinococcosis

Surgical treatment is the chief therapeutic method for Cystic Echinococcosis, and still, it is the standard gold procedure for large hydatid cysts. Despite the progress of the surgical technique, secondary Echinococcosis due to the leakage of protoscoleces may occur throughout the operation. Such re-occurrence was documented in 2-25% of patients (Ammann and Eckert 1996; Eriksen and Agopian 2017). Also, the potent anaphylactic reaction is an additional risk. Consequently, application of some protoscolicidal agent is necessary due to cyst fluid dissemination risk (Pawlowski 1997). An innovative technique that was applied includes Puncture, Aspiration, Injection, and Re-aspiration (PAIR). This procedure starts with the percutaneous puncture of the cyst under ultrasonic guidance, followed by cyst hydatid fluid aspiration, injection of protoscolicidal substance (such as ethyl alcohol), then re- aspiration of the fluid in the next twenty minutes. This method attackes the germinal layer, reducing the cyst. Lastly, collapsing and solidifying of the hydatid cyst takes nplace (Hemphill et al. 2007; Eriksen and Agopian 2017).
Several experimental studies within animal models have established the chemotherapeutics of Echinococcosis. Albendazole and mebendazole are proved to possess the same efficacy (Walker et al. 2004), with mild adverse properties (Table 1). Surgical intervension has some limitations, like recurrence of hydatid cysts. Moreover, it is not applicable for inactive symptomless and tiny hydatid cysts (Brunetti et al. 2010).

Nano-medicine

The word nano is derived from the Greek words "*Nanos*" for "dwarf." It equally indicates one billionth part (10^{-9}). 'Nanoparticles' is defined by the American Society Testing and Materials as particles with a minimum double or extra dimension with a size of 1-100 nm (Alanazi et al. 2010). Dual alternative styles for metallic NPs production include the "bottom-up" way and the "top-down" method (Kaushik et al. 2010). These techniques create nanomaterials which differ from their original material in surface-related characteristics or quantum amount (Roduner 2006). Moreover, nanotechnology represents an expanding research area and a hopeful arena due to its application in diverse scientific research disciplines (Dutta et al. 2017).
Nanomedicine includes biological and non-biological medicinal products. Biological nanomedicines are prepared from biological sources, whereas non-biological ones are called non-biological complex drugs (NBCD), and they are manufactured from different synthetic structures (Mühlebach 2018). The nanomedical products express an extensive variation in their type and structure and have been used in many remedies for acute and chronic diseases. Also, differences in toxicity, solubility, and bioavailability characters are modified by nanotechnology in medicine (Soares et al. 2018).
Disadvantages of nanoparticles include their high cost and potential risks for the human body. Consequently, biological methods, for instance, utilizing microorganisms (Li et al. 2012) and enzymes (Rangnekar et al. 2007), are advised as possible eco-friendly choices for the purpose. Nanomedicine advantages include high bioavailability and stability, diverse administration ways, organized release, and negligible toxicity. In contrast, the disadvantages consist of ethical worries, their cost-effectiveness and associated risks (Aditya et al. 2013). In their paper concerning nanotechnology and hepatic illnesses, Reddy and Couvreur (2011) have pointed out that nanomedicines are habitually used phospholipids (such as liposomes), polymers, or iron resources (like minor Fe O Nanoparticles). In a previous investigation, Alving et al. (1978) applied liposomes to increase the effectiveness of medications with *Leishmania donovani* in

experimentally infected hamsters. Also, nanobio-technology is a part of nanotechnology that presents NPs production for particular applications with minimum risk influences from bio-systems resources. Nanobio-technology is a wide-ranging term, covering the creation and subsequent utilization of particles smaller than 100 nm size (Ahmad et al. 2003). Various organisms, including, plants, algae, filamentous fungi, yeast, bacteria, and viruses, could be considered as sources for NPs production. Some researchers studied the use of microorganisms as potential eco-friendly materials to synthesize NPs, for instance, cadmium, gold sulfide and silver (Mukherjee et al. 2002; Ahmad et al. 2003). Green biosynthesis is recommended now-a-days; it depends on plants and their extracts for generating the NPs (Begum et al. 2009). Among these, leaves extract of black tea has capability to release gold and silver NPs, which is attributed to the presence of polyphenols in the black tea plant (Begum et al. 2009). Nanoparticles produced by plants are more stable, and their synthesis is quicker than that of microorganisms. Also, the NPs are more varied in shape and size than those created by other organisms (Iravani 2011).

Furthermore, AuNPs synthesized by green vegetative were more stable than NPs created by other techniques. Plants contain many phytochemical materials, like terpenes, polyphenols, carboxyl, hydroxyl, and aldehyde functional groups, that can reduce gold salt $HAuCl_4$ to AuNPs (Chanda et al. 2011). Their outcomes emphasized the ability of non-toxic cinnamon -Au NPs as a signal for identifying cancerous cells, which possibly would be clinically advantageous for diagnosis of the disease (Chanda et al. 2011).

Bacteria, Actinomycetes, and fungi were examined to produce metal NPs (Singh et al. 2016). Enzymes from bacteria and phytochemicals of plants with antioxidant activities or reducing characteristics are accountable for reducing metal materials into NPs (Durán et al. 2011).

Table 1: Anti-Echinococcosis drugs with adverse effects.

Drugs	Adverse effects	References
Benzimidazoles	Caused leukopenia, thrombocytopenia, and hepatotoxicity	Eriksen and Agopian (2017)
	contraindicated for pregnant women (teratogenic)	Moro and Schantz (2009)
	20-40% of patients failed to respond positively	
Albendazole	It amplified the transaminases.	Teggi (1995)
	20% of cases showed abnormalities in liver function.	Horton (1997)
	Teratogenicity has been stated when it is given to experimental animals during early pregnancy.	McManus et al. (2003)
	Induce hematuria, leukopenia, and teratogenic in rats. Also, it is not recommended for women during pregnancy.	Gollackner et al. (2000) Perez-Serrano et al. (1994)
	May leads to embryotoxicity and teratogenic in experimental animals. Therefore, it should be avoided during pregnancy and lactation. Also, it is slow.	Horton (1989) Hemphill et al. (2007
Mebendazole and albendazole	20-40% of hydatidosis doesn't respond to therapy.	Elissondo et al. (2008)
	High cost, lifelong consumption, parasitostatic rather than parasiticidal, high recurrence.	Kuster et al. (2014) Hemphill et al. (2007)
	Lead to hepatotoxicity, severe leukopenia, thrombocytopenia, and alopecia.	Junghanss et al. (2008)
	Result in neutropenia, liver toxicity, and alopecia. It is contraindicated during pregnancy, chronic hepatic disease, and bone marrow depression were recorded.	
	Elevated transaminases, abdominal pain, headache, vertigo, urticaria, and jaundice were observed.	Moro and Schantz (2009) Aronson (2016)

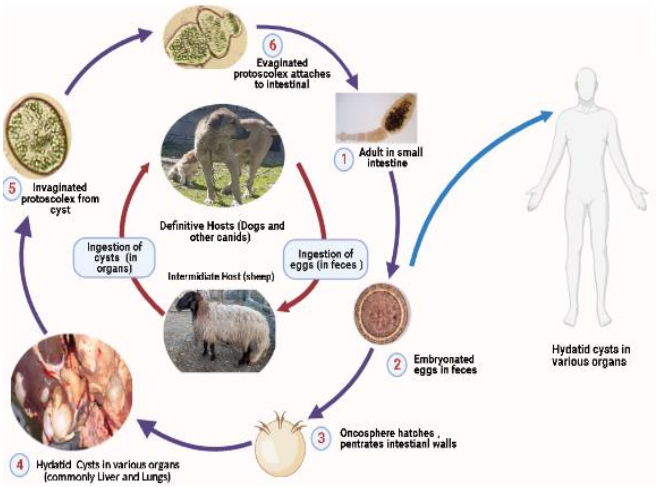

Fig. 1: The life cycle of *Echinococcus granulosus*.

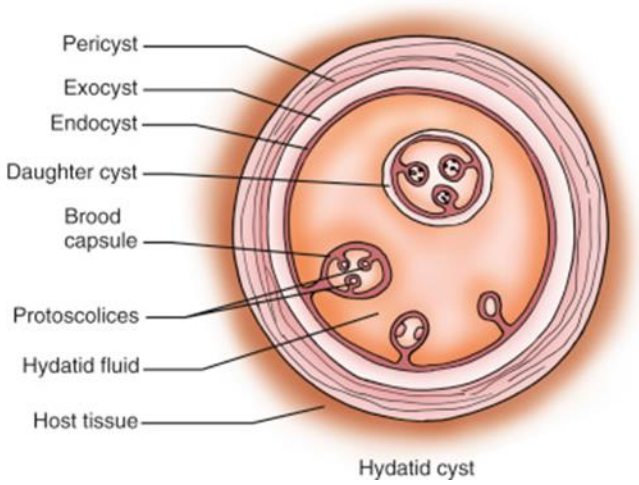

Fig. 2: Schematic diagram of Echinococcal cyst structure, illustrating the pericyst, exocyst, endocyst, and protoscolices of *E. granulosus* (Eriksen and Agopian 2017).

Table 2: Scolicidal efficacy of nanoparticles according to some recently published articles.

Compound	DIsease	Experimental design	Dosage	Treatment period	Efficacy assessment	References
Selenium N.P.s	CE	*In vitro*	500 mg/ml	10 minutes	100%	Mahmoudvand et al. (2014)
Silver NPs	CE	*In vitro*	0.15 mg/ml	120 minutes	90%	Rahimi et al. (2015)
Colloidal silver	CE	*In vitro*	4 mg/ml	60 minutes	71.6%	Lashkarizadeh et al. (2015)
Gold NPs	CE	*In vitro*	0.3 mg/ml	120 minutes	94%	Barabadi et al. (2017)
Solid lipid NPs Loaded on albendazole sulfoxide	CE.	*In vivo*	0.5 mg/kg BID 2 mg/kg	Every 48 hr for 15 days	Economic & shortening time	Ahmadnia et al. (2013)
Chitosan albendazole NPs	CE	*In vivo* & *In vitro*	25 mg/ml	21 days	Significant effects	Torabi et al. (2018)
Albendazole-chitosan microsphere	Alveolar E	*In vivo*	150 mg/kg		Efficient	Abulaihaiti et al. (2015)
Albendazole sulfoxide-loaded PLGA-PEG	CE	*In vitro*	150 & 200 µg/ml	5-60 minutes	100%	Naseri et al. (2016)
Albendazole & Albendazole Sulfoxide-Loaded Solid Lipid NPs	Alveolar E	*In vitro*	2,000 g/L 2,500 g/L	48hr. 72hr.	ABZ and ABZSO showed good physicochemical properties, regular release, higher permeability and efficiency by loading SLNPs	Soltani et al. (2017)
ABZ and PZQ loaded solid lipid NPs (SLNs)	CE.	*In vivo*	---------	Three months treatment	Treatment reduced the wet weight and size of developed cysts for the ABZ and PZQ loaded SLNs was 83% and 85%, respectively.	Jelowdar et al. (2017)
Ag NPs, Fe N.P.s, Cu N.P.s, Si N.P.s, and Zn N.P.s	CE	*In vitro*	0.25, 0.5 and 1 mg/mL	10-60min	Ag NPs showed the highest effect, followed by Si NPs, CuNPs, Fe NPs, ZnNPs	Norouzi et al. (2020)
Albendazole on Ag NPs, ABZ and Ag NPs	CE	*In vivo*	Mice were given ABZ-loaded on silver NPs orally via a gastric tube at a dose of 100 mg/kg/d	8 weeks	ABZ-loaded NPs showed high drug efficacy in experimentally infected mice, with minimum histopathological alterations,	Nassef et al. (2019)
Chitosan–Curcumin Nanoparticles	CE	*In vitro*	0.25, 0.05, 1, 2, and 4 mg/mL,	for 5, 10, 20, 30, and 60 min	Mortality % rate 68% in 4 mg/mL concentration	Napooni et al. (2019,b)
Gold NPs	CE	*In vitro*	250, 500, 1000, 2000 and 4000 µg/mL).	5, 10, 20, 30, and 60 min.	4000 µg/mL of gold NPs killed 76%of protoscoleces in 60 min	Napooni et al. (2019,a)
Zinc oxide Nanoparticles ZnO- N.P.s	CE	*In vitro*	concentration of 50,100 and 150 mg/ml	10, 30 and 60 minutes	The mortality percentage of 50 mg/ml ZnO NPs is 19.6% of protoscolices at 10 minutes.	Norouzi et al. (2019)
Albendazole-lipid nanocapsule	CE	*In vivo*	Dose of 5 mg/kg.	Daily for 30 days by an intragastric tube.	ABZ-LNCs exhibited greater chemoprophylactic efficiency than ABZ administered orally to mice. In addition, the treated group didn't show any cyst.	Ullio Gamboa et al. (2019)
Albendazole sulfoxide (ABZ-SO)-loaded chitosan-PLGA NPs	CE	*In vivo*	A daily dose of 10 mg/kg	45 days	the therapeutic influence of ABZ-SO-loaded CS-PGLA NPs in the weight and volume of cysts were statistically significant compared with the control group	Darvishi et al. (2020)
Zirconium Oxide (ZrO_2)	CE	*In vitro*	250, 500, 1000, 2000, and 4000 µg/ml)	60 min.	1000, 2000, and 4000 µg/ml were significantly effective in the killing of protoscoleces.	Ibrahim (2020)
TiO_2 Nanoparticles and *Echinometra mathaeis* gonad extracts	CE	*In vivo and invitro*	15 µg/ml gonad extract + TiO_2 Nanoparticles	60 min.	Killed 84% of the treated protoscolices	Navvabi et al. (2019)

copper NPs (CuNPs)	CE	*In vitro and ex vivo*	CuNPs 250, 500, and 750 mg/mL separately and with albendazole of 200 mg/mL	5-60 min	The mortality proportion of protoscoleces was 100 % after 10 min of incubation with 750 mg/mL of CuNPs and with albendazole	Ezzatkhah et al. (2021)
Silver Nanoparticles Ag NPs	CE	*In vitro*	AgNPs 0.05,0.1, 0.2,0.3, 0.4mg/ml	From 10-210 min	The percentage of mortality was 100% after 2hr.of incubation with AgNPs 0.4 mg/l. The effects were dose and time-dependent	Jalil et al. (2021)

Nanoparticles are essential for medical uses owing to their exceptional characters, for example, large surface to mass proportion, their quantum structures, and abilities to adsorb and transport other compounds (drugs, probes, and protein).

Presently, metal NPs have broad and diverse applications in catalysis, electronics, biosensing, photonics, cosmetics, ecological cleanup, photo- imaging, and drug transport (Nath et al. 2013; Singh et al. 2016). Studies for developing the most effective and biodegradable green techniques for metal NPs are in progress. Green production of metal NPs possesses many advantages, such as simplicity, cleanness, effectiveness, safety, and cheapness. They use biological sources (plants, fungi, algae, besides microorganisms) to reduce and stabilize function (Mukherjee et al. 2012, 2014).

Moreover, NPs are dependable as a medication for the treatment of different diseases because of their antimicrobial effects. They have been proved to express promising activities against bacteria, viruses, and parasites (Jebali and Kazemi 2013).

Presently, metal NPs, mainly AuNPs and AgNPs, are applicable in the therapy of many diseases, like malignancy, diabetics mellitus, Parkinson's, Alzheimer's, HIV/AIDS, arthritis, hepatitis, cirrhosis, spinal cord injury, tuberculosis, and cardiovascular illnesses, because of their optoelectronic and physicochemical properties (Patra et al. 2015). Moreover, Aly et al. (2018) stated that silica-coated NPs with polyclonal antibodies improved Nano-sandwich ELISA sensitivity and specificity for diagnosing *Toxoplasma gondii* in sera and urine of patients owing to their high surface to volume proportions and crystallographic surface structure.

With respect to vaccine production, NPs show many advantages in comparison with conventional vaccines and adjuvants. They improve the solubility of hydrophobic antigens, have fewer adverse effects post-vaccination, give a sustainable controlled release of the prepared antigens, target the lymph nodes or reticuloendothelial tissues directly, and require smaller volumes and fewer doses (Dobrovolskaia et al. 2016). Nanovaccines and nano-adjuvants can be administered separately or collectively in a single shot to minimize the required doses. Nanovaccines can be given by diverse routes, which offer more flexibility, making them ideal for veterinary medicine applications, especially if many birds or animals need to be vaccinated (El-Sayed and Kamel 2018; Kamel et al. 2019). Nanotechnology aids the NPs to target the immune system specifically (in vaccine preparation), or to avoid its stimulation (in other medical applications) (Dobrovolskaia et al. 2016). Nanoparticles can deliver different compounds via endocytosis, for instance, drugs, chemotherapeutic agents, and imaging substances. Also, biological materials like antigens, antibodies, RNA, or DNA could be delivered. They can even be depended on to deliver light and heat to their target cells when required (El-Sayed and Kamel 2020).

In Nanoparticles toxicity, which was raised from its high production and exposure of humans, recent results showed that NPs could be accumulated in vital organs such as the heart, liver, spleen, kidneys, and brain after ingestion or dermal contact. Nevertheless, scarce information is known concerning the toxicity mechanisms responsible for harmful/toxic effects of nanoparticles. *In vitro* and *in vivo* investigations pointed out that NPs could induce the creation of reactive oxygen species (ROS), which is a principal mechanism of their toxicity. ROS production leads to oxidative stress, inflammation, and subsequent destruction of proteins, cell membranes, and DNA. At the same time, ROS production induced by NPs is organized by size, shape, surface, composition, solubility, aggregation, and the route of NPs uptake (Sengul and Asmatulu 2020).

The Organization for Economic Cooperation and Development (OECD 2002) have recommended oral toxicity tests, eye irritation, skin toxicity, and lethal Dose 50 to assess acute *in vivo* toxicity of nanomaterials. *In vivo* toxicity studies include many parameters like dose, route of administration, metabolism, excretion, and immune reactions, which are also highlighted. The toxic effects of NPs on human health are significantly increasing their global recognition. Therefore, the ongoing nanotoxicology researches to investigate the biological pathways taken by NPs and induced toxic effects have increased noticeably during the last few years (Ashajyothi and Chandrakanthb 2019).

The absence of official regulations regarding nanomedicines and nanomaterial manufacture for clinical applications is considered a global issue (Foulkes et al. 2020). On the other hand, several nanomaterials and nanotechnologies have already been approved and organized in the clinical trials for different drugs, like antifungals, anticancer drugs, and pain management agents (Anselmo and Mitragotri 2019).

Protoscolicidal activity of Nanoparticles

Several researchers studied the protoscolicidal effects of biogenic NPs against *E. granulosus* within *in vitro* and *in vivo* models. Mahmoudvand et al. (2014) investigated

protoscolicidal effects of selenium (Se) NPs, which were produced by Bacillus sp. MSh-1. Their results documented the potent protoscolicidal activity of different concentrations of Se NPs after a short period of treatment. Moreover, other researchers recommended Se NPs as an innovative therapeutic agent for the treatment of cutaneous leishmaniasis localized lesions. They proved their effects against both promastigote and amastigote of the causative agent (*Leishmania major*) of the disease (Beheshtia et al. 2013). With respect to cytotoxicity of selenium NPs, Shakibaie (2013) pointed out that no mortality was recorded in mice injected with 2.5, 5.0, and 10.0 mg kg–1 of biogenic selenium NPs manufactured from Bacillus spp. In contrast, animals inoculated with 20 mg kg–1 of Se NPs expressed 20% deaths, with changes in biochemical and hematological parameters. Furthermore, the toxicity of biogenic Se NPs was less than that of the synthetic Se NPs, which established Bacillus sp's MSh-1 ability in converting the high poisonous Se compound to less poisonous Se NPs (Shakibaie 2013).

Furthermore, other researchers observed the ability of Se NPs in reducing *Candida albicans* biofilm. It was documented that Se could be coated on the surface of medical devices, which then would express activity against bacteria and fungi (Guisbiers et al. 2017). Selenium is a trace element essential for life (adult human needs ~40 µg Se/day). However, it is poisonous at high levels, from N3200 to 6700 µg Se/day (Navarro-Alarcon and Cabrera-Vique 2008).

Additionally, Rahimi et al. (2015) investigated protoscolicidal ability of biosynthesized silver NPs by *Penicillium aculeatum* against Cystic Echinococcosis. The findings proved that all concentrations of the AgNPs had remarkable protoscolicidal action. These investigators decided that AgNPs might be used as probable protoscolicidal because of their biodegradable source and harmlessness. Moreover, Lashkarizadeh et al. (2015) highlighted the protoscolicidal ability of amphotericin B, Ag NPs, essential oil of *Foeniculum vulgare* Mill, and hypertonic saline against Cystic Echinococcosis. They documented the antiparasitic activity of AgNPs. The maximum activity was observed in 4 mg/mL, leading to a mortality percentage of 71.6% of protoscoleces after one hour of the treatment period.

Furthermore, Ag NPs could reduce the toxic effects of albendazole, the drug of choice for hydatid disease treatment. These toxic effects of albendazole may include necrosis and degeneration, steatosis, and raised serum hepatic enzymes. As a result, coating ABZ on Ag NPs could be a promising technique to increase ABZ effectiveness against Cystic Echinococcosis (Nassef et al. 2019).

Another investigation pointed out that the greenish synthesized AuNPs by *P. aculeatum* displayed activity against Cystic Echinococcosis protoscoleces in *in vitro* model. Several concentrations of these AuNPs for different incubation times were investigated (Barabadi et al. 2017). Their findings represented a novel method in applied nanotechnology with promising results in its effects against parasites. Also, they recommended AuNPs as a probable protoscolicidal agent against *E. granulosus*. They explained these effects by the large surface area to volume proportion, which provided it with novel mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties that are missing in their original substance (Barabadi et al. 2017). A recent study demonstrated remarkable protoscolicidal effects of AuNPs. These gold NPs can be considered as an alternative treatment for Cystic Echinococcosis, eliminating side effects associated with chemical drugs (Napooni et al. 2019a).

Moreover, albendazole and Praziquantile coated solid lipid NPs represent appropriate carriers for these drugs. It is more effective than free albendazole and Praziquantile for CE's chemoprophylaxis treatment in the mouse model. This makes this compound a candidate for further investigations involving clinical practice (Jelowdar et al. 2017).

In a previous research, albendazole's loaded chitosan microspheres (ABZCS-MPs) activity as a novel carrier in experimental mice inoculated with *E. multilocularis* was assessed. ABZ-CSMPs showed higher absorption and better-quality bioavailability of ABZ in treating this infection in experimentally infected murine compared to the group given liposome–albendazole and albendazole drugs. As a result, ABZ-CS-MPs are considered as excellent applicant for treating Alveolar Echinococcosis caused by *E. multilocularis* (Abulaihaiti et al. 2015).

Another recent study pointed out that chitosan–curcumin NPs exhibited scolicidal activities, which suggested them as appropriate anti-protoscoleces agents (Napooni et al. 2019b). According to Torabi et al. (2018), chitosan albendazole (ChABZ) and chitosan praziquantel (ChPZQ) nanoparticles are more effective than albendazole and praziquantel against hydatid cyst disease in *in vitro* and *in vivo* models. According to their findings, a significant reduction in the number of hydatid cysts was detected in the murine group inoculated with ChABZ and ChPZQ NPs in therapeutic and chemoprophylactic designs. Moreover, these researchers demonstrated that ChPZQ NPs were more efficient than ChABZ in destroying the micro hydatid cysts. This may be attributed to the ChPZQ NPs smaller size and high stability than ChABZ NPs (Torabi et al. 2018).

In a recent *in vivo* study, Darvishi et al. (2020) demonstrated the activity of ABZ-sulfoxide-loaded chitosan-PGLA NPs produced by nanoprecipitation. According to this study, remarkable therapeutic effects in the weight and size of the treated cysts compared to those of the control group were observed. They concluded that ABZ-sulfoxide-loaded chitosan-PGLA NPs could improve the hydatid cyst disease treatment in the murine model. Similarly, Ibrahim (2020) investigated several levels of ZrO_2 NPs against the protoscoleces of *E. granulosus*. This study revealed that 1000, 2000, and 4000 µg/ml of ZrO_2 NPs were significantly efficient in killing the parasite after 60 minutes of incubation.

Furthermore, albendazole sulfoxide loaded with solid lipid NPs were produced and examined *in vivo* experiments against Echinococcosis (Ahmadnia et al.

2013). According to this study, cysts in treated mice were reduced in size and weight. Also, the cysts in animals treated with albendazole sulfoxide loaded with lipid NPs showed intensive ultra-structural changes. These results proved the destructive activity of the compound against the parasite (Ahmadnia et al. 2013). The microtriches structures, functions of which were related to nutrition, were shortened or even lost in many treated hydatid cysts, suggesting that the *E. granulosus* reacted to adverse environments by reducing the absorption membrane area (Ahmadnia et al. 2013).

Various mechanisms proposed for antiparasitic activity of NPs have been highlighted in the literature, including apoptosis induction. Naseri et al. (2016) assessed *in vitro* apoptotic activities of albendazole sulfoxide and albendazole sulfoxide-loaded poly (lactic-co-glycolic acid) (PLGA)-PEG as an innovative nano polymeric particle against protoscoleces. They showed that ABZs and ABZs-loaded PLGA-PEG were able to stimulate cell death of protoscoleces with the oligonucleosomal DNA fragmentation, which indicates the existence of late stages in apoptosis. These apoptotic activities of ABZs on protoscoleces were evaluated by caspase-3 mRNA expression of the *E. granulosus* genome. Similarly, it was noticed that albendazole and albendazole sulfoxide loading solid lipid NPs exhibited good physicochemical characteristics and controlled releasing by using solid lipid NPs as drug delivery carriers (Soltani et al. 2017). These workers proposed that such materials are useful for the treatment of Cystic Echinococcosis (Table 2).

Cystic Echinococcosis (CE) is still a neglected disease, for which the approved treatment is the use of Benzimidazole. This medication displays a parasitostatic, instead of parasiticidal, activity on hydatid cyst disease with low bioavailability. Consequently, many trials were performed to improve its solubility, absorption, and bioavailability. These experiments aimed at boosting the drug activity through NPs, resulting in accumulative intra-hydatid cystic drug levels (Siles-Lucas et al. 2018). Shnawa (2018) also reviewed several published articles related to biogenic NPs as potential agents against hydatid cyst disease and applied a Nano-carriers medication delivery system that hopefully extends the treatment options further.

In contrast, the main concern regarding use of nanoparticles is their toxicity; thus, the cytotoxicity issue of these nanoparticles should be highlighted. Besides, more studies are required to investigate the effects of these nanoparticles and their mechanisms of action as a treatment option for the CE, particularly in animal models and clinical management (Albalawi et al. 2020).

Conclusion

Hydatid cyst disease is a life-threatening zoonotic problem that results from the metacestodes of *E. granulosus* infection with limited treatment options. The standard treatment for CE is the surgical operation. However, one of the major problems following surgery is the recurrence of the infection owing to spilage of the hydatid fluid. Up to now, no effective drugs and scolicidal agents are available. The only synthetic chemical drug licensed for human treatment is Benzimidazole, which has a therapeutic efficacy of over 50%, underlining the need for novel drug delivery systems. Also, this medication is known to have a parasitostatic effect instead of parasitocidal activity against *E. granulosus,* with limited bioavailability and severe adverse effects. Therefore, there is a crucial necessity to progress an innovative and efficient anti-hydatid agent.

Based on the results obtained from several *in vitro* and *in vivo* studies, NPs could be considered as an up-and-coming candidate and an alternative CE treatment resource. The most of the NPs tested for CE treatment were metal NPs, metal oxide NPs, and polymeric NPs. NPs are currently receiving much attention in research concerning Echinococcosis, but their safety is still questionable.

Consequently, the cytotoxicity of these NPs should be highlighted in future investigations. Besides, mechanisms of the cellular and molecular action of these NPs against hydatid cyst disease need to be explained. These may give a new approach in the NPS aspect and perhaps present a chance for manipulating a novel and more efficient drug for hydatid cyst disease.

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PREVENTIVE MANAGEMENT OF THE PARASITIC DISEASES THROUGH TRACE ELEMENTS

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INTRODUCTION

Parasitism is still a serious threat to the livestock economy worldwide (Rashid et al. 2019). Gastrointestinal (GI) parasitic infections are considered among the major threats to livestock production all over the world due to retarded growth and productivity, and animal mortality (Githiori et al. 2004). A major impediment to maximizing production from livestock is the cumulative effects of parasitism on animals (Ahmad et al. 2017). GI tract parasitic infections of production animals have great economic impact, especially in developing countries. Helminths, especially GI nematodes and trematodes, impose severe threats to livestock in these areas in the form of morbidity, mortality, cost of treatment and control measures (Lashari and Tasawar 2011). Also, helminth infections in ruminants decrease natural resistance to diseases, result in poor weight gain and poor feed utilization (Pedreira et al. 2006). Apart from the importance of nematodes in ruminant populations, higher prevalence rates of cestodes and trematodes have also been reported (Rizwan et al. 2017; Ikurior et al. 2020). In developing countries, antiparasitic drugs are used extensively for the control of parasitic infections, especially by smallholder farmers. This extensive use may lead to the development of resistance. Other factors responsible for the development of resistance are poor efficacy of antiparasitic agents, inappropriate dose, low protein diet and environmental toxicity (Smith and Sherman 2009; Khan et al. 2017). Development of resistance to antiparasitic agents and their residual effects in animal products have stimulated scientists and veterinarians to investigate alternative sources to prevent and control parasitic infections and to improve public health (Qadir et al. 2010; Badar et al. 2017). Keeping in view the limitations of chemotherapy, alternative solutions, such as biological control of parasites, use of vaccines, phytotherapy, use of trace elements (TEs) and development of resistant host genotypes, are being considered. These strategies, based on the holistic approaches, may substitute and/or augment existing prevention and control measures for parasitic infections, which have special significance in resource-poor communities due to their increased availability and cheaper prices.

The availability of trace elements (chemical elements required in minute quantities for normal growth of animals) in an appropriate quantity is a pre-requisite for the health and productivity (milk, meat, eggs, wool, hides) of livestock, while their insufficient intake or unavailability decreases productivity (Khan et al. 2007). Under natural grazing conditions, forages are among the major sources trace elements (TEs) for herbivores; however, water and soil also contribute to supply considerable quantities of TEs. Feed sources of TEs are largely separated into a variety of base feedstuffs, like harvested forages, concentrates, range or pasture plants, and mineral supplements (McDowell and Arthington 2005). The level of TEs consumption mainly depends upon forage intake. Factors responsible for the concentration of TEs in grazing forages are: plant species, plant developmental stage, dry matter yield, soil type, and climatic conditions (Mirzaei 2012). Determination of the abundance of TEs in grazing forages and their bioavailability to animals is important for meeting the requirements of animals (Qudoos et al. 2017; Rizwan et al. 2019; Ahmad et al. 2020).

In developing countries, data related on the association of TEs in the complex systems represented in the soil-plant-animal interface are poorly available, except for a few recent investigations (Qudoos et al. 2017; Rizwan et al. 2019; Ahmad et al. 2020). Other than this, a better understanding of the association of TEs with the burden of parasites in livestock is still needed. In this chapter, the role of TEs in animal health through their influence on physiological processes, and the development of non-specific and/or specific immunity against GI parasites, are discussed comprehensively.

Strategies to Control Parasites

For the control of parasites in livestock, especially those reared by resource-poor farmers, it is important to identify the burden and types of parasites along with

specific risk factors associated with the parasitic infections in the specific area (Ayana and Ifa 2015). Breaking the life-cycles of parasites is the main goal in attempts to control parasitic infections. The use of antiparasitic drugs and appropriate management (of both animals and pastures) help to interrupt the life-cycle of parasites. The use of antiparasitic drugs for the control of parasitic infections is one of the best methods and is recommended globally (Grade et al. 2008). The strategic application of single or combination of two or more drugs for the control of parasites is very effective (Parr and Gray 2000). Integrated management through the combination of chemotherapy and other interventions usually results in the best parasite control (Atnafe and Melaku 2012). However, the development of resistance to almost all antiparasitic drug classes, due to their frequent use over the past few decades, has become a problem around the globe. In developing countries, most anthelmintics are used without registration and proper tests, which directly affect their efficacy (Atnafe and Melaku 2012).

Inadequate use of anthelmintics, poor efficacy of the commercially available products, and low protein diets result in development of resistance (Smith and Sherman 2009). Antiparasitic treatment of livestock in most countries is not practiced regularly in a systemic way, which may have a considerable effect on parasite prevalence and leads to resistance. Furthermore, sample size and the nature of sample sources (faecal or autopsies) are also important points in this regard (Githiori et al. 2004; Khan et al. 2017a).

Anthelmintic resistance is widespread, especially in nematode populations around the world (Kaplan 2004). The development of resistance to antiparasitic drugs and entrance of drug residues in the food chain have stimulated investigations into alternative ways to control parasitic infection and to improve public health. In this perspective, the search for alternative and/or augmenting tools to enhance antiparasitic activity gains currency. In Pakistan, some sheep breeds are resistant to *Haemonchus (H.) contortus* (e.g., native Lohi breed; Saddiqi et al., 2010). An alternative strategy to control resistance is manipulation of positive genetic variation. There are three ways through which one can introduce positive genetic variation: (a) selection of breeds, (b) cross-breeding and (c) selection among the breeds (Nicholas 1987). These methods are sustainable, efficient, safe and economical, but the only hindrance is the need of expertise in the field of genetics and the time needed to develop robust population of parasite-resistant sheep with suitable production value (Stear et al. 2007).

Another option is strategic grazing management, e.g., rotational grazing, which embraces stocking density and routine moving to clean pastures (Stear et al. 2007). Grazing management is related to the ecology of parasite larvae, plant species in grazing pasture, epidemiology of parasites, climatological status, schedule of using antiparasitic drugs and prevailing conditions (Hamad 2014). Another method to control parasitic infections is biological control, including the use of nematophagous fungi (e.g., *Duddingtonia flagrans*), which directly decreases the number of infective larvae (L3) in pasture (Waller et al. 2004). Addition of fungal spores to feed is also an effective method to control worms (Waller and Thamsborg 2005).

Plants have been used from ancient times for treatment of domesticated animals and humans. Plants are important sources of antibacterial, antiparasitic and insecticidal agents. Plants are being studied in different parts of the world for their ovicidal, adulticidal and larvicidal anthelmintic activities (Githiori et al. 2004; Masood et al. 2013; Tugume et al. 2016; Kebede et al. 2017). Antiparasitic agents extracted from plants have also been used in human and animal populations, but compared to commercial antiparasitic drugs, their scientific evaluation is limited (Masood et al. 2013; Badar et al. 2017). Use of botanical de-wormers is a good approach and is a possible augmentative solution to combat antiparasitic drug resistance (Jabbar et al. 2006).

Malnutrition and Parasitic Infections

Malnutrition and parasitic infections are directly related (Hailegebriel 2018). Malnutrition occurs in cases of GI parasitic infections due to induction of intestinal bleeding, impaired digestion and poor absorption of nutrients (Din et al. 2018). These parasites also lead to reduction in feed intake, fat absorption, protein usage and loss of nutrients in the form of diarrhea (Robertson et al. 1992). Likewise, malnutrition adversely affects local and systemic immune responses, resulting in increased susceptibility to parasitic infections (Koski and Scott 2001; Rajoo et al. 2017). GI parasites are one of the major risk factors which contribute to malnutrition, reduced performance and poor productivity in livestock and poultry (Yun et al. 2000). In human populations, malnourished people are primarily at risk of getting heavy parasitic infections, and helminthic infections with *Ascaris lumbricoides,* hookworms, *Trichuris trichiura* and *Schistosoma mansoni usually* cause malnutrition in humans (Papier et al. 2014; Mekonnen et al. 2014).

Among environmental factors, nutritional status plays an important role in affecting resistance to infection. Protein malnutrition in this regard is of prime consideration, as it leads to poor immunity and increased parasite burden (Clough et al. 2016). The public and veterinary health significance of helminth infections are often studied in the laboratory with the model roundworm, *Heligmosomoides (He.) polygyrus* in mice (Behnke et al. 2009). This parasitic infection may result in poor growth (Coltherd et al. 2011). The parasite load varies in different strains of the mice, representing genetic variation for resistance (Reynolds et al. 2012).

Immunity and Gastrointestinal Parasites

The GI tract of animals and humans harbors various species of nematodes, as these parasites have adapted themselves for nutrient uptake and evasion of local immune responses at the intestinal interface of the host. These parasites provide a strong challenge to the immune

system of the host, leading to repeated exposure to infective stages, resulting in high endemicity of the parasitic infection (Sorobetea et al. 2018).

On entering the body of the host, first task for a parasite is to take over its immune system to reach its predilection site. This process depends upon the exact location and type of parasite and involves crossing host tissues, membranes and blood or lymphatic barriers. This is achieved by the wide variety of proteinases which are present in excretory/secretory (ES) products (Tort et al. 1999). The larval or adult forms produce these proteinases, which hydrolyze collagen, fibrinogen and hemoglobin. Metalloproteinases, serine, aspartic and cysteine proteases have been reported for GI nematodes. Most of them are related to zinc metalloproteinases and cathepsin B-type cysteine proteinases (Dezfuli et al. 2015).

Parasites play an important role in the adjustment and formation of the immune system in humans and animals. Strong mucosal Th2, Th1, and regulatory responses have been reported in hookworm infections, resulting in up-regulation of IL-15 and a complex, ALDH1A2. The juvenile stages of parasites in most helminth infections cause robust Th2 immune responses in their mammalian hosts. *Fasciola (F.) hepatica* (liver fluke) colonizes ruminants and can down-regulate the Th1 immune responses in mice, even in strains that are unable to produce IL-4 (O'Neill et al. 2000). This indicates that Th2 cells or cytokines are not the only factors to act against helminths, and the wide spectrum of proteins such as ES components are produced by these parasites, which assist them to evade host immune responses, including IFNγ production, invasion of tissue and feeding (Lucena et al. 2017). Infection with trematodes, like *F. hepatica* and/or *Dicrocoelium dendtriticum* in ewes, can lead to the development of mastitis after parturition. It has also been observed that ewes suffering from pregnancy toxemia are presented with increased β-hydroxybutyrate concentrations when infected with trematodes (Mavrogianni et al. 2014).

Deficiency of certain minerals in animals can be identified by alterations in metabolic activity (like enhanced cell proliferation) and alterations in rapidly growing tissues, which are badly affected due to mineral deficiency (Guthrie 1986). Mineral deficiency also has a profound effect on cells having shorter half-life and should be replaced frequently, such as lymphocytes. The intestinal immune responses induced by GI parasites are both physiologically complex and redundant in nature (McClure 2000). Mucous secretions, smooth muscle, nervous system and epithelium are the local protective barrier, in addition to innate and acquired immune systems. Among these, the mucosal immune system is substantial and more active metabolically; it is more profoundly affected due to mineral imbalance. Mucosal immune responses are acquired at the cost of higher requirements of nutrients; protein loss and malabsorption of nutrients may be enhanced due to disturbances in the integrity of GI tract due to worm infection (Humphrey et al. 2002). Therefore, trace minerals are essentially required for metabolic pathways involved in intestinal immunity (McClure 2008).

Role of Trace Elements in Animal Health

Even though animals require only very small amounts of TEs to maintain homeostasis, deficiencies of these elements may lead to deformities of the skeletal system, decreased growth rate, and immunodeficiency. Trace elements have been used as an immune-potentiating tool (to enhance the immune profile) of humans and animals all over the world. There are about 52 TEs required in animals; among these, 16 are categorized as macro {i.e. phosphorus (P), calcium (Ca), chlorine (Cl), sulfur (S), sodium (Na), magnesium (Mg), and potassium (K)} or micro minerals {i.e., zinc (Zn), cobalt (Co), manganese (Mn) selenium (Se), iron (Fe), copper (Cu), molybdenum (Mo), fluorine (F) and cadmium (Cd)}. These TEs are considered essential for the proper functioning of various physiological systems of the body. The possible functions and general mechanism of TEs to maintain the health of animals are shown in Fig. 1. Other TEs are also necessary, but are under less consideration because their excess and deficiency mostly do not show severe clinical signs (Spears 1999; McDowell 2003; McClure 2008). Following are some of the TEs that are essential for development of immunity in humans and animals: Na, Mg, S, K, P, Fe, Ca, Zn, Se and Mo (McClure 2008; Arthington and Havenga 2012). Table 1 summarizes the physiological and immunomodulating roles of some selected TEs in animals.

Imbalance in TEs ratio in feed is the main issue affecting livestock populations, mostly in grazing animals (Samanta and Samanta 2002). Deficiency of TEs is among the major causes of reduced and low-quality meat production, poor quality wool, poor quality hair and death of animals around the world (Grace and Knowles 2012). Minor TEs deficiency does not cause evidently harmful effects, and remains undetectable; however, clear signs and symptoms can be observed in severe deficiency. Deficiency may thus lead to severe diseases in animals (e.g., Cu deficiency causes swayback disease, deficiency of Mg causes grass tetany and that of Ca causes rumen stasis, blindness, and death; Suttle 2010). A high rate of mortality has been reported in animals reared in mineral deficient areas (Soetan et al. 2010). Skeletal disorders may not be directly related to Ca deficiency, but may also be attributable to an imbalance in the proportion of Ca and P in the diet (McClure 2008). In most cases, excess of one TE decreases bioavailability of another (e.g., Mg deficiency may be due to excess K (McClure 2008), and excess of Fe and Mo decreases absorption of Cu (Keen et al. 2003)). The mechanism of reducing resistance against diseases due to TE deficiency is shown in Fig. 2.

TE deficiency causes severe losses to the livestock industry to an extent similar to those caused by infections. These possess a special enzyme system that on activation helps to develop resistance against diseases (Suttle and Jones 1989; McClure 2003). Zn, Cu, Co, Fe, I, Mn, and Se are important for normal immune function (Radostits et al. 2007). These minerals play a role in: (a) defense against

oxidative damage to tissues, (b) reduction of inflammatory reactions and (c) association with antioxidant enzymes (McClure 2008). Uncontrolled oxidation results in a weakened immune system in animals (Arthington and Havenga 2012). Trace elements can act as cofactors for certain enzymes (Hussein and Staufenbiel 2012), which act as antioxidants and are vital for maintaining livestock immunity (Gressley 2009). The list of TEs that act as cofactors and components of various enzymes is given in Fig. 3.

Table 1: Physiological and immunomodulatory roles of selected trace elements in animals

Sr. No.	Trace Element	Physiological role	Immunomodulatory role	Reference
1.	Sodium	absorption of different nutrients; increase appetite of animals; transmission of pulse; response to stimuli and peristalsis of gut	enhance production of granulocyte–macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor-α (TNF-α) and enhances immunity.	McDowell, 2003; Wu et al., 2013
2.	Magnesium	production of vitamin B6; takes part in oxidative phosphorylation, and metabolism of lipids, carbohydrates, and protein	combines lymphocytes to target cells; provides energy to the cell for normal functioning and development of immunity.	Keen et al., 2003; Fekete and Kellems, 2007
3.	Phosphorus	component of protein (phosphoprotein), sugar phosphates, ATP and some enzymes; formation of microbial proteins in the rumen & increased appetite	maintaining the immune response and provide energy to the cell.	Keen et al., 2003
4.	Sulfur	present in amino acids, enzymes, hormones (insulin and corticosteroid) and vitamins (biotin and thiamin).	plays a role in replication, transcription, translation, antioxidant defense and immunity; maintains the mucosa of the intestine and controls inflammation.	Grimble, 2001
5.	Potassium	production of enzymes; nerve transmission and response to stimuli	modulation of macrophage physiology	McDowell, 2003; Villalonga et al., 2010
6.	Calcium	necessary for integrity of tissues and helps in muscle contraction	stimulates production of immune cells and provides energy for normal cell functioning and controls peristaltic movements	Fekete and Kellems, 2007
7.	Vanadium	used in protein, lipid and carbohydrate metabolism; controlling glucose level in the body	stimulates immune responses and enhances immunity by providing energy to the cells of the immune system	McDowell, 2003
8.	Manganese	part of many metalloenzymes and enzymes like kinases, hydrolases, transferases, and decarboxylases; neurotransmission, metabolism of lipids and carbohydrates	activation of inflammatory cells, i.e., neutrophils and macrophages	McDowell, 2003; Zhu and Richards, 2017
9.	Iron	cofactor of many enzymes; transportation of oxygen and carbon dioxide	activates the propagation of T and B cells, antibodies and compensates the amount of Fe in case of blood loss after worm infestation	Keen et al., 2003; Cassat and Skaar, 2013
10.	Cobalt	preparation of cyanocobalamin, methylcobalamin	enhanced anti-inflammatory properties	Nagabhushana et al., 2008
11.	Copper	formation of cytochrome C oxidase, ATP and monoamine oxidase; regulation of adrenal functions in neurotransmission and acts as an antioxidant; maintain the function of the body e.g. as cofactor, metalloprotein, and metalloenzymes.	epithelium repair, energy provision to cells, T cells and antibody formation,	Keen et al., 2003; Fekete and Kellems, 2007
12.	Zinc	part of many metalloenzymes and also regulates their functions; used in Vitamin A metabolism, replication, and transcription; increases appetite and plays a role in fetal growth	acts as an antioxidant and stimulator of thymulin; increases production of lymphocytes, provides energy for cell functioning and transcribes immune cells	Park et al., 2004
13.	Selenium	part of enzymes (glutathione peroxidase) and proteins; regulates reactions, energy and arachidonate metabolism; helps in cell integrity, brain functions and endocrine maintenance	limits tissue damage due to oxidation, controls immune response and inflammation	Hoffmann and Berry, 2008; McClure, 2008; Hefnawy and Tortora-perez, 2010
14.	Molybdenum	present in enzymes like xanthine oxidase, sulfite oxidase, and aldehyde oxidase; role in purine nucleotides, vitamin B6 functioning, and metabolism of lipids and carbohydrates	multiplication of immune cells and inhibition of anti-inflammatory mechanisms	Johnson et al., 1974
15.	Iodine	production of thyroid hormone in the form of thyroxin; starts ATP production and regulates oxidation rate and protein formation	cell growth and control of immune responses	McDowell, 2003; McClure, 2008

Role of Trace Elements against Gastrointestinal Parasites

Although some TEs are required in small amounts, their deficiency may cause problems, such as decreased resistance against bacterial and parasitic infections, decreased immunity, placenta retention, abortion, reduced growth and development (Suttle 2010). Limited information is available regarding any possible association between TE deficiency and GI parasitic infections in livestock. In the abomasum, parasites present in the growing stages can affect P and Ca absorption, resulting in skeletal defects. Sheep infected with larvae of *Ostertagia circumcincta* showed a deficiency of Ca and P, but in the case of *Trichostrongylus colubriformis* infected sheep, along with Ca and P deficiency, inhibition of skeletal development was also recorded (Fekete and Kellems 2007). Low levels of Co, Zn, and Fe in sheep infected with Fascioliasis have been observed (El-Sangary 1999). Rams infected with *H. contortus* showed a significant effect on the mean levels of Zn, Co, Se and Cu (Kozat et al. 2007). It has been stated that TEs may be used for effective and quick cure against GI parasitic infection. Administration of Cu, Se and vitamin E in sheep showed a significant increase in immunity against *H. contortus* (Camargo et al. 2010; Soli et al. 2010). The roles of some essential TEs for the development of immunity and their effects against GI parasites are discussed below:

Copper

Copper plays an important role in the maintenance of various biological processes, such as antioxidant protection mechanisms due to Cu/Zn superoxide dismutase (CuZnSOD) activity, Fe homeostasis, and energy metabolism (Whittaker 2010). Due to the role of Cu in oxidation mechanisms, its proper level is necessary for the appropriate function of defense against oxidative stress and infectious diseases (Huang et al. 2012).

Copper deficiency in animals may cause symptoms related to the immune system (Suttle and Jones 1989). Most prominent symptoms reported in Cu-deficient animals are: decreased T and B cell mitogens on splenic lymphocytes, decreased T cell numbers (most importantly T-helper cells), decreased response to antibodies, and increased susceptibility to infections, ataxia, growth reduction, diarrhea, anemia, abnormal pigmentation, decreased reproductive performance and increase in bone disorders (Suttle and Jones 1989).

The mode of action of Cu against GI parasites is not clear. It may act as an intraluminal anthelmintic or affect host immune response as a nutritional supplement (Langlands et al. 1989). The role of Cu in GI parasitic infections, especially in nematode infections, has been widely explored in animals (Frandsen 1982). Different methods were used to establish the Cu requirement to reduce GI nematode burdens in dairy animals infected with *Haemonchus* sp. (Chartier et al. 2000). Copper supplementation is reported to cause a reduction in

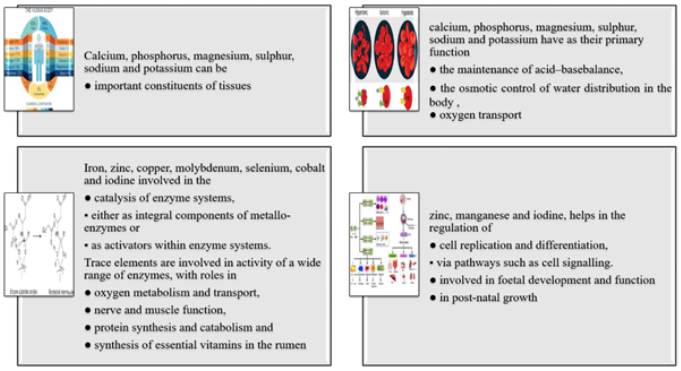

Figure 1: Possible functions and general mechanisms of trace elements in maintaining the health of animals.

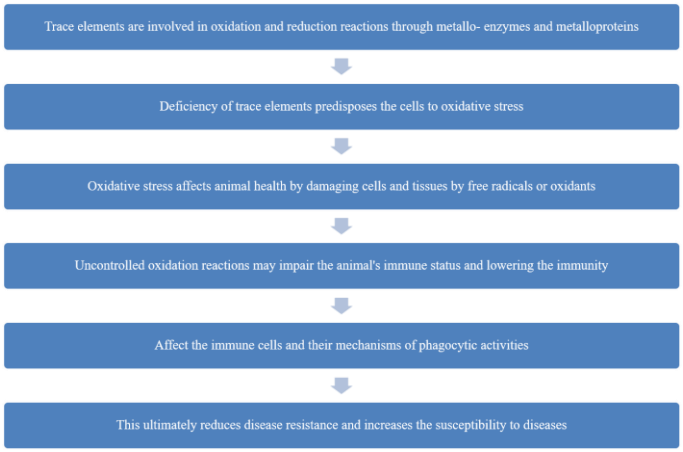

Figure 2: Showing how deficiency of trace elements impairs disease resistance.

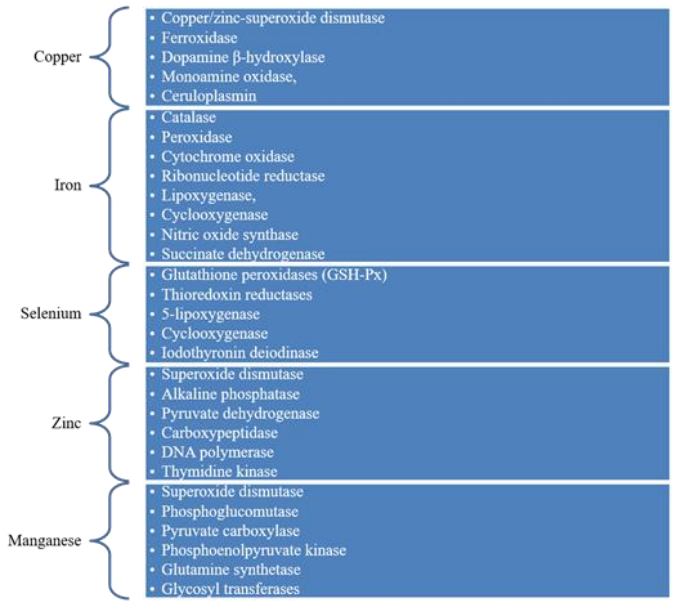

Figure 3: The list of trace elements which act as cofactors and components of enzymes in physiological processes.

abomasal nematodes (Burke and Miller 2006). Hucker and Yong (1986) noted higher worm burden and fecal eggs per gram (EPG) in Cu-deficient sheep compared to those with normal Cu levels. Animals with ostertagiasis showed a reduction in blood Cu level, probably because

of interferencewith Cu metabolism due to increased pH of the abomasal and duodenal digesta (Bank et al. 1990). The effectiveness of oral Cu supplementation is related to a high concentration of soluble Cu in abomasal digesta. In case of nematode infections, reduction in blood Cu concentration is reported (Poppi et al. 1990). Cu availability is reduced in helminth-infected animals and the magnitude of effects is not related to the dietary Cu level. Animals with low Cu levels exposed to infective larvae of *Trichostrongylus (T.) axei* and *T. colubriformis* showed decreased ceruloplasmin activities, with decreased Cu levels in the plasma and liver.

The use of copper oxide wire particles (COWP) results in a significant reduction of *H. contortus* worm load (Vatta et al. 2009). Experimental infection trials also showed the persistent efficacy of COWP against *H. contortus* (Chartier et al. 2000); however, observations of Burke et al. (2007) and Vatta et al. (2009) showed a non-persistent effect of COWP. Oral administration of COWP to animals had a direct effect on parasites and resulted in lower EPG and worm burden in livestock (Soli et al. 2010). The effect of Cu level on the immune system and health of animals is shown in Fig. 4.

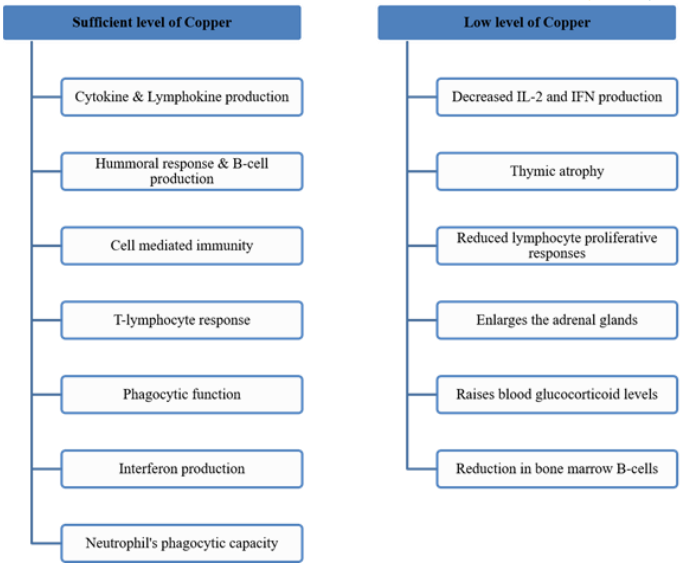

Figure 4: Effect of copper level on immune system and health of animals.

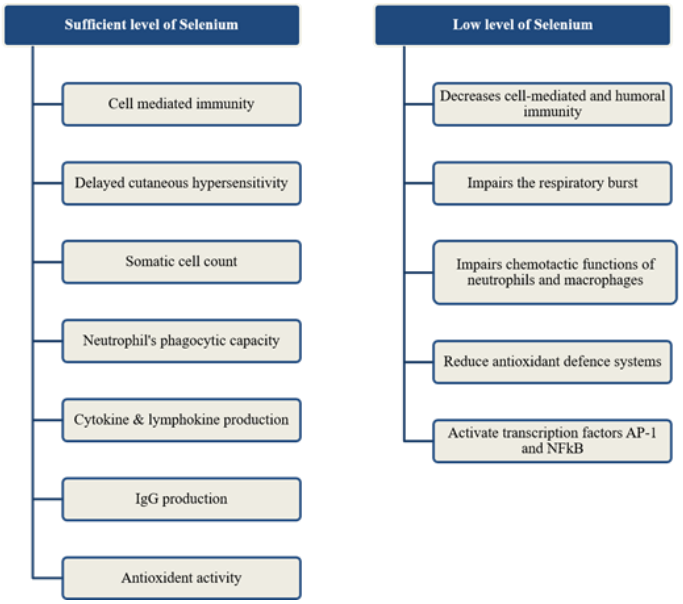

Figure 5: Effect of selenium level on immune system and health of animals.

Selenium

Selenium is a vital element and plays an important role in increasing immune competence of the host by neutralizing oxidation reactions (Hoffmann and Berry 2008). Selenium-deficient diets decreased resistance to parasitic infection. Utilization of Se may provide effective antioxidant protection against the oxidative stress experienced during *H. contortus* infection (Burke and Miller 2008). Subclinical deficiency of Se results in reduced production and immunosuppression in animals (Hefnawy and Tortora-Perez 2010). In the case of parasitic infections, the host responds to the parasite by generating reactive oxygen species (hydroxyl radical, hydrogen peroxide, superoxide anion radical, peroxynitrite and nitric oxide) that damage the parasites but also enhances oxidative stress for the host (Sorg 2004; Rosenfeldt et al. 2013). Phagocytes, such as eosinophils, macrophages, and neutrophils, are responsible for the production of these reactive oxygen species, which may cause severe damage to the host through immunosuppression (Kotze 2003).

Neutralization of reactive species can occur by antioxidant defense systems, consisting of enzymatic (i.e., glutathione peroxidase,GPx), glutathione reductase, catalase (CAT), superoxide dismutase (SOD)) and non-enzymatic defenses such as reduced vitamins A, C and E and glutathione (Sorg 2004; Rosenfeldt et al. 2013).

GPx is involved in a chain of reactions catalyzing the formation of thromboxanes, prostacyclins, prostaglandins, and leukotrienes (Leal et al. 2010; Rosenfeldt et al. 2013). Selenium is a component of GPx and there is a strong association between the level of Se and enzyme activity. Deficiency of Se results in lower amounts of functional GPx, which may lead to severe cellular damage due to changes in the structure of proteins, polysaccharides, DNA and lipids (Hefnawy and Tórtora-Pérez 2010; Ferguson et al. 2012). Therefore, Se can be seen to be directly involved in proper working of the immune system, regulating phagocyte function, cell-mediated and humoral immune responses, and induction of pro-inflammatory cells that reduce oxidative cell production (McClure 2008; Leal et al. 2010).

Increased expression of TLR-4, L-selectin, selenocysteine-containing selenoproteins glutathione peroxidase-4 and IL-8R in animal neutrophils is related to supranutritional supplementation with Se-yeast. These genes are involved in the response against parasites and bacteria. Increased expression of the selenocysteine-containing selenoproteins GPx-4 leads to the lipid hydroperoxide free radicals detoxification in the intestinal mucosa (Speckmann et al. 2014). Deficiency of Se in *He. polygyrus*-infected mice led to an increase in adult worm numbers, parasitic egg production and fecal egg count

(Smith et al. 2005). Barium selenite injections in weaned Se-deficient lambs reduced fecal egg count and increased body weight (Celi et al. 2010), while use of intraruminal Se pellets in animals infected with *O. circumcincta* and *T. colubriformis* did not reduce worm counts or fecal egg counts (McDonald et al. 1989). According to Camargo et al. (2010), Se stimulates immunity of animals infected with *H. contortus*, resulting in a reduced number of parasites. The combination of Se and Cu had a significant effect on parasitic burden in terms of reduction in EPG and worm load in sheep infected with *H. contortus* (Silva et al. 2013). Celi et al. (2010) documented that the Se status of sheep has a vital role in resistance against parasitic infection. The Se supra-nutritional availability effects *H. contortus* infection and Se-yeast supra-nutritional supply helps to control the severity of infection (Hooper et al. 2014). A comprehensive outline of the effect of selenium level on the immune system and health of animals is shown in Fig. 5.

Molybdenum

The optimal range of Mo in feedstuff is 6-10 mg kg-1 dry matter, when the level of Cu is marginal, and this range is inconsistent with the maximal permissible concentration. Mo acts both as an essential nutrient and an immunity modulator, especially for mucosal immunity (Blood and Radostits 1989). Molybdenum is also vital for boosting the immune system and its deficiency predisposes an animal to primary and secondary infections. This element is necessary for the development of immune responses and normal functioning of the rumen microbial fauna, thus indirectly improving host nutrition (Ellis et al. 1958).

A low level of Mo in diets can reduce the ability of animals to reject challenges with *H. contortus* and *T. colubriformis* (Suttle et al. 1992; McClure et al. 1999). An optimum intake of about 4–8 mg/animal/day of Mo was found to be the greatest defense against parasites, as this level was linked to an increase in parasite-specific immune responses, i.e., jejunal mast cell numbers and antibody levels, proliferation of worm-specific lymphocytes, globule leucocyte numbers and eosinophil count.

Mo also plays a role in enhancing protection against inflammatory responses in nematodes, which is achieved through increased concentration of superoxide radicals in the mucosa, hence reducing the effectiveness of Cu-dependent inflammatory responses and the function of Mo as a co-factor for xanthine oxidation (Suttle et al. 1992). A similar mechanism is likely to be present in *T. colubriformis* rejection (Bendixsen et al. 1995). Another possibility is that the involvement of Mo in pyridoxal oxidase action mediated rejection of the parasites (Lee et al. 2002). Supplementation of Mo results in 78% reduction of *H. contortus* (Suttle et al. 1992) and 23% of *T. vitrines* (Suttle et al. 1992a). A low level of Mo in the diet reduces the ability of sheep to reject infection of *Trichostrongylus* sp. (McClure et al. 1999). Effects of Mo level on the immune system and health of animals are shown in Fig. 6.

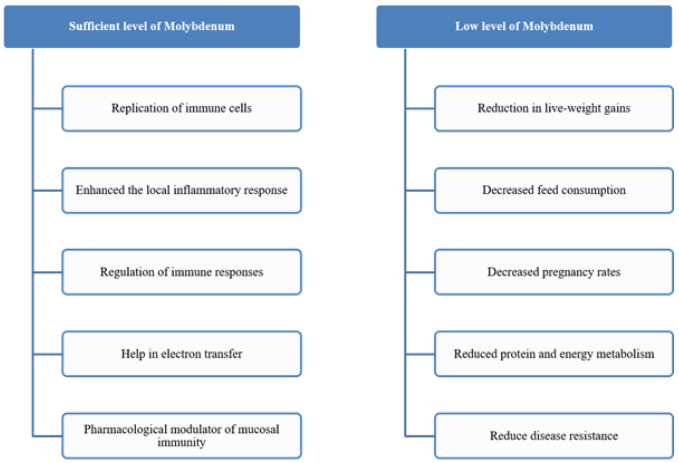

Figure 6: Effects of molybdenum levels on the immune system and health of animals.

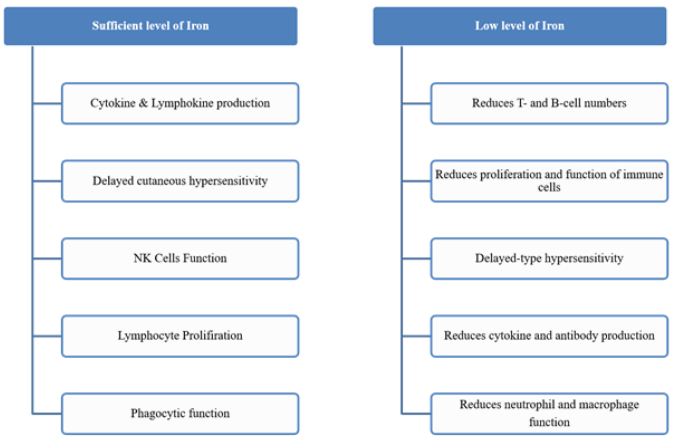

Figure 7: Effect of iron level on the immune system and health of animals.

Iron

Animals and humans are required to maintain optimum Fe concentration and Fe homeostasis based on its toxicity (Zhang et al. 2009). This mineral is a part of proteins, a cofactor for enzymes, iron-chelating proteins, and the heme group in hemoglobin and other hemoproteins (Dunn et al. 2007). Immunological processes in the host are regulated by minerals such as Fe, P, Co, and Zn, to make the immune system more responsive against parasites (Hughes and Kelly 2006).

Parasites need Fe to survive and reproduce and to produce disease in the body of vertebrate hosts, e.g., schistosomes (Jones et al. 2007). To cope with this situation, mammals have adapted themselves against these pathogens by activating iron-sequestering systems which minimize the concentration of free Fe in the body. Thus, lactoferrin and transferrin, Fe chelating proteins, lower the Fe concentration to levels below those needed for the parasite to survive (Nairz et al. 2010). Furthermore, infections are linked with hypoferremia, a host response in which free Fe in body fluids is reduced. Therefore, parasites have developed strategies to capture the Fe retained in proteins as in lactoferrin. In this way, lactoferrin acts as microbiostatic. In addition, Fe can also

disturb the functional integrity of the parasite surface of *Giardia, Toxoplasma* and *Entamoeba,* thereby acting as a parasiticide (Ordaz-Pichardo et al. 2013).
Anemia due to Fe deficiency results in *G. intestinalis* and other protozoal infections due to destruction of intestinal mucosa, leading to malabsorption of micronutrients like Fe. Gastrointestinal parasitic infections can also lead to Fe deficiency and anemia (Le et al. 2007). Fe-deficiency anemia in helminth infections is attributable to malabsorption of Fe from intestine and direct feeding on blood, hindering Fe metabolism (Adebara et al. 2011). Fe and vitamin B_{12} have positive relation with respect to the host and its parasite (Marcelo et al. 2007). Effects of Fe levels on the immune system and health of animals are shown in Fig. 7.

Zinc

Zn plays an important role in minimizing pathogenesis by up-regulating the immune system through activating defensive mechanisms (Hughes and Kelly 2006; Ahmad et al. 2020). It is an essential element in cell-mediated cytotoxicity, T- and B-helper cell function, and other immune responses that enhance the efficiency of gut epithelial barriers and hence intestinal immunity against GI parasites, specifically nematodes. Zn deficiency can lead to down-regulation of these immune responses, resulting in increased susceptibility of animals to parasites (Scott and Koski 2000; Hughes and Kelly 2006). Zn is also necessary for the immune system and can reduce parasite numbers (Bundy and Golden 1987).
It has been reported for mouse models that parasites can better evade immune responses in Zn-deficient hosts than in healthy mice because IL4 production is suppressed in the spleen of the former, leading to lowered titers of IgE, IgG1, and eosinophils, as well as poor performance of APCs and T-cells (Scott and Koski 2000). Zinc supplementation can prevent lambs from infection with *H. contortus* by disturbing its life-cycle and causing oxidative stress. Zn has also been proposed to possess anthelmintic activity against nematodes (Váradyová et al. 2018; Rizwan et al. 2019).
Studies of the effect of Zn on intestinal nematodes in animals in laboratory setting have shown variable results. Rats given feed deficient in Zn at 3 mg kg^{-1} showed higher *Trichinella (Tr.) spiralis* burden for longer periods of time than those in the control group (Fenwick et al. 1990). Similar results were reported in Zn-deficient rats infected with *He. polygyrus* and *Strongyloides ratti* fed on the Zn-deficient diet with Zn concentration of 3 mg kg^{-1} (Fenwick et al. 1990a). On the contrary, mice fed Zn at 5 mg kg^{-1} efficiently controlled *H. polygyrus* infection. Although Zn at 3 mg kg^{-1} increased survivability of the above-mentioned helminths, *Nippostrongylus brasiliensis* remained unaffected in mice at this concentration (Minkus et al. 1992). However, *H. polygyrus* survival was enhanced in mice fed Zn in diet at 0.75 mg/kg. It indicates that Zn has a role in intestinal immunity against helminths in laboratory animals. Zinc also has been reported to play an important role in the activation of

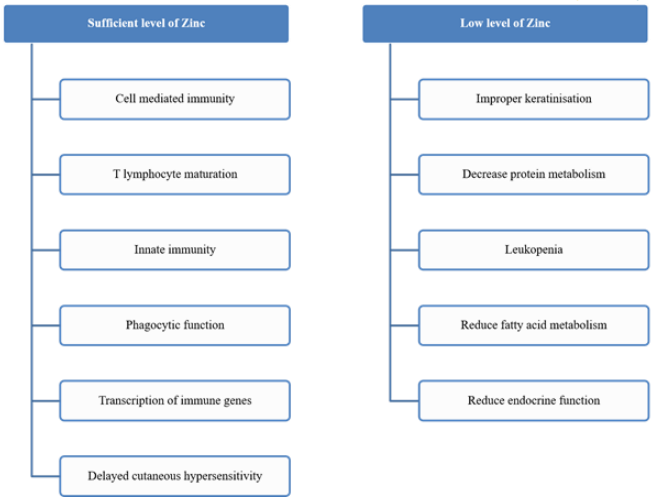

Figure 8: Effect of zinc level on the immune system and health of animals

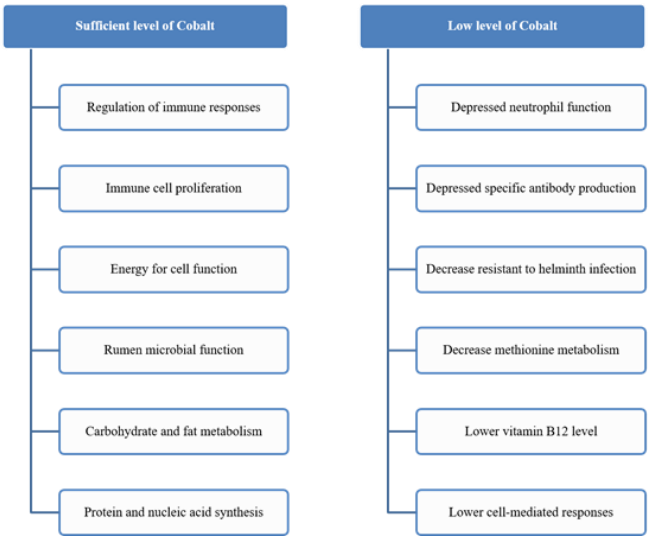

Figure 9: Effect of cobalt level on the immune system and health of animals

humoral immune response in mice infected with *Giardia* (Astiazarán-García et al. 2015).
A reduced level of serum Zn was observed in animals infected with GI parasites. Animals infected with *T. colubriformis* showed 17% Zn reduction in serum (Symons 1983). Zinc supplementation resulted in a reduction of the worm burden of *S. mansoni* in hamsters (Mansour et al. 1983). Severe deficiency of Zn resulted in increased worm burden of *Tr. spiralis* (Fenwick et al. 1990). This element laos has a positive impact on the immune system against GI parasites in livestock and laboratory animals (Koski and Scott 2001). Effects of Zn level on the immune system and health of animals are shown in Fig. 8.

Cobalt

Cobalt (Co) is an important TE in the diet of ruminants, as it is required for vitamin B12 synthesis (National Academies of Sciences, Engineering, and Medicine 2016; Qudoos et al. 2017). Methylmalonyl-CoA mutase and 5-methyltetrahydrofolate homocysteine-methyltransferase

are the two main enzymes which are dependent on vitamin B12 and play a major role in the formation of methionine and tetrahydrofolate (National Academies of Sciences, Engineering, and Medicine 2016). However, their relation to parasitic infection is not fully understood. Deficiency of Co results in a reduction of resistance against parasitic infections. Cobalt can help control parasitic infections, as cattle suffering from Co-deficiency are more prone to GI parasite infections (Silva et al. 2020). In a trial, lower cell-mediated responses to vaccination and higher EPG of nematodes were observed in animals fed low level of Co in the diet as compared to those having sufficient Co supplementation (Vellema et al. 1996). Effects of Co levels on the immune system and health of animals is shown in Fig. 9.

Molybdenum

The role of Mo in immunity is well-known and results in decreased *H. contortus* burden (McClure 2003). Parenteral administration of Mo results in worm rejection by the involvement of immune cells (Miller 1984).

Manganese

The relationship of Mn with parasitic infections in livestock is not well explored; however, excess Mn showed an increase in worm burdens in *Ascaridia galli*-infected chicks (Gabrashanska et al. 1999).

Conclusion

For the physiological functioning of the immune system, several micro and macro TEs are required. Essential TEs are important for cell metabolism and the immune system. Adequate levels of TEs, such as selenium, molybdenum, copper, iron, zinc and cobalt have significant effects on animal health through reduced GI parasitic infection. Other TEs are either directly or indirectly involved via physiological pathways that regulate mucosal immunity. The likelihood that minerals can affect gut immune responses of livestock against endoparasites can be predicted through implications generated in epidemiological and experimental studies in ruminants and monogastric species. Research is still needed to determine the effects of these minerals on mucosal immunity and determination of pathogenesis and control measures of already identified minerals and potentially involved minerals in humans and livestock species. Understanding immunity and nutrition is necessary for prevention of diseases. A systematic veterinary research approach is required to investigate the principles of mammalian resistance to gut diseases through epidemiological and clinical observation, followed by confirmation through experiments and elucidation of pathogenesis. This will allow the formulation of basic principles regarding physiological status and gut diseases across mammalian species.

It has been reported that deficiency of TEs in animals is directly associated with depressed immune system. Nutrition has the potential to affect GI parasites because it directly affects the degree of expression of the immunity and rate of acquisition of the infection, which can reduce the survival, fecundity, and establishment of GI parasites. A gap between TE deficiencies and parasitic infections in animals is reviewed in this chapter, which confirms the requirement for further research to explain their possible role against GI parasites. Due to sub-clinical deficiencies of TEs, animals use feed less efficiently, which may lead to a decrease in growth rate, low reproductive performance, and immunodeficiency. However, acute deficiencies of TEs cause huge economic losses in the sense of mortality. Therefore, it is important to identify TE-enriched pasture and soil to improve the immunity in animals.

Animals having inadequate supplies of nutrients are more prone to GI parasitic infections, which reduce their productivity. To determine the sufficient amount of TEs for animals, appropriate analyses of soil, forages, and animals are essential. Nutrition of animals depends upon soil-plant-animal complex, although season can strongly influence the dietary requirements for TEs. Deficiencies of TEs in soil and forages affect animal production adversely. Analysis of a particular area is imperative to assess its TE profile and to compare their availability for grazing animals.

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PARASITIC DISEASES OF FISH

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INTRODUCTION

The fishes (Phylum: Chordata) are the most diversified group of aquatic organisms known as cold-blooded vertebrates. They have gills and fins for breathing and swimming, respectively. Different kinds of fishes protect themselves with scales and have an efficient body for convincing movement in water (Tedesco et al. 2017). Moreover, fish is considered as an important part of human food and playing a significant role in the economy of various states worldwide (Essetchi et al. 2003; Andronova and Yakimovich 2019). There are around 28,900 species of fish present in the world, 13,000 species are known as freshwater, having 170 families and 2,513 genera, which are found in rivers and lakes (1% of the total water available on earth), whereas the remaining 16,000 species are found in saline medium (covering 70% of the earth) (Leveque et al. 2008). This chapter describes the parasitic threats to profitable fish farming around the globe.

Parasitic fauna of fish

Fish is directly or indirectly affected by different kinds of parasites, which cause high mortality in this species. Four major groups of parasites that cause infections in fish are: Protozoa (ciliates, flagellates, microsporidians, myxozoans), platyhelminthes (monogenean, digenean, cestodes), nemathelminthes, and acanthocephala. The physiology of fish facilitates the occurrence of various lethal diseases, which lead to mass mortality (Al Marjan and Abdullah 2009; Lerssutthichawal et al. 2015). Furthermore, fish act as hosts for numerous parasites; especially gastrointestinal (GI) helminths which are considered major fish parasites, causing intensive losses to the fish industry. Parasites affect the diet, metabolism, and secretory functions of the digestive system, which causes severe damage to the nervous system and interrupts the normal reproduction of the fish. The parasitic threats are the major reason for the reduction in the fish population (Habib 2007). Parasitic fauna infecting various systems of fish is given in Table 1.

Protozoan parasites of fish

Protozoans are the significant parasites of fish kept under intensive fish culture. The major groups of protozoa infecting the fish are myxozoans, microsporidians, ciliates, and flagellates (Wang et al. 2019). These parasites can increase their number when the host fish is overcrowded, resulting in weight loss, emaciation, and mortality (Gomes et al. 2017). Among different groups of protozoans, ciliates and flagellates have a direct life cycle and mostly infect the pond water fish. On the other hand, microsporidians are intracellular and require tissue of the host for reproduction (FAO 2015). The fish became infected by ingesting spores from the infected fish or food sources (Manbe et al. 2020). The cells having the parasites start to increase in size to accommodate the proliferation (merogonous and sporogonous development) of the parasite (Wang et al. 2019; Agboola et al. 2021). The multiplication of these sporozoites inside the cell causes the development of tumor-like masses in various tissues of the fish (Saha and Bandyopadhyay 2017). The pathological lesions that occur due to these cancer-like growths include multiple whitish nodules on the tissues, and thickening of the wall of gall bladder (FAO 2015). Acute anemia may result in the case of microsporidian infection of haemopoietic cells (Maciel et al. 2018).

Myxozoan parasites infect many groups of fish, including Cichlidae, Cyprinidae and Mugilidae (FAO 2015). In Africa, more than 135 species of myxozoans are known to cause infection in fish of all habitats i.e., freshwater, brackish water and marine water habitats (Santos et al. 2018). More than 17 species from four different genera of parasites including *Henmeguya*, *Myxobolus*, *Myxobilatus* and *Parahenmeguya* have been reported to infect the fish. Infra-communities and component communities are being formed by the myxozoan parasites which can surpass the colonies formed by other parasites (Balta et al. 2019). These myxozoan parasites can cause histozoic and coelozoic infections (FAO 2015). The signs of the former include, whitish cyst containing milky fluid with microscopic spores, which can be seen on the scales, fins, lips, and around eyes. It has been further reported that the infection may cause breaking of the cartilage of gills, resulting in disturbed breathing and inadequate gaseous exchange (Maciel et al. 2018). In cultured fish, several Myxozoan infections have been reported i.e, skeletal deformities, locomotory disturbances, emaciation, sunken eyes in the brain, and circulatory dysfunctions at the base of the gill lamellae (Santos et al. 2018; Manbe et al. 2020). The simplified life cycle of protozoan parasites infecting the fish has been elaborated in Fig. 1.

Table 1: List of parasitic fauna harboring various fresh and salt-water fish fauna around the world

Sr. #	Parasite Name (Common name)	Site of Infection	Host	Disease	Source
Parasites of integument & skeletal system					
1.	*Amyloodinium Ocellatum*	Gills & Skin	All types of fishes	Amylodiniosis/Marine velvet disease	(Moreira 2017)
2.	*Trichodina*	Gills & Skin	All	Trichidiniasis	(Nofal 2017)
3.	*Cryptocaryon irritans*	Skin, Fins, Gills	Marine Fishes	Cryptocaryonosis/White spot disease	(Liu 2020)
4.	*Ichthyophthirius multifiliis*	Skin	All	Ichthyophithiriasis/white spot disease / Ich	(Buchmann 2020)
5.	Monogeneans	Skin & Gills	Goldfish, channel catfish, angelfish	Skin and gill monogean disease	(Neves 2020)
6.	Isopods	Gills & Body surface	*Epinephalus (Ep.) coidois, Ep. malabaricus*	-	(Purivirojkul 2020)
7.	*Brooklynella hostilis*	Skin & Gills	Damselfish, Amphiprioninae	Brooklynellosis/Clownfish Disease	(Anshary 2020)
Parasites of eye					
1.	*Diplostomum spathaceum* (Eye Fluke)	Eye	Fresh water and brackish water fishes	Diplostomiasis	(Vyhlídalová 2020)
Parasites of vascular system					
1.	*Sanguinicola occidentalis*	Heart	*Perca jlavescens*	Sanguinicoliasis	(Muzzall 2000)
2.	*Paradeonta cylix*	Heart	Greater amberjack	Blood fluke disease	(Repullés-Albelda 2008)
3.	*Cardicola*	Blood	Northern Bluefin tunna thynnus	-	(Shirakashi 2016)
4.	*Trypanoplasma sp.*	Vascular system, kidneys	Blue tilapia fish	-	(Carrington 2017)
5.	*Crassiphiala bulboglossa*	Blood	Vermont fish	-	(Achatz 2019)
6.	*Aporocotylid digenean*	Heart, Gills, Cranial & mesenteric Blood vessels	Fresh and marine water fish	Black grub	(Poddubnaya 2021)
Parasites of the central nervous system					
1.	*Tetrahymena corlissi*	Skin, Eye & Muscles	Fresh water fishes' guppies	-	(Imai 2000)
2.	*Uronema Nigricans*	Connective tissues (Skin, Fins & Nervous Tissues)	Bluefin tuna · Thunnus maccoyi	-	(Arévalo 2018)
3.	*Myxosoma (My.) cerebralis/ Myxobolus (M.) cerebralis*	Head, Cartilage & backbone	Salmon and trout	Whirling disease	(Alexander 2020)
Parasites of viscera and musculature					
1.	*Contracaecum rudolphii*	Body Cavities & visceral organs	All types of fishes	-	(Baruš 2001)
2.	*Sphaerospora renicola*	Kidneys	Cyprinids	Renal dropsy	(Eszterbauer and Székely 2004)
3.	*Posthodiplostomum cuticula*	Viscera, Heart & Posterior kidney	Freshwater fishes	Black spot disease	(Ondračková 2004)
4.	*Diphyllobothrium latum* (Broad fish tapeworm)	Viscera & Musculature	Fresh water fishes	Diphyllobothriasis	(Scholz 2009)
5.	*Proteocephalus ambloplitis*	Ovary	Freshwater fishes	-	(Scholz 2019)
6.	*Bolbophorus confuses*	Skeletal muscle & Viscera	Channel catfish	Channel virus disease or enteric septicemia like condition	(Doffitt 2020)
7.	*My. cerebralis / M. cerebralis*	Head, Cartilage & backbone	Salmon and trout	Whirling disease	(Alexander 2020)
Parasites of the alimentary canal					
1.	*Glugea (Gl.) hertwigi & Gl. stephani,*	Submucosa of GI & Mesentery	Smelt, marine flatfishes	Non-functional, granulomatous bulged out abdomen	(Ogawa 1998)
2.	*Bothriocephalus acheilognathi*	Intestine	Carps and other cyprinids	Haemorrhagic enteritis/ Gowkongensis	(Salgado-Maldonado 2003)
3.	*Cryptobia iubilans*	Stomach	African cichlids	Cryptobiosis	(Woo 2003)
4.	*Balantidium*	Intestinal lumen	Marine and Fresh-water fish	Catarrhal enteritis and ulceration	(González 2005)

5.	*Goussia (G.) subepithelialis, G. Carpelli*	Intestinal epithelial cells	Carps, marine and freshwater fishes	Coccidiosis	(Pasnik 2005)
6.	*Cryptosporidium*	Stomach	Marine tropical fish	Cryptosporidiosis (necrosis of gastric epithelium)	(Méndez-Hermida 2007)
7.	*Ceratomyxa shasta*	Posterior Intestine	Salmonids	Ceratomyxosis	(Bjork 2010)
8.	*Pseudophyllid Triaenophorus*	Intestine	Wild and cultivated salmonids	Chronic haemolytic Anaemia	(Hoole 2010)
9.	*Spironucleus*	Intestine	Salmonids, All cichlids, bettas, gouramis, other aquarium snd freshwater fishes	"Hole in the head" disease	(Williams 2011)
10.	*Caryophyllidea*	Intestine	Cyprinid and catostomid fish,	Intestinal nodules and ulcers	(Barčák 2014)
11.	*Contracaecum*	Alimentary tract	Largemouth bass, centrarchids	Anisakidosis	(Younis 2017)
12.	*Camillanus*	Alimentary tract	Largemouth bass, other centrarchids	-	(Manickam 2018)
13.	*Philometra*	Body cavity, Tissues & Ovaries	Marine fish	"Parasitic Castration" in female fish	(Ali 2018)
14.	*Eustrongylides*	Viscera & Muscles	Angelfish, other aquarium and freshwater species	Eustrongylidosis	(Guagliardo 2019)
15.	*Capillaria*	Intestine	Angelfish, discus, other aquarium fish	Capillariasisis	(Abdel-Rahman 2019)
16.	*Crepidostomum*	Intestine	Salmonids In the Nearctic and Palearctic regions	Enteritis	(Faltýnková 2020)
17.	*Enteromyxum (E.) leei, E.scophthalmi*	Intestinal Epithelium	Tiger puffer fish, marine and fresh-water fish	Enteritis	(Picard-Sánchez 2020)
18.	*Acanthocephalus*	Intestinal lumen	Wild-caught freshwater fishes, wild-caught marine fish	Necrotic haemorrhagic Ulcers	(Nakao 2021)

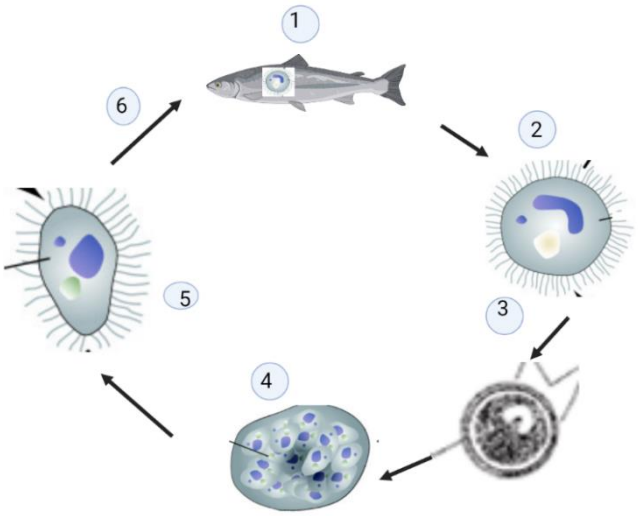

Fig. 1: Simplified life cycle of protozoan parasites infecting the fish: 1=Trophont feeds on fish, 2=mature tomont leaves the host, 3=Tomont secretes gelatinous cyst wall, 4=Tomont undergo cell division and produce daughter tomites, 5=Tomites differentiate into infective Theronots, 6=Theronts bore through the cell wall and infect the fish (Source: Modified from Roberts 2012).

Helminths parasites of fish

Helminths are the biggest group of parasites infecting the fishes. More than 30,000 helminth species are infecting the sea and freshwater fish and some of them are known to cause serious fish diseases or may represent an important public health concern (Williams and Jones 1994). Platyhelminthes (flatworms: cestodes, monoge-neans and digeneans); Nemathelminthes (roundworms: nematodes) and Acanthocephalan (thorny-headed worms) are major groups of helminths parasites (Nguyen et al. 2020).

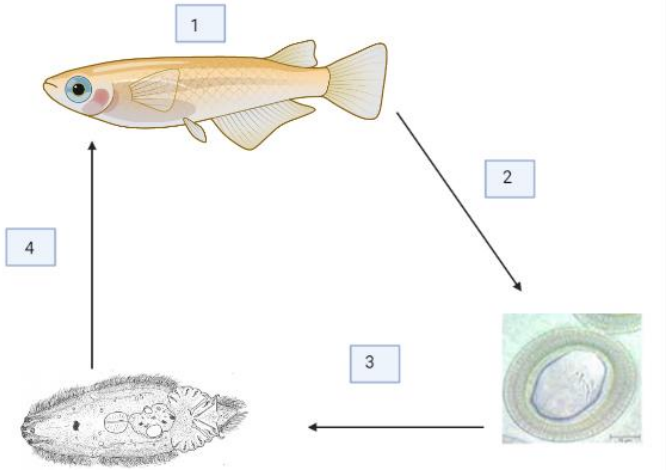

Fig. 2: Simplified life cycle of Monogeneans parasites infecting the fish: **1**=Adults present on fish skin and gills, **2**=Eggs laid by adult, **3**=Eggs hatch ad release oncomiracidium, **4**=Free swimming oncomiracidium attaches to the host (Source: Modified from Roberts 2012).

Trematodes

Trematodes, also known as flatworms or flukes, are a class of helminths which are further classified into monogeneans and digeneans. Their site of infection is the skin, fins and gills of the teleost that inhabit freshwater and brackish water (Antar and Gargouri 2018).

Monogeneans

Naturally, monogeneans are host-specific (Saad-Fares 1992). They are hermaphrodites and at the anterior end possess sensory structures, a mouth with or without accessory suckers, special glands and clamps for the

attachment to the host. Mostly they reside on the body surface of the host like epidermis, scales, fins, gills, lip folds, nares, and branchiostegal membranes. They have a direct life cycle, without the involvement of an intermediate host (Buchmann 2002).

There are three main taxa of monogeneans; Dactylogyroidae, Caspaloidae and Polyopisthacotylea. Dactylogyroidae family members are relatively smaller in size and are usually present in inland fishes as compared to the Caspaloidae and Polyopisthacotylea, which are larger and mostly found in marine fishes. Due to their different taxa, they are slightly different in their structure and nature of infection like Dactylogyroids are oviparous, while Gyrodactylidae is viviparous. The former consists of one or two anterior-dorsal pairs of eyes and posterior-ventral opisthaptor, while later possesses no eyespots and two pairs of anchor hooks. Dactylogyroids inhabit the gills of fish, while gyrodactylidae reside on the skin and fins. Some of them occasionally infest nasal cavities, stomach, or urethra of freshwater fishes (Šimková 2017). In their natural habitats, fish can co-exist with monogeneans but some of them like gonodactylids can be pathogenic, predominantly to the young fishes.

Dactylogyrus (D.) vastator is the parasite of carp fry fish and infests the gills of the host, causing epithelial hyperplasia intervening in the respiratory system that leads to death of the host. *D. extensus* is also a deadly parasite of the young, as well as adult, fishes (Dzika 2009). *D. groschefti* has shown more than 90% mortality rates in young *Clarias gariepinus* (Hansen 2003). Macrogylodactylus mostly affect aquaculture fishes viz *Clarias*, *Lates niloticus,* and Anabantidae species (Iyaji 2008). Parasitic infestation is dependent on several physical and chemical factors. Physical components may include temperature and depth of the pond, while oxygen and salinity levels are the main chemical features. Temperature is generally the main element for seasonal parasitic prevalence (Gopko 2020).

Digeneans

Digeneans trematodes are a very diverse group of fish parasites, belonging to 15 families and more than 50 species (Selbach 2020). They mostly parasitize freshwater fishes and can act as internal or external parasites involving several organs. They have complex, indirect life cycle and are heteroxenous in nature due to the involvement of multiple intermediate hosts and larval stages in their life cycle. Bivalves and gastropod mollusks act as their intermediate hosts. Their metacercariae are present in large numbers within-host and affect several tissues and organs. Lesions appear depending upon the organ involved. *Sanguinicola* species are parasites of *Synodontis schall* and *Auchinoglanus occidentalis*. It is commonly known as blood fluke because worms and eggs are present in blood and may cause thrombosis and subsequent necrosis. On the other hand, migration of miracidia through the gills epithelium leads to direct blood loss, followed by anemia. They cause damage to the heart, brain, eyes, and other soft organs (Kirk 2012). *Syphodera ghanensis* and *Aspidogaster africanus* infect the intestines of *Chrysichthyes nigrodigitatus* and cause intestinal destruction of the host (Kohn et al. 2007). The general life cycle of trematode parasites is elaborated in Figs. 2 and 3.

Cestodes

Cestodes are commonly called as tapeworms due to their ribbon-like and multisegmented body structure. Their characteristic feature is host specificity; cestodes generally affect members of siluriform. They are mainly divided into two forms namely, the monozoic forms notably *Caryophyllaeidae,* and the amphilinid represented by the segmented Pseudophyllideans and Proteocephalideans.

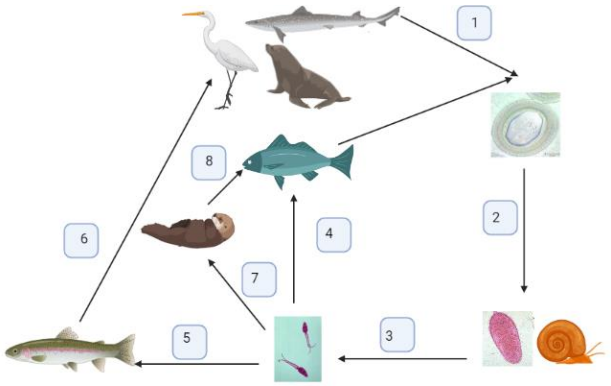

Fig. 3: Simplified life cycle patterns of Digeneans parasites infecting the fish: 1=Eggs released from final host, 2=Egg hatch into miracidium which invades molluscan host, 3=Larval development resulting in the production of cercariae, 4= Cercariae direct invaded final host, 5=Cercariae released invades fish intermediate host, 6=Cercariae develop into metacercaria in intermediate host and this metacercaria invades final host, 7= Cercariae invades invertebrates intermediate host, 8=Encysted metacercaria eaten by final host (Source: Modified from Roberts 2012).

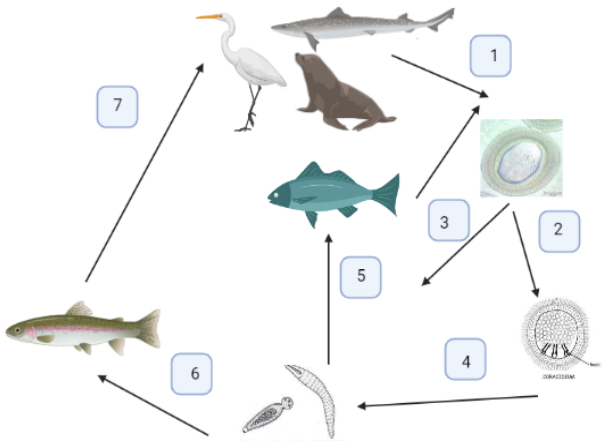

Fig. 4: Simplified life cycle patterns of cestodes parasites infecting the fish, **1**=Eggs laid by final adult host, **2**=Egg hatch and release coracidium, **3**=Eggs eaten by invertebrate intermediate host, **4**=Coracidium eaten by intermediate hots and develop into procercoid/plerocercoid stage, **5**=Plerocercoid stage is eaten by fish final host, **6**=Procercoid eaten by fish intermediate host and development to plerocercoid occurs, **7**= Eaten by final hosts (Source: Modified from Roberts 2012).

They are predominantly found in farmed and wild fish in Asia, Africa and Europe (Rocka 2017). Cestode infestation occurs in the alimentary tract, muscles, or other internal organs. Some of the most damaging parasites of freshwater fish are larval cestodes called Plerocercoids. If Plerocercoids are present in muscles, they damage the skeleton system, and when they are present in gonadal tissue, they damage the reproductive system. Cestodes also become a major issue when they affect vital organs such as the brain, eye, or heart (Dove and Fletcher 2000). The digestive tract is the predominant site for their residence, but they do occur in other parts of the host body. For instance, *Bothriocephalus acheilognothii* resides in the digestive tract of host fish families Cyprinidae, Poecillidae, Cichlidae and Centrachidae (Pérez-Ponce de León et al. 2018), while *Nesolecithus africanus* resides in the coelomatic cavity of mormyrid *Gymnachus niloticus* host and *Polyonchobothrium clarias* resides in the gall bladder of *Clarias and Oreochromis* specie *(*Ali 2020).
Adult cestodes do not cause much damage to their host but some of them cause severe damage to fish, resulting in compromised fish quality. Like *Eubothrium*, chronically affect the quality of farmed fish. *Polyoncho-bothrium clarias* infests the gut mucosa of host fish and causes an inflammatory response in the gut. It also migrates towards the gall bladder and produces granulomatous nodules and fibrous tissues. Larval forms of plerocercoids and cysticercoids cause damages in freshwater fishes. In African fish, predominant infection is due to ligula plerocercoids in the body cavity of *Barbus*, *Cyprinid* species and *Cyclophyllidean cysticercoids* infect the mesenteries of siluriform, *Clarias* and *Bagrus* species (Song 2018). They have piscivorous birds such as gulls and cormorants as their definitive hosts. *Ligula plerocercoid* infects *Barbus* species and causes abdomen distention along with the formation of hemorrhages in the abdominal wall of the host and may also result in reproductive impairment (FAO 2015). The general life cycle of cestode parasites is elaborated in Fig. 4.

Nematodes

Nematodes are important parasites of several species of both aquaculture and wild fish. They use fish as intermediate or transient hosts and result in heavy infection, involving almost all organs of their hosts (Ali et al. 2014). Nematodes have a distinctive shape, having resistant cuticles which make them hardy and relatively last longer as compared to platyhelminths in post-mortem conditions (FAO 2015). There are 40 species of nematodes, severely infecting the digestive system of various fish species in Africa. Adult nematode usually resides in the fish digestive tract. However, different life stages may be present in internal organs, coelomic cavity, external muscle layers, the swim bladder, and inside the skin or fins, depending upon different species of nematode and infected fish (Dick and Choundury 1995). In Pakistan, 13 species of nematodes, 8 of which are new, were recovered from 15 species of fish at the Karachi coast. *Camallanus cotti, Tetrahymena corlissi,* and *Anisakis simplex* were the most common nematodes found in the intestine of wild marine (Khan and Begum 1971). In healthy fish, nematodes are often present in low numbers but can increase in number to produce serious illness or even death. If the fish is the final or conclusive host, then the nematode will go into another organism, generally a marine invertebrate like a side swimmer, side copepod, or insect larva, in which it will grow before being eaten by a fish. Upon ingestion, nematode species sexually mature and reproduce in the fish. In this situation, the fish is named as the definitive or final host (Ali et al. 2014). Nematodes, that have an indirect life cycle, use the fish as intermediate hosts. The conclusive host (that comprises the propagative adult phase of the nematode) could be a fish-eating fish, mammal, or bird. Fish nematodes have

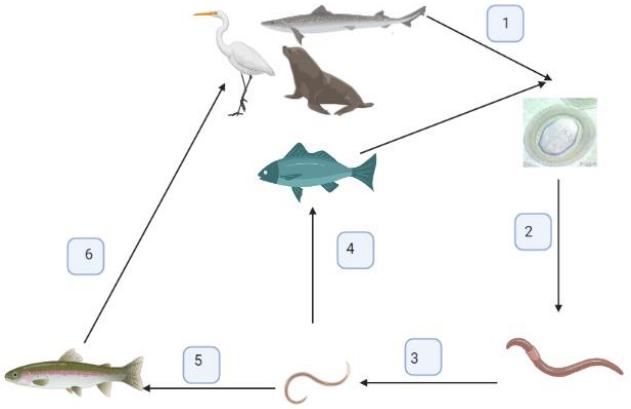

Fig. 5: Simplified life cycle patterns of nematodes parasites infecting the fish: **1**=Eggs laid by adults hatch to release free-swimming larvae, **2**=Larvae eaten by invertebrate intermediate host, **3**=Further larval development occurs in invertebrate host, **4**=Infective larvae eaten by fish final host, **5**=Larvae encyst in fish intermediate or paratenic host, **6**=Infective larvae eaten by final host (Source: Modified from Roberts 2012).

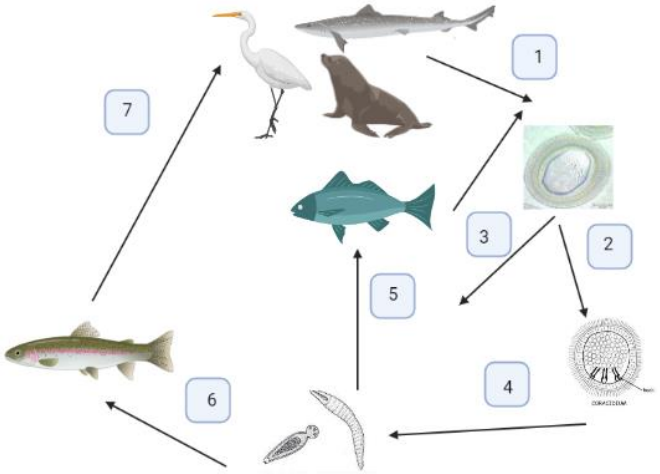

Fig. 6: Simplified life cycle patterns of acanthocephalan parasites infecting the fish: **1**=Eggs laid by final adult host, **2**=Eggs hatch and release coracidium, **3**=Eggs eaten by an invertebrate intermediate host, **4**=Coracidium eaten by intermediate hots and develop into procercoid/plerocercoid stage, **5**=Plerocercoid stage is eaten by fish final host, **6**=Procercoid eaten by fish intermediate host and development to plerocercoid occurs, **7**= Eaten by final hosts (Source: Modified from Roberts 2012).

the capability to persist in alternate individuals, recognized as paratenic hosts. Such hosts are not necessary for the completion of the life cycle, but they can comprise infected nematode life stages and be a basis of infection. They can be worms, fish or further marine organisms which feed on the nematode larvae or eggs (Pazooki et al. 2012). The general life cycle of nematode parasites is elaborated in Fig. 5.

Acanthocephala

Acanthocephalans are internal parasites of fish and amphibians and are characterized by the presence of an armed and eversible proboscis used to pierce the gut wall of fishes. These gastrointestinal parasites can cause pathological changes which may result in deterioration of the host health (Taraschewski 2008). Parasites have the characteristic feature of a proboscis which can evaginate and is crowned with many rows of recurved hooks. Acanthocephalans are heteroxenous and they don't have alimentary canal. Eggs laid by the adult worm in the intestinal lumen are passed out through feces and enter the body of the first intermediate hosts, such as amphipods, isopods, and copepods by ingestion. Here, the hatching of eggs takes place into acanthella or acenthar, which is the first-stage larva (Kennedy 2006). Larvae develop into adult worms when an intermediated host is ingested by the definitive host, such as fish amphibians or reptiles. Due to the encapsulation of larvae in the tissue and penetration of the proboscis of adult worms in the muscular layer of the digestive tract of the host, pathological changes take place (Oniye 2004), such as widespread granuloma, followed by fibrosis inflammation, peritonitis, obstruction, and perforation of the digestive tube (Sanil 2011). African fishes *(Tilapia, Barbus, Heterotis* niloticus and *Citherinus, Synodontis)* are the final host of Acanthochala. *Acanthocephlus, Paragorgorhynchus, Termisentic* and *Neoechinorhynchus* (Valladao 2020) are the genera of acanthocephalan, which infect *Tilapia, Barbus, Heterotis niloticus and Citherinus synodontis* species of fish, respectively (Taraschewski 2008). The general life cycle of acanthocephalan is elaborated in Fig. 6.

Some significant parasites of fish

Cryptocaryon irritans

Cryptocaryon (C.) irritans is the cause of white spot disease in wild and cultivated marine fishes (Yin et al. 2018). It is a ciliated protozoan parasite that can survives at temperatures, ranging between 15°C and 30°C (59°F–86°F). Cryptocaryon is known to infect several fish species and its different strains have been isolated from various parts of the world. Although, some of them possess many similar characteristics like life cycles and salinity tolerances, however, many of them are beyond the previously described "normal" ranges (Yambot et al. 2003). The parasite has a direct life cycle and transmission of infection within a group of fish does not require any other animal or host for its development (Colorni and Burgess 1997). However, complex life cycle phases develop on and off the fish host, which eventually leaves the cyst as theronts, the infective and the free-swimming stage. Theronts dynamically seek fish hosts. The trophont is the "nourishing step" and the parasite is embedded inside the tissues of the fish at this step. After leaving the fish, the trophont becomes a protomont before encysting and converting into a tomont, which is the reproductive stage. The tomont matures and divides into various tomites. The span of the whole life cycle may differ, depending upon several factors including, strain of the parasite, temperature, salinity and fish host (Yambot et al. 2003; Rigos et al. 2013). An average duration of life cycle is recorded around 1 to 2 weeks; however, it may extend up to 11 weeks. This variation in the life cycle is attributed to the uncertainty of tomont maturation (Dickerson 2006). Time required and the size at different life cycle stages may vary due to the variety of strains of *C. irritans,* temperature and salinity level of water, or maybe due to different fish species acting as hosts. The temperature range for the ideal development of most of the strains of Cryptocaryon is 23–30°C (Wang et al. 2018).

Infection with Cryptocaryon in most cases shows small white spots, nodules, or patches on the skin, fins, or gills of infected fish. Other symptoms include ragged fins, cloudy eyes, pale gills, increased mucus production, or changes in skin colour, and skin may also appear thin (Raga 2007; Yin et al. 2014). Behaviourally, fish may flash (scratch), swim unusually, hang at the surface or on the bottom, act lethargic or breathe more speedily in distress (Colorni and Burgess 1997).

The parasite can be diagnosed through microscopic examination (wet mount) of tissue taken from gill arch, tail fin or the body surface of the infested fish. Within a host population, Cryptocaryon may increase mortalities for several days. However, the degree of pathology may vary, depending upon the strain of parasite, the species of host (fish), previous exposure to the parasite and the water temperature. Affected fish can be transferred to freshwater for 1 hr for 2-3 days. The other method can be used as the addition of 0.5 ppm $CuSO_4$ in the treated water with strong aeration for 5-7 days. The water must be replaced daily during the treatment. The infected fish must be shifted to parasite-free water storage tanks 2-3 times at 3 days intervals (Martin 2015; Jiang et al. 2016).

Trichodinas

Trichodina is one of the common parasites of fishes (wild or cultured) that resides in the marine and freshwaters. These parasites are popularly known as ecto-commensals and ciliated protozoans (Martins and Ghiraldelli 2008). So far, approximately 300 species have been reported from different environments in the world (Tang and Zhao 2012). This ciliated parasite can infest the host in a very short time frame due to its direct transmission and mainly infests fish kept under sub substandard conditions (Lom 1995). It replicates by binary fission and has been the topic of great interest since the previous century (Kruger et al.

1995). Not all trichodinids are pathogenic, most of them are non-pathogenic, but when the relationship among host/parasite/environment is broken by any of the given factors, like nutritional deficiency, low water quality, infectious and/or parasitic diseases, trichodinids proliferate and become responsible for severe epidermal lesions and disease outbreaks, as reported by Huh et al. (2005). The lesions mostly seen due to the infestation of this parasite are hyperplasia and necrosis of the epidermal cells (Hassan 1999). Trichodina is the most dominant parasite in Nile tilapia. The disease caused by *Trichodina* sp., is termed as Trichidiniasis and its clinical signs are abnormal coloration, lethargic movement, loss of appetite, weight loss, skin lesions, fin erosion, elevated mucus production, and gill necrosis. Gills are the main targeted organ for infestation (Biagini et al. 2009); thus, the epithelial tissue of gills is an outstanding factor to assess the properties of environmental variables, toxins, and water quality (Mazon et al. 2002). Formalin and sodium chloride are used as chemotherapeutic agents and may harm branchial tissue because these are ingredients that have a toxic influence on the fish, as detected by Mert et al. (2014). Color changes also occur in fish exposed to different doses of sodium chloride and formaldehyde.

Ichthyophthirius multifiliis

Ichthyophthirius (I.) multifiliis is an obligate parasite, ciliated protozoan of freshwater fish present in warm and humid climates. The temperature range for its outbreaks is 15–25°C (Noga 2014). It causes high mortality and economic downfall towards various aquaculture food fish. It also affects several aquaculture species, including grass carp, rainbow trout, channel catfish, snow trout (Mallik et al. 2015), striped catfish (Kumar et al. 2018), ornamental fish, and hatcheries (Mohammadi et al. 2012). It is present in 3 progressive stages: (1) a reproductive tomont, (2) an infective theront and (3) a parasitic trophont (Nigrelli et al. 1976). The trophont is an obligate parasitic stage and depends upon vulnerable fish for its proliferation. The theront penetrates fish epithelium and causes infection. The trophont grows in fish epithelium for 5 to 6 days; after its development the adult trophont leaves the host. Infected fish show various clinical signs including, swimming promptly and rubbing their bodies against the sides of the tanks, gasping at surface water. They become more lethargic and eventually stop feeding. Infection may occur due to lower water temperature (Ling et al. 2013). Histo-pathologically, large trophont stuck in the gills can be seen. The parasite has a C-shaped, large-sized macronucleus. The outer layer of the infected gills appears as inflamed, clubbed, and deformed. The presence of multiple trophonts at the gill lamella might be because of their rapid multiplication inside host fish. Hyperplasic and hypertrophied secondary gill lamellae can be observed. Management of water temperature is another effective way to control the multiplication of the parasite. Salt is an effective treatment for Australian warm water fish against Ich. It is inexpensive, safe, and easy to handle. A solution of 2% salt in 250 ppm formalin has also proved very effective in lowering the burden of trophonts in Snow trout (Mallik et al. 2015). Many other chemicals like malachite green, malachite green in combination with salt or formalin, formalin, chloramine-T, potassium permanganate, and copper sulphate have been tested to prevent Ichthyophthiriasis (Kinnunen et al. 2005).

Brooklynella hostilis

Brooklynella (B.) hostilis is the causative agent for Brooklynellosis, which is also recognized as slime-blotch or clownfish disease. In the marine aquarium, *B. hostilis* infects most teleosts. It is a kidney shaped organism and has a length of about 60-80 µm, with bands of cilia. *B. hostilis* belongs to the order Hartmannulidae, and genus monotypic *Brooklynella,* which includes only one species. It replicates by binary fission, depends on dead skin, and causes severe damage to gills (Fioravanti and Florio 2017). Common symptoms are discoloration and abnormal breathing. It can also cause cast aside skin and congestion of gills. After its reproduction, the newly formed protozoa can swim freely by using their ciliates. The parasite spreads swiftly and can get transferred to a novel host effortlessly. These tiny protozoa can get entry into an advanced host or generally they become attached to the already existing host, on which their parents were residing (Anshary 2020). While feeding upon the fish, the parasite also releases toxins that can be fatal to the fish. Free swimming protozoa can survive without a host for a short time. To make the aquarium free of this parasite, it must remain fallow (fishless) for at least four weeks to kill the remaining free-swimming parasites that will not be able to find a host to feed upon.All fish within the aquarium must be put into a quarantine tank and kept out of the main aquarium during these four weeks (Hirazawa et al. 2016).

Trypanoplasma

The kinetoplast genera *Trypanosoma* and *Trypanoplasma* are common marine and fresh-water fish parasites. Similar blood flagellates are present in Europe, Asia, and North America in freshwater and occasionally marine fishes. *Trypanoplasma (T.) borreli* and *T. bullocki* are pathogenic blood parasites, affecting cyprinids in Europe and marine flatfish in the Atlantic United States, respectively (Carrington 2017). Leeches feed on infected fish and act as an intermediate host. Infected leeches then act as vectors to infect other fishes. *T. borreli* possesses an indirect life cycle in leeches, especially *Hemiclepis marginata*. Like other flagellates, *T. borreli* has a large variety of parasitic forms present within the blood. It infects the vascular system, kidneys, and other organs. Heavily infected hosts are lethargic, emaciated, and have sunken eyes. Infected fish are anemic with damage of the excretory part of the kidney, causing osmoregulatory problems (Lom and Dykova 1992). *Trypanosoma carassi* can induce far-reaching damage to hematopoietic tissues in goldfish, causing anemia, whilst Kamińska-Gibas et al. (2018) described a long-running decrease in hematocrit, hemoglobin, and protein levels in sculpins analytically

infected with *T. bullocki*. Removal of leeches is the best way to elucidate this problem.

Myxobolus cerebralis

Myxobolus (M.) cerebralis is a parasite of the Salmonidae family, including salmon and trout (Hedrick and El-Matbouli 2002). Myxosporean causes a disease known as whirling disease. It was first described in rainbow trout in Germany, and also appeared in Europe, United States, and South Africa. There is no zoonotic importance of this parasite. Skeletal deformation and neurological damage occur by myxosporean infection. Invertebrates and vertebrates are the definitive and intermediate hosts of this parasite, respectively; either host can be called alternate host for *M. cerebralis* (El-Matbouli and Hoffmann 1998). Younger fish are more susceptible to the disease before ossification of cartilage and full development of the central nervous system (Ryce et al. 2005). Susceptibility depends upon the age of the fish. Parasites can attack fish within 2 days after hatching (Markiw 1991), this could be different for different fish species as in the case of rainbow trout. It infects until 9 weeks after hatching, while chinook salmon can become infected 3 weeks after hatch (Sollid et al. 2003). Several reports are unable to differentiate between entry of parasite and setting of the life cycle. So, in most situations, recognition is based on the transport of infected fish coming from Europe, but it may be due to the reason that the parasite does not easily inhabit the new area and it does not need further confirmation of its occurrence (South Africa, Lebanon, Morocco). In North America, its presence was first reported in 2006 in Alaska (Arsan 2007), while in Arizona, its presence was first reported in 2000 (Bartholomew and Reno 2002). Myxospore develops within the oligocheate host (*Tubifex (Tu.) tubifex)*. The *M. cerebralis* ingestion by *Tu. tubifex* occurs, which leads to protuberance of the polar filaments and their spores anchor into the gut lining. Shell valves get open, binucleate sporoplasm runs away and sticks in the middle of epithelial cells. Then, inter-epithelial schizogonic multiplication of binucleate sporoplasm takes place. The plasmogamy of 2 uninucleate cells occurs to develop 1 binucleate cell stage. Both nuclei divide mitotically to produce the 4-nuclei stage. The emergence of the 4-cell stage through plasmotomy; 2 cells enfold the other 2 cells which in turn leads to the generation of early pan-sporocyst with 2 somatic and 2 generative cells. As a result of 3 mitotic divisions of both generative cells and 2 mitotic divisions of the somatic cells, 16 gametocytes (8α and 8β) masked by 8 somatic cells emerge. Then the meiotic division of the 16 diploid gametocytes, 16 haploid gametocytes, and 16 polar bodies results, leading to the formation of 8 zygotes after copulation of each pair of α- and β-gametes. The sporoblast emergence occurs after 2 mitotic divisions of the zygote, 3 pyramidally arranged cells, and one inner cell are produced. As a result of the mitotic division of the 3 peripheral cells, 3 capsulogenic and 3 valvogenic cells are formed. The valvogenic cells outstretch the capsulogenic cells, while internal cleavage of the developing sporoplasm cell generates one generative cell enfolded by one somatic cell. The sporoplasm persists unfolded in the pansporocyst till its final number of germs by recurrent mitotic divisions. The inflated mature triactinomyxon spore is formed (El-Matbouli and Hoffmann 1998).

Whirling disease is identified predominantly by persistent inflammation of the cartilage, although other clinical signs are also present. Cartilage degradation occurs after the development of lesions. Although, in mature fish, parasites are present in isolated pockets in bone and are less likely to be associated with inflammatory lesions. Parasites digest the cartilage, destroying tissue structure, outcome of which is irregular bone formation and skeletal abnormalities (Hedrick et al. 1999). Any cartilage can get an infection and it differs among salmonid species, cranial regions develop lesions (Baldwin et al. 2000) and in Yellowstone cutthroat trout lower jaw (Murcia et al. 2011). Lesions originate as small foci then get developed with time into necrosis, destroying cartilage along with tissue damage and inflammation (Baldwin et al. 2000; MacConnell and Vincent 2002). Several stages of parasites are present at a single time-older stages locate themselves enclosed and younger stages at the leading edges (Baldwin et al. 2000). No drug treatment is specifically recommended for *M. cerebralis*, many drugs have been evaluated but no drug is found satisfactory. Several drugs reduced infection and suppressed disease, but none prevented or eliminated the infection. Most of the drugs cause toxicity and reduce growth.

Myxozoan parasites

Myxozoans are protozoan parasites that largely affect the gut region of various kinds of fish, living both in fresh and marine water. The most common of these parasites is *Enteromyxum (E.) leei*. It is a cause of disease in cultured sea bream and other species which commonly inhabit the Mediterranean region. Parasites affect the digestive tract, leading to severe enteritis which may in worse situations lead to emaciation and death. It also causes a similar type of disease in tiger pufferfish Rea. *E. leei* can get transferred between hosts without the need of an intermediate host, leading to the rapid spread of the disease in the fish population (Diamant 1997). Another important myxozan parasite is *E. scophthalmi* that causes similar intestinal conditions in the intestinal cavity of turbot fish species (Redondo et al. 2004) and is known to have direct transmission.

Neobenedenia girellae

Neobenedenia (N.) girellae is a monogenean, warm water ectoparasite of marine cultured fish species, which affects significantly greater amberjack, representing the main bottleneck for its production (Shirakashi et al. 2013). It causes high mortality and the range is 70-100% (Ogawa et al. 1995; Shinn et al. 2015). In early infection, the parasite gets attached to the fins and the cranial skin region, while for long-term infections it moves towards ventral and

dorsolateral skin regions (Hirayama et al. 2009; Hirazawa et al. 2011). The life cycle is shown in Figure 4. The adult parasites attach to the epithelial surface of the fish and deposit eggs into the environment. The ciliated larvae hatch from the embryonated eggs, which are called oncomiracidia. These oncomiracidia reinfect the fish and continue the life cycle. The symptoms of fish ectoparasites are host and parasite-specific. For instance, sea lice-infected Atlantic salmon skin shows an ulcerative process resulted from the second antennae and dermatitis characterized by varied epidermal thickness, detached cells, necrosis, and mobilized leucocytes (Jones et al. 1990). Other monogenean infection, as *N. amelleni,* shows surface epithelium denudation and interstitial oedema in red hybrid tilapia (Oreochromis spp.) (Robinson et al. 1992). Previously, it has been stated that *N. girellae* infection affects amberjack skin and alters epidermis thickness and density of goblet cells due to distorted osmoregulatory and respiratory skin functioning (Hirayama et al. 2009; Hirazawa et al. 2016). Additionally, it causes secondary bacterial infections to the skin, observed by the revamped fish conduct; scratching its skin with the tank, resulting in major skin injuries (Ogawa et al. 1995; Hirayama et al. 2009). Hydrogen peroxide solution, when used as bath treatment along with oral administration of praziquantel, has proved to be effective to control the parasite. Moreover, acquired protection against secondary infection with the parasite was demonstrated in primed Japanese flounder (Hirazawa et al. 2016).

Bolbophorus confuses

It is a digenetic fish trematode and affects skeletal muscles and viscera of the host fish, and therefore, adversely affects fish production. It affects channel catfish (Doffitt 2020) and has a complex life cycle, involving different hosts; white pelican being the definitive host, while snail, and catfish being the first and second intermediate hosts, respectively (Outa 2020). Metacercariae affect the skin and skeletal muscles of the host, but in severe cases, they penetrate the visceral organs, like the kidney and liver, and cause channel virus disease or enteric septicemia like condition. Visceral organ involvement increases mortality rates (Kahn 2010).

Posthodiplostomum cuticula

It is also a digenean that hosts freshwater fish and causes black spot disease in them. Like other digenean, it also possesses a complex life cycle, involving three hosts (Ritossa 2013). Adult parasite affects the bird (definitive host) and eggs are released in feces that contrive the miracidia which penetrate the snail (the first intermediate host). These give rise to sporocysts which evolve into Furco-cercariae, released from the snail, penetrate the skin of fish (second intermediate host) and develop into metacercaria. They infect the skin, fins, and muscles, producing visible black cysts (melanin surrounded meta-cercaria so-called as black spot disease) (Ondračková 2004).

Proteocephalus ambloplitis

It is a tapeworm, belonging to a large group of cestodes that infect a variety of species. It is of serious health concern (Scholz 2019). The larval stage inhabits the ovary of the host, while adult *Proteocephalus* worms reside in the inner wall of the gut and shed eggs (egg-filled proglottids: body segments) with the fish feces. Eggs hatch and get dispersed in water, then taken up by the crustaceans, where they develop into larvae, procercoid. This gets entry to fish gut when fish eat crustacean and develops to plerocercoid within the host.

Contracaecum rudolphii

Contracaecum rudolphii is a nematode parasite, affecting several fish species such as *Alburnoides bipunctatus*, *Anguilla anguilla*, *Barbatula barbatula*, *Cyprinus carpio*, *Gobio gobio*, *Perca fluviatilis*, *Phoxinus phoxinus*, *Poecilia reticulata* and *Tinca tinca* (Moravec 2009). It infects the fish directly or indirectly i.e., by infecting the copepods that are ingested by the fish. Its life cycle involves three larval stages, the third larval stage being the infective stage of the parasite. It penetrates the intestinal wall to get access to the body cavity and to encyst the visceral organs (Baruš 2001).

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Section B

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MEAT BORNE BACTERIAL PATHOGENS

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INTRODUCTION

Animals encounter various emerging and re-emerging diseases caused by various infectious pathogens. A few of these diseases can cross the species barrier and pose a serious public health concern, leading to economic burden as well. Foods of animal origin like milk, meat, eggs and shellfish are essential components of human diet (Newell et al. 2010; Wiwanitkit 2018). However, food products containing pathogens like bacteria, viruses, parasites, or chemical substances, are responsible for causing more than 200 diseases globally. The severity of these diseases ranges from self-limiting diarrhea to fatal cancerous conditions (Oliver et al. 2005). It has been estimated that 1 out of 10 people in the world suffer from a disease resulting from eating contaminated food (Dhama et al. 2013). The persistence of foodborne diseases hampers socioeconomic development by overloading health care systems, ultimately damaging the national economy. Children under 5 years of age contribute to majority (40%) of cases caused by these foodborne pathogens (Addis and Sisay 2015).

Among five categories of foodborne diseases, allergies, metabolic food disorders and idiosyncratic illnesses affect only a limited number of the population. Contrarily, infections and intoxication can cause pathological conditions in almost every person (Butler et al. 2015). Because the pathogens are ingested with food, these foodborne diseases mainly affect small intestine, a part of gastrointestinal tract (GIT). The food digestion and absorption are carried out mainly in the small intestine, which receives secretions from liver and pancreas. These secretions constitute various enzymes to digest carbohydrates, proteins and fats (Abebe 2020).

Diarrhea

Most of foodborne bacterial diseases cause severe debilitating infections like meningitis or severe diarrhea. Diarrhea is an acute syndrome of GIT caused by various pathogens and their toxins (Camino et al. 2017). The word “diarrhea” is derived from the Greek term “diarrhein” meaning “to flow through”. It is a symptom of gastroenteritis and can be defined as increase in fecal volume, fluid content, and frequency of the bowel movements (Tortora et al. 2021). Physiologically, diarrhea can be induced due to secretion of solutes, increased intestinal motility, intestinal structural abnormalities, and unabsorbed solutes (Curtis et al. 2000). Diarrhea can also be caused due to virulence factors produced by the replicating bacteria or due to ingestion of preformed toxins (intoxication) (Pearmain and Moor 2016). Infectious diarrhea can be caused by a variety of different pathogens, including bacterial pathogens, as described in Fig. 1 (Rouger et al. 2017). The bacterial pathogens cause diarrhea by two basic mechanisms:

Toxigenic diarrheal disease

In this disease, enterotoxins secreted by bacterial pathogens disrupt physiological mechanism of small intestine, after attaching with epithelial cells. These enterotoxins enhance the secretion of electrolytes and result in loss of water. In toxigenic diarrheal disease, bacterial pathogen does not invade the epithelial tissues, thus results in a condition known as secretory diarrhea (Chess 2020).

Invasive diarrheal disease

Sometimes, a pathogen invades the tissues of the small or large intestine, damaging the epithelial lining and under lying tissues (Ribet and Cossart 2015). Invasion of the deeper tissues results in severe form of dehydration, which is a major cause of death in children (under 5 years age) worldwide. Ulceration of the lining, and damage to deeper tissues, may lead to appearance of blood in the stool, a condition known as dysentery (Chess 2020).

Meat Borne Diseases

Flesh of some animals, which is used as food by humans, is known as meat. Meat is commonly derived from mammalian, avian, aquatic, reptilian and amphibian species. There are two main types of meat, commonly known as red and white meat, depending upon the concentration of myoglobin in muscle fibers. Meat is an excellent source of high-quality proteins, vitamins, bioavailable minerals, and contains all essential amino acids, which cannot be synthesized endogenously (Dilger 2017).

Among food products, raw foods of animal origin are most likely to be contaminated with pathogens. These foods include unpasteurized milk, raw eggs, raw meat, and raw shellfish (Artursson et al. 2018). Depending upon the health status of the animals and sanitary condition during meat processing, meat can harbor a wide variety of pathogens that can be transmitted to humans (Vongkamjan et al. 2015). These pathogens, including

viruses, bacteria, fungi, and parasites, can enter the food chain by directly infecting the animals or by contamination during meat processing due to poor sanitation practices and personal hygiene (Gómez et al. 2020). The meat borne diseases (MBDs) are commonly classified into meat borne infections, meat borne intoxication, and meat borne toxico-infection, as described in Fig. 2 (Bintsis 2017).

Several bacterial pathogens are involved in majority of meat borne infections. There are many bacterial species known for contaminating domestic meat species like beef, lamb, pork, and poultry or there are some bacteria entering during the meat processing. These bacterial species include, *E. coli*, non-typhoidal *Salmonella*, *Campylobacter*, *Shigella dysenteriae*, *Listeria monocytogenes, Brucella species*, *Yersinia enterocolitica*, *Mycobacterium bovis*, and bacterial species responsible for food intoxication like *Staphylococcus aureus*, *Clostridium* species, and *Bacillus cereus* (Bauerfeind et al. 2016).

The sources of these bacterial pathogens in meat and meat-related products include animals itself, environmental sources, human handlers, water used during processing, or contaminated equipment (Castro et al. 2016). Therefore, only preventing diseases in food animals may not be sufficient to control the MBDs but a strict hygienic precaution should also be implemented during meat processing to minimize the MBDs (Burlage 2011).

The correct source of the diseases is usually difficult to know because it will take several days or weeks to develop signs and symptoms of the MBDs. So, commonly the most recent meal receives the responsibility, but it may not be the contaminated meal. The detection of the bacterial pathogen from the sample of food can help to confirm the causative agent. However, it may not be possible always because mostly the food samples are not available for detection of the pathogen to confirm the exact source of infection (Christine et al. 2017).

Red and white meat are commonly contaminated with variety of mesophilic and psychrotrophic bacteria. These bacteria may be pathogens of animals and birds itself or might have entered during processing of raw products (Lund and O'Brien 2011). The majority of these diseases are zoonotic in nature and these are transmitted to humans directly or indirectly (Lim et al. 2010). Like foodborne diseases, MBDs are also categorized into two main types i.e., meat borne infections and meat borne intoxications (Noor 2019). The concept of "farm to fork" must be implemented to minimize contamination of meat or meat products (Collineau et al. 2020). The meat supply chain from farm to consumers has been illustrated in Fig. 3 (Bhunia 2018). This chapter will discuss the important meat borne bacterial pathogens and diseases caused by these bacterial pathogens with respect to public health.

Escherichia coli

The bacterium *E. coli* was first isolated from a fecal sample by Theodor Escherish in 1885. *E. coli* is a Gram-negative, non-spore forming, and facultatively anaerobic bacillus. It is a commensal microbe, habituating the human and animal intestinal tracts (Croxen et al. 2013). The strains of *E. coli* are classified based on O-antigen (Somatic), H-antigen (Flagella) and K-antigen (Capsule). There are 174 somatic-antigens, 53 flagellar-antigens and 80 capsular-antigens (Tuo et al. 2020).

The most of serotypes of *E. coli* are responsible for causing diarrhea or intestinal diseases, while others are responsible for causing non-intestinal diseases (Chitarra et al. 2014; Moxley et al. 2020). Animals act as primary reservoirs for this bacterium and the pathogen can be transferred to humans via animal products that act as vehicle, or by direct contact (Avery et al. 2004; Roche et al. 2010). The transmission of *E. coli* to humans from various sources is summarized in Fig. 4.

There are several pathotypes of *E. coli* on the basis of occurrence of certain virulence factors. These pathotypes are capable of causing diseases in humans and animals, as described in Table 1 (Ahmed and Shimamoto 2015; Manges 2016). The virulence factors responsible for causing diseases may vary among different pathotypes (Fleckenstein and Kuhlmann 2019). However, there are some common pathological mechanisms shared by all pathotypes to induce diarrhea, which are as under:

1. Causing damage to epithelial cells after adherence
2. Affecting ion pumps
3. Increasing fluid loss
4. Altering cytoskeletal assembly
5. Causing cell death

Although, most strains of *E. coli* are harmless inhabitants of human and animal GIT, but a small percentage of these strains are pathogenic to both humans and animals (Munns et al. 2015). The outbreaks of *E. coli* are usually attributed to contaminated food products, including meat (Gyles 2007; Pereira et al. 2014). Among pathotypes of *E. coli*, EHEC can cause life-threatening diseases like hemolytic uremic syndrome and hemorrhagic colitis (Callaway et al. 2009). The main serotype associated with EHEC is O157:H7 (King et al. 2014). The inability to ferment sorbitol is commonly used to distinguish O157:H7 from other commensal strains. Being primary reservoir of EHEC, intestines of cattle can harbor EHEC without any disease. EHEC can also be present in GIT of other domestic animals (Byrne et al. 2020). Generally, foods of animal origin have been associated with several outbreaks in developing and developed countries. It has been demonstrated that EHEC is present in various animal food products, like ground beef, poultry, lamb, pork, and raw milk (Baran et al. 2020). The diarrheal diseases are usually preventable through avoiding potentially contaminated foods, proper hygienic measures during food preparation and by thorough cooking (Irshad et al. 2020).

Salmonella

This bacterial pathogen causes Salmonellosis, which is the third most common cause of mortality among foodborne diseases. Microbiologically, this enteric bacterial genus includes Gram-negative facultative anaerobic bacilli. It is a member of the family *Enterobacteriaceae*. The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori* (Guerrini et al. 2021).

Table 1: Pathotypes of *E. coli*, important toxins, and diseases

E. coli pathotypes	Disease	Important toxins
Intestinal pathogenic *E. coli*		
Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) O157:H7 is the most important pathogen of *E. coli*	Diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome	Shiga-like toxin (Stx), enterohemolysin (EHly)
Enterotoxigenic *E. coli* (ETEC)	Acute watery diarrhea	Heat-stable toxin (ST) Heat-labile toxin (LT)
Enteroinvasive *E. coli* (EIEC)	Acute dysentery	Shiga-toxin
Enteropathogenic *E. coli* (EPEC)	Acute and/or persistent diarrhea	
Enteroaggregative *E. coli* (EAEC)	Persistent watery diarrhea	Enteroaggregative heat-stable (EAST), Plasmid-encoded toxin (Pet), Enterotoxin
Diffusely adherent *E. coli* (DAEC)	Watery diarrhea in children	Secreted autotransporter toxin (SAT)
Adherent invasive *E. coli* (AIEC)	Diarrhea, inflammatory bowel diseases	
Extraintestinal pathogenic *E. coli* (ExPEC)		
A. Uropathogenic *E. coli* (UPEC)	Urinary tract diseases	Hemolysin (Hly)
B. Sepsis-associated *E. coli* (SepEC)	Sepsis	Invasion (lbe)
C. Neonatal meningitis *E. coli* (NMEC)	Meningitis in newborns	
D. Avian pathogenic *E. coli* (APEC)	Colibacillosis in fowls	

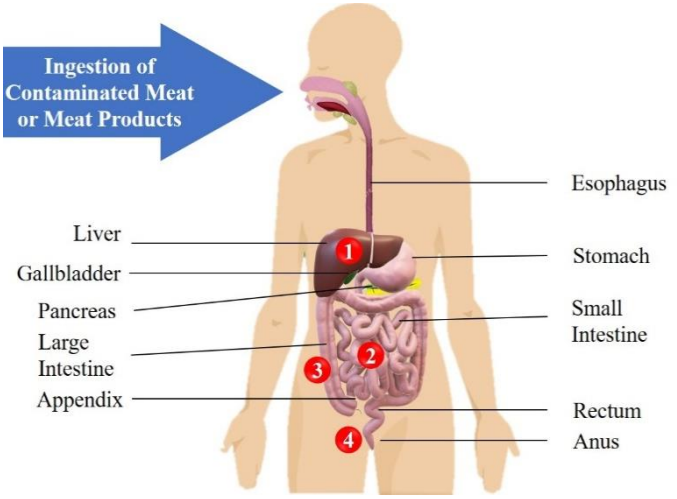

Fig. 1: Parts of human GIT affected by meat borne bacteria
After the ingestion of contaminated meat or meat products, bacteria or bacterial toxins can induce infections in different parts of the human GIT (Noor 2019). (1) Infection in the liver. (2) Bacteria are adsorbed into the intestinal epithelial cells that leads to induction of inflammatory immune response. (3) Replicating bacteria replicate without penetration or produce an invasive disease due to intestinal penetration. (4) Combined effects of bacterial invasion, bacterial virulence factors and inflammatory immune response lead to diarrheal stool or dysentery.

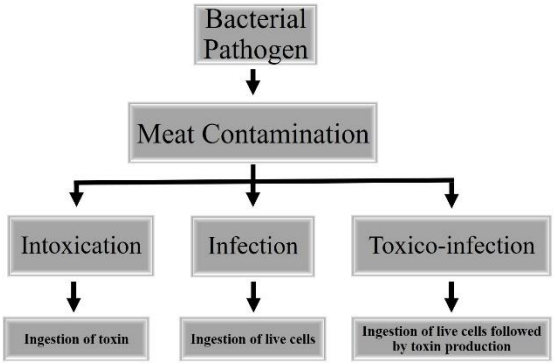

Fig. 2: Flow diagram showing various forms of MBDs
Meat contaminated by bacterial pathogens can induce intoxication, infection, or toxico-infection in humans.

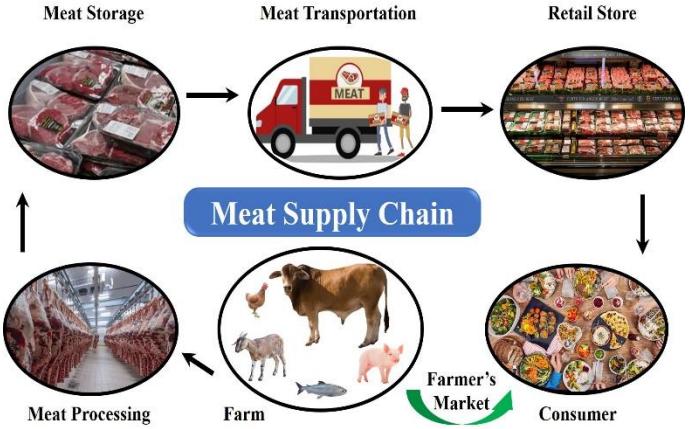

Fig. 3: Meat production and supply chain network
MBDs can be minimized by controlling the contamination by pathogens at all levels of meat supply chain (Zwirzitz et al. 2020).

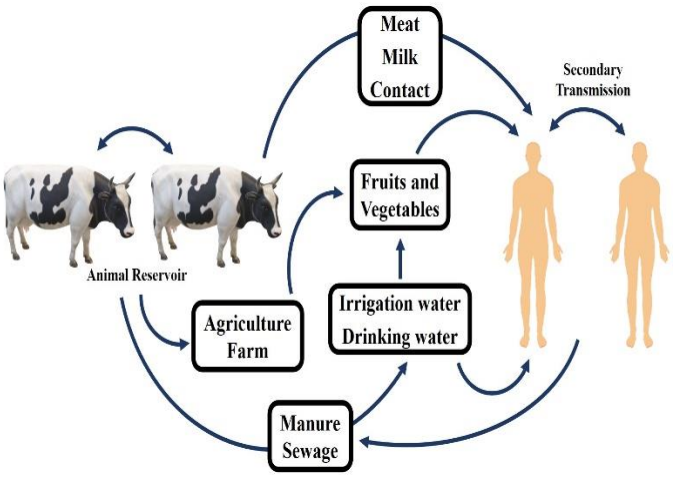

Fig. 4: Transmission of *E. coli* to humans from various sources (adapted from Bhunia 2018).
Meat act as a primary vehicle to transmit the pathogen to humans. However, humans can also get infected via animal-to-person contact, contaminated water, milk and contaminated fruit and vegetables (Steele and Odumeru 2004). Moreover, secondary transmission (person-to-person) can also spread the disease within a population.

Salmonella enterica is further divided into about 2659 serovars, depending upon vertebrate hosts. Among these serovars, 1547 belong to subspecies *enterica*, almost all of these serovars (99%) are capable of infecting humans and animals (Issenhuth-Jeanjean et al. 2014; Radhika et al. 2014). *Salmonella* serovars can be classified on the basis of host restriction, as under:

Host-restricted serovars

These serovars are capable of infecting single host species, causing typhoid like disease. The examples of this group include *S. pullorum* and *S. gallinarum* in poultry (Alali et al. 2010) and *S. typhi* and *S. paratyphi* in humans (Gal-Mor et al. 2014).

Host-adapted serovars

These serovars are usually capable of causing disease in one host species, however these can also infect other host species. The examples of this group include *S. typhisuis* and *S. choleraesuis* (Barco et al. 2013).

Broad-host-range serovars

These serovars are capable of colonizing in the GIT of a number of different animals but they rarely cause systemic infections (Nawaz et al. 2020). Among a large number of zoonotic serovars in this group, nontyphoidal serovars (NTS) are the most common serovars infecting different animals and humans. The examples of NTS include *S. enteritidis* and *S. typhimurium* (Ahmad et al. 2020).

The diseases caused by *Salmonella* are of major concern for public health, especially in low-income countries due to high mortality in these countries (Chousalkar and Gole 2016). A WHO study has depicted that NTS had a great impact on global health due to foodborne transmission, especially in south-east Asia, Africa, and Eastern Europe (Marzel et al. 2016). The scientists used different nomenclature for this genus but now CDC has approved a nomenclature, as summarized in Table 2 (Issenhuth-Jeanjean et al. 2014).

Salmonellae are Gram-negative, non-spore forming, non-lactose fermenting, facultative anaerobe, and flagellated enteric bacilli, causing systemic diseases in variety of different hosts. The most subspecies of *Salmonella* can grow at a wide temperature range, with optimum temperature range of 35-37°C. *Salmonella* species possess three main antigens: Flagellar antigen (H antigen), Somatic antigen (O antigen) and Vi antigen (Boyen et al. 2008; Bahramianfard et al. 2021).

NTS rarely cause severe disease in healthy adult animals, while young animals are at higher risk of getting severe Salmonellosis. Both NTS and typhoidal *Salmonella* are naturally acquired by ingestion (Hoelzer et al. 2011). After ingestion, stomach acidity reduces the number of the ingested microbes. The survived pathogens can establish themselves in the small intestine to induce an infection (Santos 2014). The typhoidal *Salmonella* bacteria spread to lymphoid tissues to cause systemic infection after interacting with microfold (M) cells of the intestine (Jepson and Clark 2001). These pathogens can survive within dendritic cells and macrophages after phagocytosis due to their ability to resist respiratory burst within these phagocytes (Nix et al. 2007).

Eventually, they are disseminated to various body parts via lymphatic system or blood circulatory system. NTS serovars remain confined to intestinal lumen and produce strong immune response in the intestinal lumen (Winter et al. 2010). While typhoidal serovars have the ability to cross this intestinal barrier and these serovars remain relatively undetected by the immune system, as summarized in Fig. 5.

The antimicrobial drugs can inhibit (bacteriostatic) or kill (bactericidal) the bacterial pathogens by disrupting essential mechanisms of the bacterial replication. The ability of the bacteria to resist the effect of these antimicrobial agents is known as antimicrobial resistance (AMR). The bacterial pathogens can acquire this resistance horizontally by gene transfer or become antimicrobial resistant due to chromosomal mutation (Cosby et al. 2015). Some of the bacterial strains are intrinsically resistant to some antimicrobial agents due to inherent functional or structural characteristics (Atabey et al. 2021).

The persistence of AMR in *Salmonella* serovars is alarming for public health system (Rahman and Mohsin 2019). The level of expression of AMR in *Salmonella* serovars may also greatly differ (Cummings et al. 2013). Some serovars are capable of resisting against multiple types of drugs, thus named as multidrug resistant (MDR) bacteria (Call et al. 2008; Pinheiro 2020). It has been reported that about 5% of NTS isolated from human infections were resistant to five or more antimicrobial drugs. The emergence of MDR serovars of the *Salmonella* has exacerbate the situation regarding AMR worldwide (Bahramianfard et al. 2021; Yasmeen et al. 2020).

The continuous surveillance is considered primary action to control the persistence of zoonotic diseases. The early detection and removal of the input source of the *Salmonella* may help to lower the cases of meat borne Salmonellosis cases (Hume et al. 2017). Although control plans may differ among countries, they are based on the comparable goals and principles (Khen et al. 2014). These are comprised of implementing minimum preventive measures to control infection in the flocks, detection of *Salmonella* infection through continuous surveillance and implementing the stringent control measures once *Salmonella* is detected in the flock (Fraser et al. 2010; Nawaz et al. 2021). So, it is recommended that flocks are tested to detect *Salmonella* serovars at various stages of production during farming and implementing strict control measures if the pathogen is detected in the flock or farm. Lack of general farm hygiene may increase the prevalence of *Salmonella* in animals (Stevens et al. 2009). Therefore, strict biosecurity measures are considered the main barrier against entry, spread and transmission of *Salmonella* on farms (Andres and Davies 2015).

Table 2: *Salmonella* nomenclature and number of serovars reported

Species	Subspecies	Number subspecies	Number of serovars
Salmonella enterica	*Enterica*	I	1586
	Salamae	II	522
	Arizonae	IIIa	102
	Diarizonae	IIIb	338
	Houtenae	IV	76
	Indica	VI	13
Salmonella bongori		V	22
Total			2659

Table 3: *Shigella* serogroups and their characteristics

Species	Serogroup	Number of serotypes	Geographic distribution	Distinctive characteristics
Shigella dysenteriae	A	15	Asia, Africa, Central America	Most severe dysentery with high mortality rate
Shigella flexneri	B	8	Most common in developing countries	Causes less severe dysentery than *S. dysenteriae*
Shigella boydii	C	19	Indian subcontinent mainly, scarcely in developed countries	Only serologically different from *S. flexneri*
Shigella sonnei	D	1	Most common in developed countries	Causes mildest shigellosis

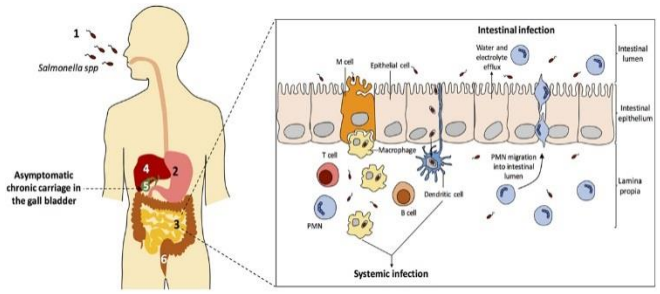

Fig. 5: Pathogenesis of *Salmonella enterica* (Urdaneta and Casadesus 2017).
(1) Ingestion of *Salmonella* via contaminated food or water. (2) Salmonellae survive the stomach environment and invade epithelial cells of the intestine. (3) The infection may lead to systemic infection or intestinal infection. (4) Bacteria may colonize the liver, bone marrow and the spleen during systemic infection. (5) Infected person may become chronic carrier due to persistence of infection in the gall bladder. (6) *Salmonella* pathogen cells are excreted from gall bladder to intestine and lead to fecal-oral transmission of disease.

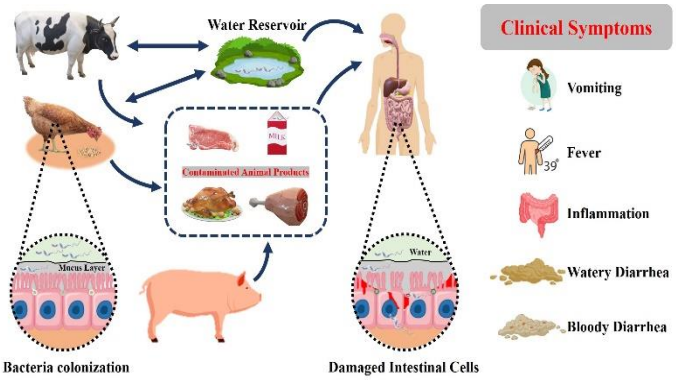

Fig. 6: Reservoirs and outcomes of *Campylobacter jejuni* infection in human (Elmi et al. 2021).
Campylobacter jejuni contaminates animal products, including meat, by colonizing in GIT of domestic animals. The consumption of contaminated foods can lead to gastroenteritis in humans where *C. jejuni* damages the intestinal epithelium by invasion. The combine impact of replicating bacteria, and bacterial virulence factors result in inflammation and diarrhea.

Campylobacter

Campylobacteriosis is a foodborne GIT disease caused by *Campylobacter* species. It has a significant impact on public health and economic burden in this 21st century (Kaakoush et al. 2015). Globally, the number of Campylobacteriosis cases is believed to be on the rise in both developed and developing countries (Haruna et al. 2013; Zbrun et al. 2020). The majority of Campylobacteriosis cases are sporadic and self-limiting. Therefore, the exact quantum of the disease is unclear. The documented disease outbreaks are usually linked to contaminated water or animal products (Dasti et al. 2010; Mourkas et al. 2019).

Campylobacter species are Gram-negative, spirally curved shaped flagellated bacterial pathogens with single polar flagellum at one or both ends. The members of genus *Campylobacter* are microaerophilic, catalase positive, oxidase positive with optimum growth at 37-42°C (Gharst et al. 2013). Among a total of 26 species of *Campylobacter*, two species are responsible of majority of human infections *i.e.*, *C. jejuni* and *C. coli* (Hameed et al. 2020; On 2013). The majority of foodborne infections (~90%) are caused by *C. jejuni*. These two *Campylobacter* species have been commonly isolated from variety of different domestic, as well as wild, animals (Newell et al. 2011). Therefore, these two *Campylobacter* species are most important regarding MBDs (Garcia-Sanchez et al. 2018). The majority of *Campylobacter* species are involved in causing GIT disease, except a group that is able to cause reproductive disorder in cattle and sheep (Burnham and Hendrixson 2018).

Campylobacter jejuni is able to cause human GIT disease with relatively low infectious dose (500-800) (Black et al. 1988). *C. jejuni* is capable of replicating in several environmental reservoirs that can lead to human infections (Teh et al. 2019). Being poultry as natural host for *Campylobacter* species, *C. jejuni* is capable of colonizing the intestinal tract of chicken and transmit within flock via fecal-oral route (Bahrndorff et al. 2013). *C. jejuni* can contaminate water reservoir and survive due to

association with other protozoa. It can survive within other domestic animals, including cattle, pork etc. (Guyard-Nicodeme et al. 2013). The consumption of contaminated unpasteurized or undercooked animal products lead to GIT inflammation by invading the intestinal epithelial cells (Fravalo et al. 2009; Mari et al. 2012). This infection leads to appearance of various signs and symptoms withing 1-10 days of ingestion of the pathogen. Various virulence and survival factors enable the pathogen to survive within these hostile conditions and cause a disease (Bolton 2015). These factors include motility, adhesion factors, bile resistance, invasion factors, antioxidant defense, and several different cytotoxins like cytolethal distending toxin (CDT). All these virulence factors lead to production of various symptoms of gastroenteritis, including vomiting, abdominal pain, fever, inflammation, diarrhea, or dysentery (Young et al. 2007), as summarized in Fig. 6.

The gastroenteritis caused by *C. jejuni* and *C. coli* is usually self-limiting, and rarely leads to mortality. However, immunocompromised patients are prone to severe systemic complications (Igwaran and Okoh 2019), such as inflammatory bowel disease, reactive arthritis, Miller Fisher syndrome (eye muscle weakness), and Guillain Barré syndrome (rapid muscle weakness) (Facciolà et al. 2017).

The use of antibiotics is usually not required to control the infection, except in immunocompromised patients (Alanis 2005). The emergence of antibiotic resistant *Campylobacter* strains can be ascribed to nonjudicial antibiotic use in human, animal, and agriculture sectors (Olkkola et al. 2016; Wysok et al. 2011). The incidence of Campylobacteriosis can be prevented by adapting hygiene measures while handling the animals and animal products (Nichols et al. 2005). The chances of disease transmission can be lowered by full cooking of poultry meat (Hansson et al. 2018). The chicken "juice" is particularly important for disease transmission and is responsible for cross-contamination of foods which are consumed uncooked (Cogan et al. 2002; Llarena and Kivistö 2020). Therefore, the prevalence of Campylobacteriosis can be reduced by hygienic measures, and careful handling of meat and meat products distinctly from other foods (Abd El-Ghany 2019).

Shigella

Shigella species are among important human pathogens, causing dysentery. It was first identified as etiological agent of dysentery in 1898 by Kiyoshi Shiga. The species of Shigella are Gram-negative, facultative anaerobes, nonmotile bacilli which are lactose non-fermenter and are unable to produce hydrogen sulfide. There are four groups on the basis of somatic O antigens of the lipopolysaccharides of this genus. These include serogroup A (*Shigella dysenteriae*), serogroup B (*Shigella flexneri*), serogroup C (*Shigella boydii*) and serogroup D (*Shigella sonnie*), as summarized in Table 3 (Christine et al. 2017). These species of Shigella can cause invasive and human specific bacterial disease, known as bacillary dysentery (Shigellosis). The complications of Shigellosis include hemolytic uremic syndrome (UHS) or reactive arthritis (Besbas et al. 2006). Among the species of this genus, *Shigella dysenteriae* is responsible for the most severe form of Shigellosis (Hendriks et al. 2020). Moreover, occurrence of drug resistant *Shigella* infections makes it more difficult to treat (Klontz and Singh 2015).

The Shigellosis is commonly considered as foodborne or waterborne disease, which can also spread from person to person by fecal-oral route. The transmission of Shigellosis is commonly associated with poor hygiene of food handlers because humans are the only known host for *Shigella* (Kingombe et al. 2005, Zaib et al. 2019). Improper storage, use of contaminated equipment and inadequate cooking can further aggravate the problem. Mechanical vectors like houseflies may also contribute to spread the pathogen. Foods, fingers, feces, flies, and fomites are the 5 "Fs" that contribute to the spread of Shigellosis (Yang et al. 2015).

Listeria monocytogenes

The majority of species of genus *Listeria* are classified as non-pathogenic, as they do not encode the virulence factors that are required to induce infection (Ivanek et al. 2006). Among these species, on the basis of public health significance, *Listeria monocytogenes* has been described as major human pathogen. *L. monocytogenes* is a rod-shaped Gram-positive bacterium, found in a wide range of environments (Schoder et al. 2011; Bell and Kyriakides 2015). This facultative anaerobic pathogen can ferment glucose without production of gas (Law et al. 2015). It can grow at a wide range of temperature (0-45°C). The ability to grow at 4°C enables this pathogen to multiply in different food products under refrigeration, thus causing foodborne infections (Almashhadany et al. 2021).

Historically, it was first described in 1926 as a causative agent of abortions and encephalitis in rabbits and guinea pigs. It took more than 50 years to establish that it can also be a foodborne pathogen (Schlech 1983). Currently, it is considered as a foodborne pathogen, causing gastroenteritis in healthy people, abortions in pregnant women and meningitis in immunocompromised individuals, with high mortality (20-30%), as described in Fig. 7 (Cossart 2011; Ranasinghe et al. 2021).

The disease caused by *Listeria monocytogenes* is known as Listeriosis in animals and humans. Listeriosis is commonly acquired by consumption of contaminated food products but rarely it can also be acquired by direct contact with animals and during birth in neonates (Linke et al. 2014; Gohar et al. 2017). The virulence of this pathogen is attributed to its ability to multiply in the cytoplasm after inducing phagocytosis (Ferreira et al. 2014; Vivant et al. 2013). The multiplying pathogen can move directly from cell to cell to avoid humoral immune response. It can survive within phagocytes by escaping before the maturation of phagosome due to the action of listeriolysin O (LLO) and phospholipase (PlcA) (Carpentier and Cerf 2011). This pathogen is known to cause a variety of different infections in humans, ranging from mild diarrhea to fatal meningitis. The various illnesses caused

by *Listeria monocytogenes* in humans are summarized in Table 4 (Bell and Kyriakides 2015).

Brucella abortus

Brucella species are nonmotile, aerobic, non-sporulating, and small Gram-negative coccobacilli. These pathogenic species are facultative, intracellular and capable of causing infection in wide range of animals that can be transmitted to human population also. The species of *Brucella* are classified on the basis of their primary host preferences. The important species of this genus include; *B. melitensis* (sheep and goat), *B. ovis* (sheep), *B. abortus* (cattle) and *B. suis* (pigs). *Brucella abortus*, *B. suis*, and *B. melitensis* are the only three species considered as zoonotic (Capparelli et al. 2009).

Brucellosis is a multicultural zoonotic bacterial disease, which is endemic in Asia, Middle East, South America, and Africa. More than 500,000 cases of human Brucellosis are reported worldwide annually (Luelseged et al. 2018). People working with animals, animal products, meat, and in the laboratory are at higher risk of acquiring Brucellosis (Ali et al. 2018; Islam et al. 2020). The majority of cases are usually attributed to consumption of raw milk and milk products. Various possible routes of transmission of Brucellosis to humans are described in Fig. 8 (Luelseged et al. 2018).

This intracellular pathogen can cause debilitating chronic disease in humans. The pathogen can cause the disease with very low infectious dose (10-100 cells). The pathogen can invade and multiply in diverse host tissues. In humans, the disease is also known as Malta fever or Undulant fever, due to fluctuating pattern of fever (Yoo et al. 2015). Other symptoms of Brucellosis include flu-like symptoms, fatigue, body aches, joint pain, profuse sweating, and weakness. The control measures for Brucellosis include proper sanitization measures, pasteurization of milk and milk products, safe handling of animal and animal products, adequate cooking and controlling the disease in animals (Borriello et al. 2013).

Yersinia enterocolitica

Yersinia enterocolitica is among three human pathogens belong to genus *Yersinia*. The causative agent of plague is *Yersinia pestis*, while causative agents of foodborne enteritis are *Y. enterocolitica* and *Y. pseudotuberculosis* (Laukkanen-Ninios et al. 2014). *Yersinia enterocolitica* is a member of *Enterobacteriaceae* family that can cause foodborne disease, known as Yersiniosis (Bonardi et al. 2014). Yersiniosis is usually a self-limiting disease, however immunocompromised patients are at risk of severe post-infection complications (Chung and Bliska 2016).

Animals act as main reservoirs of human pathogenic *Y. enterocolitica*. However, the pathogen rarely causes symptomatic disease in animals (Tan et al. 2014; Joutsen and Fredriksson-Ahomma 2016). The human infection starts after the ingestion of pathogen via contaminated food or water. The infections are usually sporadic in nature without any visible source. The common sources of pathogen include pork, salad, and milk, as described in Fig. 9 (Shoaib et al. 2019).

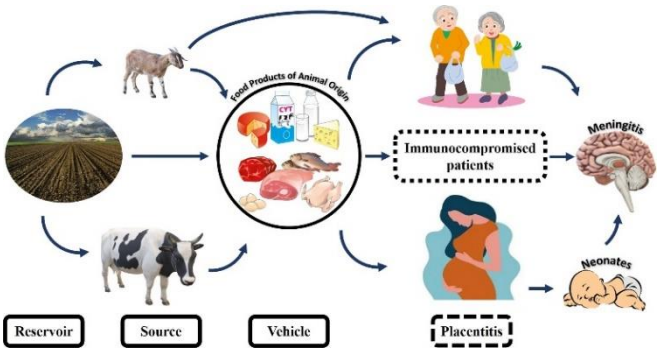

Fig. 7: Transmission dynamics and chain of infections of *Listeria monocytogenes*
Listeria monocytogenes can enter the food chain by contaminating the animal food products. The consumption of contaminated food products can cause fatal disease in immunocompromised patients, neonates, and pregnant women.

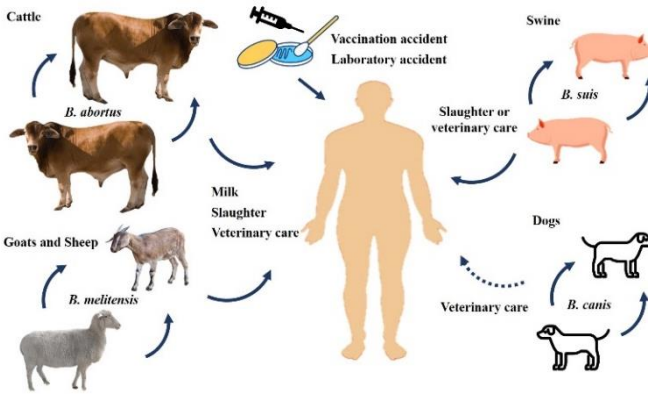

Fig. 8: Transmission of *Brucella* to humans from various sources.
Humans can acquire Brucella infection from various animals including cattle, sheep, goat, swine and rarely from dogs. Moreover, there are also reported cases of accidental transfer of Brucella during laboratory culturing or vaccination.

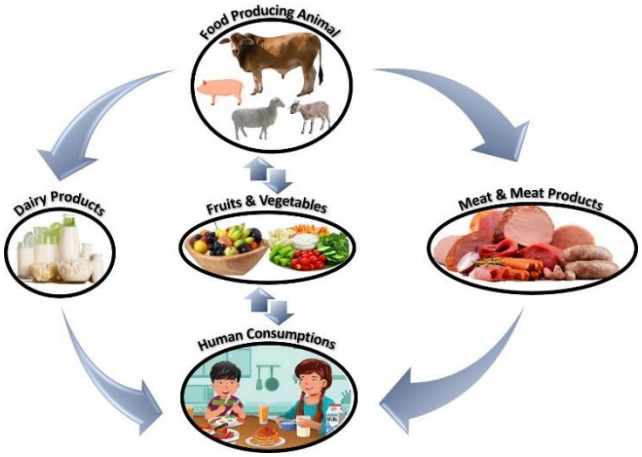

Fig. 9: Transmission of *Y. enterocolitica* in humans.
Y. enterocolitica can enter the food chain from infected animals. The humans can acquire the infection due to consumption of contaminated food and water.

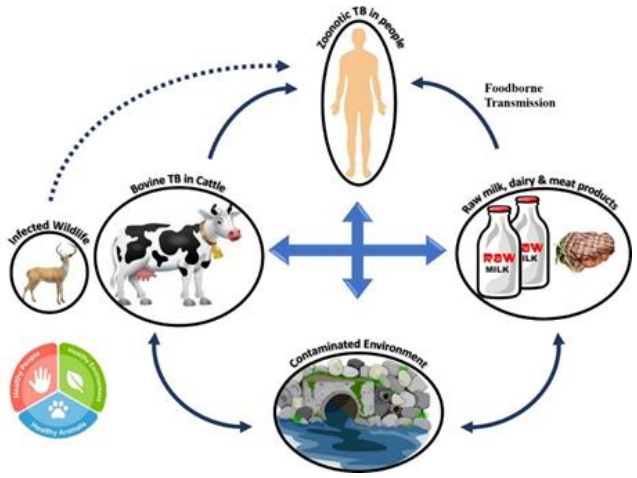

Fig. 10: Vicious cycle of transmission of tuberculosis.
Human population can acquire *Mycobacterium bovis* from animals, environment, and contaminated food products.

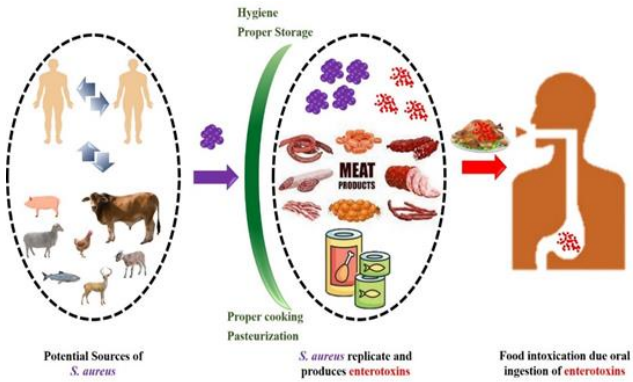

Fig. 11: Staphylococcal food intoxication.
S. aureus can enter the food products from various animal or human sources. Ingestion of preformed toxins lead to gastroenteritis. The food intoxication can be reduced by hygienic measures, proper cooking, and adequate storage.

Table 5: Enterotoxins of *S. aureus* and associated pathology

Superantigens	Associated pathology	Associated gene
Enterotoxin A	Enteritis, food poisoning	*sea*
Enterotoxin B	Enteritis, food poisoning	*seb*
Enterotoxin C	Enteritis, food poisoning	*sec*
Enterotoxin D	Enteritis, food poisoning	*sed*
Enterotoxin E	Food poisoning	*see*
Enterotoxin G	Food poisoning	*seg*
Enterotoxin H	Food poisoning	*she*
Enterotoxin I	Food poisoning	*sei*
Enterotoxin F/TSST-1	Toxic shock syndrome	*tst*

The pathogen enters the lymphatic system via M cells of the epithelium of small intestine. The antiphagocytic virulence factors like plasmid-positive strains help to circumvent the immune response. Generally, the Yersiniosis is an uncomplicated enteric disease. The severity of the disease depends upon the host age, and immune status (Valentin-Weigand et al. 2014).

Mycobacterium bovis

The association between human and animal Tuberculosis has been best known to us since centuries. *Mycobacterium tuberculosis* is the primary pathogen causing Tuberculosis in humans. *M. bovis* is the primary pathogen causing Tuberculosis in cattle, but it is also capable of causing zoonotic Tuberculosis (Anaelom et al. 2010). The ability of the *Mycobacterium* species to multiply in different hosts makes the situation abhorrent. Humans can also acquire zoonotic Tuberculosis from direct contact with infected animals, consumption of contaminated dairy or meat products, and from contaminated environment (Atabey et al. 2021). The increase in cases of zoonotic Tuberculosis infections in human population is alarming. The immunocompromised patients are relatively at higher risk of acquiring zoonotic Tuberculosis. One health approach should be adapted to mitigate the Tuberculosis situation by controlling the disease in animals, environment and humans, as described in Fig. 10 (Refaya et al. 2019).

Meat Borne Intoxications

Intoxication is the ingestion of pre-formed toxins produced by replicating bacteria in the food. The majority of bacterial pathogens are killed during the process of cooking or pasteurization but some of the bacterial pathogen can survive these conditions or can enter during the handling of meat products after cooking. These bacterial pathogens can replicate in the cooked food at room temperature and produce enterotoxins or neurotoxin (Chajęcka-Wierzchowska et al. 2019).
The bacteria responsible for production of enterotoxins include *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* (Khan et al. 2021). The neurotoxin is produced by *Clostridium botulinum,* causing the disease botulism, a disease associated with processed canned meat products (Sobel 2005). The most common source of meat borne intoxications is *S. aureus*.

Staphylococcus aureus

Members of genus *Staphylococcus* have long been associated with food poisoning and been termed as Staphylococcal food poisoning (SFP). To date, more than 50 species and subspecies of *Staphylococcus* have been identified. Most of these are non-pathogenic and food grade in nature. Members of genus Staphylococcus can be classified in two groups: Coagulase negative Staphylococci (CNS) and Coagulase positive Staphylococci, based on the capability to produce coagulase enzyme. Coagulase is tightly membrane bound enzyme that helps bacteria to convert prothrombin to staphylothrombin and plasma fibrinogen to fibrin. These help bacteria to clot blood and ultimately evade from immune cells (Zell et al. 2008).
Staphylothrombin provides antigenic disguise and in combination with fibrin clot bacterium can divert macrophages. In addition to coagulase (which is mostly produced by pathogenic strains), *Staphylococcus aureus* also produces several other virulence factors and toxins.
Among these, *Staphylococcus* enterotoxins (SEs) are responsible for food poisoning. Mostly SEs are produced by CPS rather than CNS. Very few reports have been published that highlight production of SEs by CNS (Chajęcka-Wierzchowska et al. 2019).

Among a family of about 23 different Staphylococcal enterotoxins (SEs), some cause pyrogenic diseases such as enteritis and food poisoning. As these toxins are present on mobile genomic elements, these can easily be horizontally transferred to other non-virulent strains (Varshney et al. 2009). SEs (A-E) are termed as classic enterotoxins, except SEF which is superantigen TSST-1 due to its sequence and structural homology to toxic shock syndrome toxin (Zhao et al. 2019).

The most common Staphylococcal related food poisoning cases are attributed to SEA and SEB, followed by SED and SEE as the second most important food poisoning toxins. However, toxic shock syndrome is associated with SEF/TSST-1 (Bergdoll et al. 1981). In few cases, SEC, SEG, SEH and SEI are involved in causing emetic type of food poisoning (Chen et al. 2004). The various types of SEs along with associated pathology and genes involved are summarized in Table 5.

The majority of food borne intoxications are associated with consumption of SEs contaminated food (Altaf et al. 2020). Staphylococcal food intoxication is commonly characterized by a shorter incubation period of 2-6 hrs. The amount of the toxin required to cause is very small, ranging from 5-20 µg per person or animal, but it also depends upon individual's sensitivity (Meyrand et al. 1998). Implementation of proper hygienic measures during food processing can minimize the chances of food intoxication, as depicted in Fig. 11.

Conclusion

The foodborne illness from consumption of foods of animal origin is a serious public health threat globally in developed and developing countries. The sharing of foodborne pathogens at the animal-human-environment interface suggests integrated and stringent production, hygiene, processing, and packaging measures to decrease foodborne diseases. The increasing prevalence of AMR bacterial strains in the food chain poses a serious public health concern. Judicial use of antibiotics in animal production, treatment and prevention is recommended to circumvent issues of AMR through food chain or its dissemination in the environment. Vaccination of food handlers, animals, and implementation of Codex Alimentarius Commission need to be followed to decrease burden of food infections and intoxications. The current One Health approach approved by the FAO-WHO-OIE tripartite can provide solutions for foodborne diseases of public health by policy implementation and political commitment.

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AVIAN CHLAMYDIOSIS

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INTRODUCTION

Avian Chlamydiosis is caused by *Chlamydia psittaci* (*C. psittaci*), which is a Gram-negative obligate intracellular bacterium. It causes respiratory or systemic infection, known as psittacosis in humans or ornithosis in birds. Transmission of *C. psittaci* is mainly through inhalation of contaminated aerosol. Avian/mammalian hosts and humans primarily show respiratory distress, lesions are manifested as congested lungs, fibrous exudations, and multiple inflammations at various tissues. *Chlamydia* can be detected and diagnosed by nucleic acid amplification tests, direct antigen immunostaining or serological analysis. Tetracycline is the most effective drug, but long-term use of such antibiotics can increase risks of generation of resistant strains. Therefore, natural antimicrobial extracts and herbal formulae seem to be ideal alternative therapies. Although vaccines against *C. psittaci* infection are developed and commercialized in poultry, vaccine-elicited full protection still needs more investigation.

Morphology and development cycle

Chlamydia psittaci is an obligate intracellular bacterium, which has the unique biphasic life cycle. It passes through two morphological forms during developing process: Infectious extracellular elementary bodies (EBs), and metabolically active intracellular reticulate bodies (RBs). EBs are small, round, electron dense, 'spore-like' particles (200-400 nm), while RBs are larger than EBs (800-2000 nm), their cytoplasm appears granular with diffuse, fibrillar nucleic acids, in contrast with the highly condensed nucleic acid content of the EBs. Infectious EB first attaches to host cell membrane, using bacterial proteins to bind host receptors on the surface, then injects pre-loaded effectors inside the host cytosol through type III secretion system (T_3SS). EB is subsequently internalized and form a vesicle called the inclusion inside host cell. Residing inside the inclusion, EB is transformed into RB, which is metabolically active and proliferates through binary fission. Inclusion travels along microtubules until reaches the nutrient-rich peri-Golgi region, the organism utilizes metabolites from host cells to support its own growth and expands the size of inclusion to harbor more RBs. At the end of development cycle, the expanded inclusion fills up most of the host cell cytoplasm and then RBs are transformed back to EBs. Then these newly formed EBs exit the host cell by cell lysis or extrusion and infect other cells (Escalante-Ochoa et al. 1998; Gitsels et al. 2019), as has been shown in Fig. 1.

Taxonomy and History

Chlamydia psittaci belongs to the genus *Chlamydia,* family *Chlamydiaceae*, order *Chlamydiales* and class/phylum *Chlamydiae*. For decades, the nomenclature and classification of *Chlamydia* has evolved in parallel with the increasing knowledge on its biology. In 1879, Ritter Jacob first described an outbreak of pneumonia and identified its association with imported exotic birds. The disease was named as pneumotyphus. Parrots and finches were considered as infectious vectors but there was no sign of person-to-person transmission (Ritter 1879). In 1895, Morange first applied the term 'psittacosis' which was derived from the word 'parrot' in Greek. Edmond Nocard isolated a bacterium from the bone marrow of parrot died of psittacosis and named it as *Bacillus psittacosis*. However, it was later recognized as *Salmonella typhimurium*, a conditioned pathogen (Sara et al. 1930). In 1930, Levinthal, Coles and Lillie reported a filterable virus from organ emulsion as the cause of psittacosis (Bedson et al. 1930;-Krumwiede et al. 1930; Levinthal 1930). The virus was named after Levinthal-Coles-Lilly (L.C.L.) body. Agents of so-called psittacosis-lymphogranuloma venereum-trachoma (PLT) group were unified into the genus of *Chlamydia* in 1945 (Page 1966). After that, two species, *Chlamydia trachomatis* and *Chlamydia psittaci,* were differentiated based on their relative stable morphology and chemical characteristics. The term 'virus' is no longer used when these bacteria are mentioned (Page 1968). In 1971, a new order, *Chlamydiales ord. nov.*, for *Chlamydia* species was named and species were considered independent from the order of *Rickettsiales* according to chlamydial unique biphasic developmental life cycle by Bedson and Bland (Storz and Page 1971). By phylogenetic analyses of the 16S and 23S rRNA genes in 1999, the genus of *Chlamydia* was divided into *Chlamydophila gen. nov.* (including *Chlamydophila pecorum comb. Nov.*, *Chlamydophila pneumoniae comb. Nov.*, *Chlamydophila psittaci comb. Nov.*, *Chlamydophila abortus sp. nov.* and *Chlamydophila caviae sp. nov.)* and

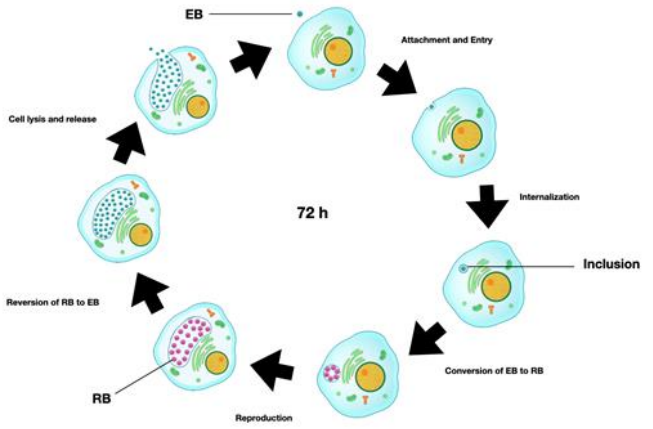

Fig. 1: Development cycle of *Chlamydia psittaci* (Scheme drafted by Zonghui Zuo).

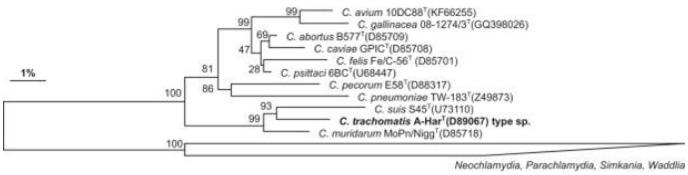

Fig. 2: Phylogeny of 11 *Chlamydia* species in single genus of *Chlamydia* based on almost complete 16S rRNA genes (Sachse et al. 2015).

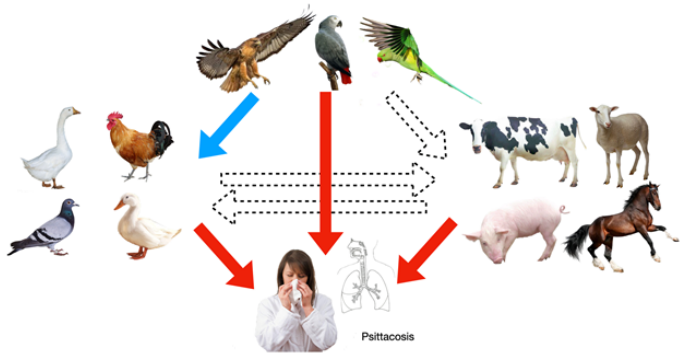

Fig. 3: Transmission of *C. psittaci* among wild birds, domesticated animals, and humans. Red arrows indicate zoonotic transmission. Blue arrow indicates confirmed transmission. Arrows with dotted line are assumed transmission without solid evidence at present (Schema drafted by Zonghui Zuo).

Chlamydophila gen. nov. (including *Chlamydia trachomatis*, *Chlamydia muridarum sp. nov.* and *Chlamydia suis sp. nov.*) (Everett et al. 1999).

In 2015, Sachse et al. (2015) proposed a single genus to include all eleven identified *Chlamydia* species (Fig. 2). Based on genomic similarity, neither cut-off values (94.5%) of 16S rRNA sequence identity nor parameters were used to distinguish species from two genera.

Transmission and Epidemiology

C. psittaci is a natural focus pathogen, which possesses a wide spectrum of hosts. It spreads all over the world and infects avian and mammalian hosts, as well as human beings (Fig. 3). Birds are primary targets. By far, at least 465 avian species, ranging from domesticated birds (chicken, duck, geese, turkeys) to pet birds (psittacines, pigeons, ratites, peacocks and many other species), can be infected (Kaleta and Taday 2003). Birds infected with *C. psittaci* are major sources of infection, because they can excrete infectious agents via feces or respiratory discharges. Bacterial shedding period is associated with strain, route, dose and individual immune status, which may last from a few days to several months (Vanrompay et al. 1995). Chlamydial shedding in feces is usually intermittent and influenced by stressors from external environment, such as cold weather, overcrowding, migration, long transportation and improper handling; or internal factors like malnutrition, breeding, egg laying and presence of other infections or inflammation. Excreted bacteria can stay infectious in moist soil or water habitats for long time (Harkinezhad et al. 2009). In general, direct inhalation of contaminated aerosol is considered as the primary transmission route in avian hosts (Vanrompay et al. 1995). At first, epithelial cells of the upper respiratory tract are infected, then *C. psittaci* descends to infect epithelium of lower respiratory tract, as well as macrophages in the airway cavity. Intense propagation happens after colonization, *Chlamydia* enters blood stream (septicemia) and uses monocytes as shuttles to travel and spread to various tissues all over the body (Page 1959). Ingestion of contaminated feed also plays a critical role. Oral infection can lead to a systemic dissemination of *C. psittaci* in spleen, liver, lungs and segments of digestive tract (Thierry et al. 2016). In the wild, raptors and scavenger birds can spread *C. psittaci* by preying on infected animals or carcasses as well. Although transmission through arthropod is believed not likely, recent studies show a high prevalence of *Chlamydiales* DNA within bloodsucking ectoparasites, like mites or ticks, which suggests possibility transmission (Page et al. 1975; Pilloux et al. 2015). Vertical transmission has been described in several avian species, even though this is not commonly reported (Lublin et al. 1996; Wittenbrink et al. 1993). An experimental study has demonstrated that the transmission is due to penetration of *C. psittaci* through eggshell (Ahmed et al. 2017).

C. psittaci can also affect domestic animals, wild mammals and human beings. Reports of infection of *C. psittaci* in this group of hosts are rare, with pathogenesis and pathology are not completely clear (Radomski et al. 2016). An experimental model has shown that *C. psittaci* respiratory-infected calves can show bronchopneumonia, from mild to severe, depending on infectious doses (Reinhold et al. 2012). Prevalence studies, either on molecular or serological level, have indicated *C. psittaci* to be ubiquitous in cattle (Domeika et al. 1994; Kaltenboeck et al. 1997; DeGraves et al. 2003). However, these infections are clinically inapparent. Due to the lack of association between infection and clinical disease, as well as the tendency to produce persistent infection, the pathogenic significance of non-avian *C. psittaci* in cattle is intensely discussed. Therefore, one theory suggests that the virulent of *C. psittaci* might sharply reduce in the process of passaging bacteria from avian hosts to non- avian hosts

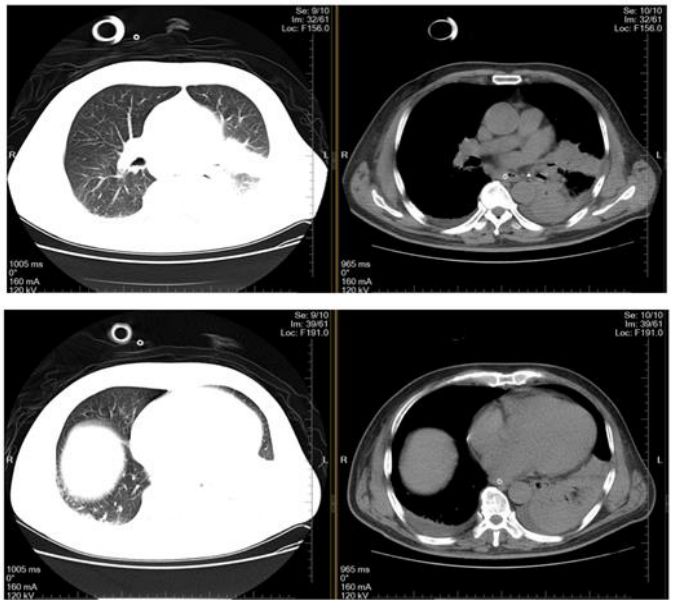

Fig. 4: Serial chest computed tomography (CT) scans of a patient with severe Psittacosis pneumonia. The CT scans showed air-space consolidation with inflammatory exudation (unpublished data).

(Non-avian host strains of *C. psittaci* are classified as BSL-2, but avain strains are classified as BSL-3) (Radomski et al. 2016).

Zoonotic hazards

C. psittaci is an important zoonosis. Humans can get infected through inhalation of contaminated aerosols, as well as close contact with sick birds or avian carriers. The first case of human psittacosis was reported by Ritter Jacob (Ritter 1879). After that, between the late 19th century and the early 20th century, there were several pandemics reported in multiple regions of Europe and America, which were all mainly caused by handling exotic birds (Chu et al. 2021). *C. psittaci* infects humans via respiratory system, then it spreads to whole body through blood circulation (West 2011). The incubation period is around 5 to 14 days or even longer. Vital organs, such as lung, spleen, liver and central nervous system, can be affected (Fig. 4). Clinical signs are variable from case by case, depending on individual immune status and the virulence of the causative strain. Typical symptoms of Psittacosis in humans consist of abrupt onset of headache, malaise, chills, fever, nonproductive coughing, myalgia, and dyspnea (Balsamo et al. 2017). Further observed complications comprise hepatitis, endocarditis, arthritis, myocarditis, encephalitis, keratoconjunctivitis and atypical pneumonia (Balsamo et al. 2017). Psittacosis can be lethal as well, most dead cases are due to late or mis-diagnosis. Now-a-days, timely treatment with antibiotics makes death extremely rare. Although the prevalence of *C. psittaci* is rather low in ordinary populations, it raised occupational hazards among poultry workers, pet bird owners, butchers and veterinarians (Laroucau et al. 2009a, 2009b; Dickx et al. 2010; Dickx and Vanrompay 2011; Lagae et al. 2014;Hulin et al. 2015). Awareness of the danger and proper handling of infected birds or cultures by following biosafety regulations are critical to protect susceptible population from infection. Moreover, *C. psittaci* is also reported related to community-acquired pneumonia and ocular adnexal MALT (mucosa associated lymphoid tissue) lymphomas (Ferreri et al. 2004; Dumke et al. 2015).

Pathology

Pathological impact of *C. psittaci* on avian hosts is largely associated with the virulence of strain and route of infection, as well as the species of host. *C. psittaci* strains can be categorized as highly virulent strains, which cause acute infection with a mortality of 5–30%, and low virulent strains that cause asymptomatic and progressive infections (Vanrompay 2020). Major outer membrane protein (MOMP) is a key virulent factor and fundamental structural component of *C. psittaci*, which is coded by the outer membrane protein A (*ompA*) gene (Baghian et al. 1990). The *ompA* genotyping with real-time PCR or DNA microarray method is commonly applied to differentiate and classify *C. psittaci* strains, as certain genotypes occur more regularly in a specific order of birds (Van Lent et al. 2012) (Table 1). Inhalation of *C. psittaci*-contaminated aerosols is regarded as the natural route of infection. Most infections caused by low virulent strains are subclinical, with inapparent signs and long incubation time. Highly virulent strains are able to result in quick death in hosts, with lesions characterized by extensive vascular congestion, fibrinous discharges and inflammation of vital organs (Vanrompay et al. 1994). Typical symptoms and lesions of various tissues are summarized in Table 2 and gross lesions are shown in Fig. 5.

Both types of strains adversely affect egg production and hydrosalpinx or oviduct cysts can be observed in some cases by autopsy (Zhang et al. 2008; Lin et al. 2019). Further descriptions of pathological changes in turkeys, chicken, ducks, pigeons, and other avian species are well reviewed (Vanrompay, 2020; Fang et al. 2021).

Diagnosis

There are multiple ways for diagnosis of avian Chlamydiosis in the laboratory. These assays detect either the existence of bacteria or the presence of *Chlamydia*-specific antibodies. Cell cultures and immunofluorescence staining are the golden standards for defining infection and the most recommended methods with respect to both sensitivity and specificity.

Sample collection

Quality of samples is essential for accurate diagnosis. Swab sampling is commonly applied in live birds. Pharyngeal or conjunctival swabs are preferred when birds showing respiratory symptoms or conjunctivitis. Cloacal swabs are less used due to intermittent shedding of *Chlamydia* in some birds. Collection of organs with lesions in dead birds postmortem is viable as well. Lungs, air sacs,

Table 1: Mainly affected hosts of different genotypes of *C. psittaci* strains

Genotype	Subgroup	Primary infected species	Other infected species	Reference
A	VS1, 6BC, 8455	Psittacidae	Turkey, Duck, Pigeon, Passeriformes	(Vanrompay et al. 1994)
B		Columbiformes	Chicken, Turkey, Duck, Psittacidae, Passeriformes	(Vanrompay et al. 1997)
C		Anseriformes	Chicken, Duck, Pigeon	(Vanrompay et al. 1993)
D	NJ1, 9N	Turkey	Chicken, Pigeon	(Vanrompay et al. 1997)
E (Cal-10/MN/MP)			Turkey, Pigeon, Duck, Ostrich, Rhea	(Geens 2005)
F	VS225, Prk Daruma, 84/2334, 10433-MA	Psittacine	Turkey	(Everett et al. 1999)
E/B	E30, 859, KKCP	Duck	Parrot, Pigeon, Turkey	(Vanrompay et al. 1997)
M56		Muskrat, Hare		(Spalatin et al. 1966)
WC		Cattle		(Everett et al. 1999)

Table 2: Lesions of avian hosts infected with highly virulent *C. psittaci*

Tissues and organs	Lesions	Reference
Lungs	Congestion, Hemorrhage, Fibrinous exudation	(Vanrompay 2020)
Air sacs	Thickened membrane, Fibrinous exudation	
Pericardium	Thickened and congested membrane, Fibrinous exudation	
Heart	Enlargement, Thick fibrin plaques or exudation	
Liver	Enlargement, Decolorization, Thick fibrin coating	
Spleen	Enlargement, Tissue darken and soften, gray-white focus	
Peritoneal serosa and mesentery	Congestion, Fibrinous exudation	
Oviduct	Follicular inflammation, peritonitis, salpingitis	(Fang et al. 2021)

spleen, liver and exudations in body cavity are the most suitable for identification and isolation of *Chlamydia*. Aseptic handling of samples is necessary during the process. Antibiotics, such as gentamycin, streptomycin, amphotericin and vancomycin, which are not affective against *Chlamydia* can be used for reducing contamination by other microorganisms. Specimens containing *Chlamydia* should be stored in 2-sucrose-phosphate (2-SP) solution at 4°C before analysis (Dubuis et al. 1997). For longer preservation, specimens should be soaked in sucrose–phosphate–glutamate (SPG) buffer and stored at -80°C (Warford et al. 1984).

Antigen Detection

Immunofluorescence staining is extensively used in the laboratory to detect and identify *Chlamydia*. This technique can detect *C. psittaci* in tissue smears/sections and single-layer cultured cells. *C. psittaci* in clinical samples can be propagated in embryonated eggs or cells. Anti-LPS and anti-MOMP monoclonal antibodies conjugated with fluorescein isothiocyanate are mostly used in commercial kits (Fig. 6). Commercial kits specific for *C. psittaci* are limited; most of them are designed and developed for the detection of *Chlamydia trachomatis* in human samples. But some of them can also be applied to detect *C. psittaci* due to the cross-reactivity of anti-LPS antibodies. In order to illustrate the relation between *C. psittaci* and pathological focus, immunohistochemical staining with anti-LPS antibodies is used to detect *Chlamydia* in tissue slices embedded in paraffin. LPS-coated ELISA kits are used in the diagnosis of human *Chlamydia trachomatis*, and these kits are able to detect *C. psittaci* as well. Chlamydial LPS shares some epitopes with other Gram-negative bacteria, and these epitopes can show cross-reaction, resulting in a high number of false-positive results (Vanrompay et al. 1994).

Conventional PCR and Real-time PCR

DNA samples can be easily extracted or prepared from all kinds of specimens with commercialized kits. Reagents for DNA stabilization or preventing DNA from degradation are recommended during sampling or storage. 16S-23S rRNA or *ompA* genes are commonly used in *C. psittaci* detection. Detailed information is well summarized by Sachse et al. (2009). Application of PCR techniques increase the sensitivity of detection and reduce risks of people getting infected in laboratories. It has been the replacement of traditional isolation and identification of *C. psittaci* from clinical tissues. In recent decade, Real-time PCR has become the preferred diagnostic method due to its rapidity, precise quantification and high sensitivity. Conventional PCR can show similar high sensitivity by using relative short DNA segments or nested procedure, but it also increases the risk of contamination during the reaction (Van Loock et al. 2005). Real-time PCR can detect the amplification of products, as the products are synthesized, in a bounded system. It needs a florescent dye or florescent-labelled probe and thermocycler equipped with fluorescent- detection capability. A series of progressive detections based on different requests are recommended. Amplification of 23S-rRNA gene is used for identifying *Chlamydia*-positive cases (Geens et al. 2005; Heddema et al. 2006; Pantchev et al. 2010). *C. psittaci*-specific detection targets at *ompA* gene or *incA* gene (Ménard et al. 2006; Opota et al. 2015). In the former *ompA*-based assay, minor groove binding probes are applied to exclude possibility of cross-reactions with *ompA* from *Chlamydia abortus* (Opota et al. 2015). There are other *ompA*-based real-time PCRs for further differentiating genotypes of *C. psittaci* and assisting to trace chains of zoonotic transmission (Geens et al. 2005; Heddema et al. 2015). Protocols are available for the specific detection of newly emerged *Chlamydia avium* and *Chlamydia gallinacea* (Laroucau et al. 2015; Zocevic et al. 2013).

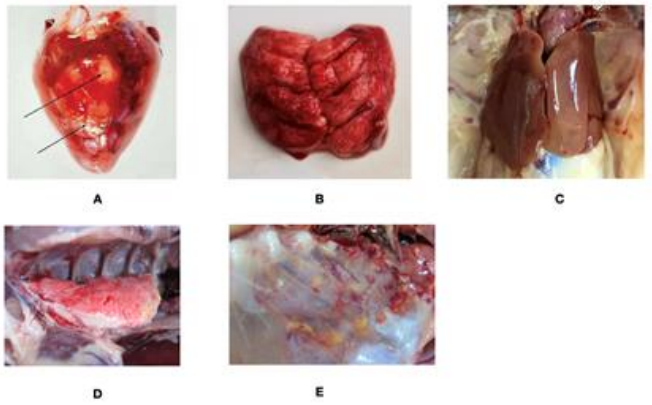

Fig. 5: Gross lesions caused by *C. psittaci* infection in birds. (A) Fibrin plaques on heart surface in turkey (Vanrompay 2020). (B) Lung hemorrhage and congestion in specific pathogen free (SPF) chicken (unpublished data). (C) Liver decolorization in SPF chicken (unpublished data). (D) Fibrinous exudation in broiler's lung (Zuo et al. 2018). (E) Fibrinous discharge in air sacs of broilers (Zuo et al. 2018).

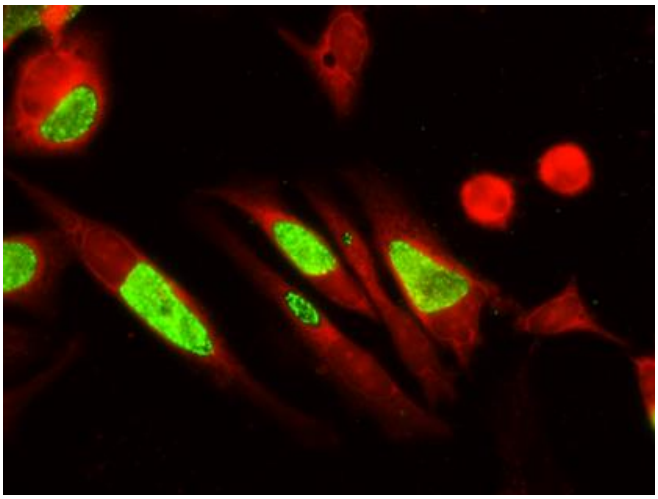

Fig. 6: *C. psittaci* was propagated in Vero cells for 36 hours at 37°C. *Chlamydia* and cells were stained with anti-MOMP fluorescent mAb and Evans blue, respectively (unpublished data)

DNA microarray and metagenomic next-generation sequencing (mNGS)

DNA microarray is a powerful tool for high throughput screening and detection of Chlamydial infections. This method is capable of identification *Chlamydiaceae spp.* by 23S rRNA gene amplification, then identification of certain Chlamydia species by hybridisation with species-specific probes(Borel et al. 2008; Sachse et al. 2005). Furthermore, it is able to detect genotype *C. psittaci* from clinical samples based on *ompA* genotyping system (Sachse et al. 2008). It also shows the advantage of differentiating mixed *Chlamydia* infections and direct identification of specific species.

Metagenomic next-generation sequencing (mNGS) has been applied to diagnose human clinical Psittacosis (Chen et al. 2020; Gu et al. 2020). This technique can detect all nucleic acids from the same sample and reassign DNA sequence data based on reference genomes. Physicians are able to understand the presence and relative proportion of each kind of microbe in an unbiased and universal perspective. At present, mNGS can improve the accuracy and reduce the delay in diagnosis of rare disease.

Antibody Detection

Serological tests are not suitable for diagnosis of avian Chlamydiosis, because of ubiquitous Chlamydial infections in birds, and antibodies against *C. psittaci* can remain persistent for several months. Therefore, serological test should be combined with antigen or gene detection assays. Beyond that, anti-Chlamydial antibodies in serum can be influenced by many factors, such as sampling time and antibiotic treatment, which may give a false negative result. Meanwhile, a positive serum only indicates that the bird was exposed to the pathogen before but does not indicate the presence of active intracellular infection.

Antibody detection tests are frequently applied for epidemiological studies (Vanrompay et al. 1995). But the complement fixation test (CFT), the immunofluorescence (IF) and a latex agglutination test targeted at IgM are currently used in routine diagnosis (Andersen 1991; Moore et al. 1991). ELISAs based on recombinant MOMP, PmpD and Pmp20G have been developed and evaluated. The sensitivity and specificity of these ELISAs reached 97.9-100%, and 100%, respectively (Verminnen et al. 2006, 2008; Liu et al. 2016; Cui et al. 2021).

Strategies for the control and treatment of *C. psittaci* infection in poultry

Quarantine of suspicious or diagnosed birds is necessary; all birds should be isolated and kept in clean and uncrowded places. Any stress, which may cause the development of infection or activation of Chlamydial shedding, must be eliminated (Balsamo et al. 2017). Sick birds may become inappetence, initial treatment with medication should be directly delivered through mouth or injection. In general, recommended treatment period will last about 30 to 45 days, and shorter treatment lasts for 14 days (Balsamo et al. 2017). But recovered birds must be monitored with PCR-related methods to make sure complete resolution of infection post treatment. During treatment period, birds should be observed daily and weighed every 3-7 days. Calcium and mineral supplements in feed or drinking water are not recommended, as these may interfere with absorption of tetracyclines, or at least these products should be administrated after an interval of 4-6 hours. Fresh food and daily cleaning and disinfection are also critical to preventing from opportunistic infections (Balsamo et al. 2017). Recovered birds are still susceptible to *C. psittaci* reinfection. Therefore, poultry yards and every piece of gear should be thoroughly cleaned and disinfected several days before treatment ends. Detailed case history and treating procedures need to be well recorded in case of further transmission.

Antibiotic therapy

Tetracyclines (chlortetracycline, oxytetracycline, doxycycline) macrolides and azithromycin are in the

Table 3: Herbal formulae for preventing or treating avian *C. psittaci* infection

Year & inventor	Prescription (parts by weight, pbw)	Therapeutic effect	References
(Bingbing 2016)	Cone of the lacebark pine 10-15 pbw Great burdock achene 1-3 pbw chlorite schist 1-3 pbw turpentine 1-3 pbw fruit of *Carthamus tinctorius L.* 2-6 pbw sargent gloryvine stem 2-6 pbw fossil shell of spirifer 2-6 pbw tortoise plastron 1-2 pbw	For prevention 5-30 days of oral administration in pigeons contributed 100% resistance when they were exposed to *C. psittaci*-infected birds	(Bingbing 2016)
(Feng 2014)	Radix scutellariae 65-75 pbw *Cyrtomium fortune* 11-13 pbw fructus mume 30-35 pbw fructus forsythiae 25-30 pbw Polygonum cuspidatum 20-25 pbw rhizoma bletillae 9-12 pbw ginseng 14-18 pbw white atractylodes rhizome 17-20 pbw radix sophorae flavescentis 12-15 pbw the root of Chinese pulsatilla 16-20 pbw *Caulis Spatholobi* 12-16 pbw Kudzuvine Root 7-10 pbw Optional: pericarpium citri reticulatae 2-5 pbw *Radix Aucklandiae* 1-3 pbw	For treatment one thousand 23-day-old layers infected with *C. psittaci* were orally administrated with compound (5 g /bird) for 35 days, the recovery rate reached 92%.	(Feng 2014)
(Ming and Junqing 2017)	*Belamcanda chinensis* 5-25 pbw Baphicacanthus root 5-20 pbw main fructus arctii 3 pbw lithospermum 5-18 pbw Dracocephalum moldavica 3-15 pbw Papilionaceae Abrus mollis 3-15 pbw subprostrate sophora 5-20 pbw Lilium brownii 4-18 pbw Ficus stenophylla 5-20 pbw Codonopsis convolvulacea kurz 3-18 pbw white peony root 5-20 pbw red halloysite 3-15 pbw opium poppy capsule 5-18 pbw pummelo peel 3-20 pbw Gyrophora hypocrocina Jatta 5-20 pbw Honey liquorice 3-18 pbw Platycodon grandiflorum 5-20 pbw	The compound can be manufactured as powder or solution by following the instruction. Diseased pigeons were orally treated with powder or solution at the dosage of 2 g or 2 ml per kg weight for 5 to 10 days. The cure rate was 83%-88%.	(Ming and Junqing 2017)
(Ming and Junqing 2017)	dogtooth violet 5-30 pbw Hibiscus mutabilis L. 5-25 pbw Scabiosa comosa Fisch 5-25 pbw Commelina paludosa 3-18 pbw *Humifuse Euphorbia* herb 3-20 pbw folium isatidis 5-20 pbw main fructus arctii 3-20 pbw Terminalia chebula Retz 5-25 pbw fructus mume 5-25 pbw pericarpium granati 5-20 pbw Adenophora stricta 3-18 pbw parched hawthorn fruit 3-20 pbw Honey liquorice 5-25 pbw Platycodon grandiflorum 5-20 pbw	The compound can be manufactured as powder or solution by following the instruction. Infected chickens were orally treated with powder or solution at the dosage of 3 g or 2 ml per kg weight for 5 to 10 days. The cure rate reached 93%.	(Ming and Junqing 2017)

frontline of anti-*Chlamydia* infection. Chlortetracycline, doxycycline, and fluoroquinolone enrofloxacin are the most frequently used antimicrobials in poultry and pet birds. These products can be administered orally via feed or drinking water. Alternatively, they can be administrated parenterally through intramuscular or subcutaneous routes (Flammer 1989; Butaye et al. 1997). These drugs are highly effective against infection and dissemination of *C. psittaci*. Therefore, resolution of respiratory symptoms can quickly occur post treatment. However, in some cases, *Chlamydia* can keep shedding without significant decrease for up to 14-days post-treatment of antibiotics (Prohl et al. 2015). The extensive use of tetracycline and long-term treating periods can result in plasma drug concentrations below therapeutic range, promoting the emergence of drug-resistant *C. psittaci* strains (Tell et al. 2003; Guzman et al. 2010; Krautwald-Junghanns et al. 2013).; Moreover, the

widespread abuse of penicillin G makes *Chlamydia* stay in a persistent state and contributes to the occurrence of chronic infections (Goellner et al. 2006). Resistance to chlortetracycline in the avian strains has been reported, but it does not raise serious problem. Routine screening and surveillance of *C. psittaci* isolates with antibiotic-resistance in poultry yards or cattle farms are urgently needed, which is of great help for assessment of actual situation (Bommana and Polkinghorne 2019).

Alternative treatment

Abusive use of antibiotics in poultry is a worldwide phenomenon. Now-a-days, it raises many animal and human health problems, endangers food safety and environment. Many countries have completely banned or restricted the use of antibiotics in animal industry. Routine prophylactic antibiotic treatment is no longer recommended because it may cause adverse effects and generate resistant strains of *C. psittaci* and other bacteria. Consequently, birds become more vulnerable to *C. psittaci*. Extracts from natural materials offer potential solutions to control *Chlamydia* infection (Brown et al. 2016). Polyphenolic substances disrupt membranes to inhibit Chlamydial growth, promote cell apoptosis or improve immune surveillance (Daglia 2012). Lipids, such as fatty acids, monoglycerides or peptides can directly disrupt the cell membrane of EBs (Peter 2010). Transferrin interferes with Chlamydial attachment and internalization (Van Droogenbroeck et al., 2008, 2011). Cellular metabolites and probiotic bacteria can inhibit Chlamydial growth by modulating host immunity. Moreover, formulae of traditional medicine prescriptions are alternative ways to tackle with this challenge as well. Several prescriptions have been proved remarkably effective in poultry (Table 3). More descriptions of natural antimicrobials are well reviewed by Brown et al. (2016).

Vaccination

In the early time of exploration of vaccines against *C. psittaci*, the most successful vaccines targeted at infections in sheep and companion cats. These vaccines are developed based on inactivated or live attenuated elementary bodies (Shewen et al. 1980; Wills et al. 1987; Anderson et al. 1990; Chalmers et al. 1997), but they can only provide adequate protection against diseases resulting from *Chlamydia* instead of infection itself (Entrican et al. 2001; Gruffydd-Jones et al. 2009). Inactivated vaccines are unable to prevent Chlamydial shedding and usually cause local inflammation at administration site. Attenuated vaccines seem ideal, but recurrence of virulence of live bacteria may occur in future. *Chlamydia abortus* 1B strain, a temperature-sensitive mutant, is wildly applied to fight against ovine enzootic abortion (OEA) (Wheelhouse et al. 2010). However, it is reported that epidemical outbreaks of OEA in 1B-vaccinated sheep across Europe are related to 1B strain, and genomic evidence has also been found(Laroucau et al. 2018; Longbottom et al. 2018). The development of vaccines against avian *C. psittaci* started in the end of 1990s. Vaccine candidates were related to major outer membrane protein (MOMP), MOMP-based DNA vaccine (Vanrompay et al. 1999), recombinant MOMP subunit vaccine or a combination of DNA and MOMP, viral-vectors (Zhou et al. 2007), transgenic rice (Zhang et al. 2009), HVT-PmpD vaccine (Liu et al. 2015) and inactivated whole EBs vaccine (Zuo et al. 2021). The detail of efficacy and type of vaccines in poultry has been well addressed in a review article (Quilicot et al. 2017). Overall, although veterinarians have made progress, there is still no effective licensed commercial vaccine against *C. psittaci* infection.

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CANINE BACTERIAL ZOONOSIS

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INTRODUCTION

The degree to which people find pleasure in the companionship of dogs is evident by the number of household dogs in the human community. With the ever-increasing ownership of dogs, the likelihood of infection with dog bacteria and other pathogenic agents is prevalent among canines and has steadily increased as well. Dogs are a common host species to a number of zoonotic diseases, which have a significant impact on public health. While some of these diseases are widely distributed but mild in nature, others, mostly caused by bacterial agents, can cause serious or even fatal illness. To compound matters, antimicrobial use (and abuse) for treating both human and zoonotic illness has led to antimicrobial resistance. Such antimicrobial resistance inevitably leads to 'epidemics' of resistant bacterial infections, from which dogs are not exempted. The spread of certain multidrug-resistant bacteria from people to their dogs represents a fascinating example of the one-medicine principle. This chapter focuses on bacterial zoonoses linked to dogs. Evidently, clear guidance, stemming from current knowledge of the epidemiology, pathobiology and diagnosis of bacterial dog-related human diseases is needed in order to provide dog owners with objective knowledge of best practices pertaining to dog care.

Salmonellosis

Overview

Salmonellosis is a bacterial disease caused by a number of bacteria of the genus Salmonella, which includes over 2300 serovars; often transmitted to humans through the fecal-oral route. The infection leads to enteric and multisystem disease in both humans and animals. Human salmonellosis is an essential zoonosis with broad economic and public health implications. While salmonellosis is most frequently caused by foodborne infection, Salmonella's zoonotic transmission from dogs has also been documented (Leonard 2014).

Etiology

Salmonella spp. are ubiquitous opportunistic, usually motile, aerobic, and facultative anaerobic, non-spore-forming gram-negative rods. They belong to the family Enterobacteriaceae; members of this family are capable of colonizing the gastrointestinal tracts of humans, dogs, a large number of other mammalian species, as well as birds and reptiles. Recent findings suggest that Salmonella is rarely detected in healthy dogs, 0–2.9% of pet-household dogs and 6.3% of stray dogs. Detection rates for Salmonella are considerably higher in dogs on a raw-meat diet, with 23 times higher Salmonella shedding, compared with those not fed raw meat. Moreover, in healthy dogs, one single Salmonella contaminated raw meat meal can contribute to fecal shedding of Salmonella for up to one week (Joffe and Schlesinger 2002; Morley et al. 2006; Finley et al. 2007; Tsai et al. 2007; Lefebvre et al. 2008).

Clinico-pathological features

Adult infected dogs tend to shed Salmonella asymptomatically, while younger, aged and pregnant dogs experience clinical symptoms after 3-to-5-days incubation period. Symptoms typically range from mild self-limited diarrhea to symptoms of severe hemorrhagic gastroenteritis and septicemia. Following the ingestion of contaminated food, Salmonella bind to, colonize and penetrate enterocytes and proliferate both freely and inside macrophages, provoking mucosal necrosis with proprial and submucosal hemorrhage, edema, and leukocytic infiltration. The affected mucosa is typically hyperemic or hemorrhagic, thickened and covered with a red, yellow, or gray exudate. In young animals infected with virulent strains, macrophages may transport Salmonella to the mesenteric lymph nodes and blood vessels, leading to circulatory invasion and septicemia.
Dogs start to become anorexic and develop fever. Diarrhea, in the form of mucoid, watery, or hemorrhagic feces, usually follows. Vomiting, dehydration, and potential hypovolemic shock and septicemia can occur, particularly in severe cases. Once clinical symptoms subside, dogs tend to shed Salmonella for a short period that may extend up to eight weeks in some cases. Pregnant females infected with Salmonella show vaginal discharge, followed by abortion.
In humans, the infection may be asymptomatic. In clinical cases, acute gastro-enterocolitis develops after an incubation period of 4–10 days in children, the elderly, and those who suffer from immunosuppression. Symptoms typically include fever, nausea, abdominal pain, and mucoid diarrhea. Bloody diarrhea is more common in children than in adults. Possible non-gut sequalae include skin lesions, arthritis, and septicemia (Weese and Fulford 2011; Greene 2012; Macpherson et al. 2013; O'Neil 2018).

Diagnosis

The standard diagnostic test for Salmonellosis requires early isolation of the organisms from feces after the initial signs of illness. In cases of disseminated disease, bacteria can be isolated from the blood or other sterile body sites. Selective media, such as selenite or tetrathionate broth, are necessary for cultivation. As a means of rapid diagnosis, PCR testing is becoming more popular. Unfortunately, validation of PCR conducted to detect Salmonella is currently lacking, and the efficacy of Salmonella PCR tests is still unclear. MALDI-TOF mass spectrometry and DNA microarrays have recently become additional methods for diagnosis of Salmonella infections (Singhal et al. 2015; Bauerfeind and Krauss 2016).

Therapeutics

In dogs, there is no antibiotic therapy indicated for uncomplicated cases. However, antibiotic therapy should be prescribed in severe cases or those with extra-intestinal complications and in animals that are chronic carriers for up to 6 weeks after checking for antibiotic resistance. Ampicillin, amoxicillin/clavulanate, fluoroquinolones, 3rd-generation cephalosporin are antibiotics of choice for the treatment of Salmonella infections.
In human, patients who do not belong to a risk category, mild infections need no antimicrobials, with only fluid and electrolyte replacement is required as treatment. In patients at high risk of invasive infection, including neonates, elderly and immunosuppressed persons, antimicrobials, such as ciprofloxacin or trimethoprim-sulfamethoxazole, should be administered for 3 to 7 days; also ciprofloxacin may be given for up to 10 days in permanent excretors. However, a high percentage of Salmonella has developed multiple antibiotic resistance and the treatment should only be given according to the antibiogram findings (Wiebe 2015; Bauerfeind and Krauss 2016; O'Neil 2018; Weese and Evason 2019).

Campylobacteriosis

Overview

Campylobacteriosis is a widespread bacterial disease that affects both humans and a number of domesticated animal species. It is caused by several bacterial species of the genus Campylobacter (formerly Vibrio). Campylobacter, a major cause of human enteritis, generally colonizes in the gastrointestinal tract of wild and domestic animals and can be isolated from both healthy, as well as diarrhea-affected dogs. Most human cases of Campylobacteriosis are foodborne, typically occurring after the handling or consumption of contaminated, untreated surface water, unpasteurized milk, or undercooked meat. Dogs and other companion animals are also possible sources of human infection, and their true roles may be currently underestimated. Around 6% of cases of human Campylobacteriosis have been identified to have occurred due to contact with pets (Tenkate and Stafford 2001; Koene et al. 2004; Adak et al. 2005; Mazick et al. 2006; Rossi et al. 2008).

Etiology

Campylobacter spp. are motile, slender, spiral or comma-shaped gram-negative rods that grow best under microaerophilic 5% O_2, 10% CO_2 and 85% N_2 atmospheric conditions. They are obligately parasitic inhabitants of mucous membranes and remain infectious for only a few weeks outside their hosts. There are at least 37 known species and subspecies of this genus, with just one subset of clear pathogens. The zoonotic agents *C. jejuni* subsp. jejuni, *C. coli, C. lari* and *C. upsaliensis* are thermophilic species, that can grow at 42°C. They may be directly or indirectly transmitted from other vertebrates to humans. There is sufficient evidence to believe that the risk of acquisition of *C. jejuni* increases due to the contact with infected dogs, particularly those with diarrhea (Marks et al. 2011; Percival 2014; Poxton et al. 2015).

Clinico-pathological features

In dogs, clinical signs of Campylobacteriosis are non-specific and depend on the severity and duration of infection. Incubation period is often short, with symptoms of enteritis mostly develop within three days. The main sign is mild diarrhea with blood or mucus. Fever, vomiting, anorexia, and lethargy are less frequent signs. In young and physically weak animals, dehydration is more prevalent. Abnormally high fluid in colonic contents, as well as thickening, congestion, and edema of the colonic mucosa, are common macroscopic findings in both naturally and experimentally infected dogs. Microscopically, thickening of the mucosa is caused by epithelial glandular hyperplasia. Findings of subepithelial congestion, hemorrhage, as well as inflammatory cells infiltration have also been reported. The colon and cecum show diminished epithelial cell heights and brushed borders, with a reduction in the number of goblet cells. Staining samples with sliver staining to help detect the filamentous bacteria in intestinal crypts may be needed to confirm a diagnosis of Campylobacteriosis, as the pathological findings in many cases of Campylobacter infections are non-specific. Common extra-intestinal sequalae of Campylobacter infections in dogs include abortions, perinatal death, icterus, and other liver disorders.
In humans, the incubation period usually lasts from 3 to 5 days, with the eventual development of bloody or mucoid diarrhea in most cases. In 30% of patients, Campylobacteriosis may initially be manifested by an influenza-like symptoms rather than diarrhea. In such cases, the patients may complain fever, headache, weakness, dizziness, and myalgia. In immunosuppressed patients, extra-intestinal symptoms such as bacteremia, meningitis, and reactive arthritis can also be seen (Skirrow 2002; Marks and Kather 2003; Marks et al. 2011; Sahin et al. 2014; Kreling et al. 2020).

Diagnosis

Reaching a definitive diagnosis of Campylobacteriosis in dogs is often challenging. This is largely due to high prevalence of colonization in healthy animals. Fecal cytology with a finding of curved, "Campylobacter-like" species is used by some workers, leading to possible false-positive diagnoses. All Campylobacter spp. Are not pathogenic, and some other harmless bacteria morphologically resemble to Campylobacter. Consequently, fecal culturing has become the gold standard diagnostic test. Although difficult to interpret at time, fecal culturing has the benefit of supplying isolates for species identification and antibiotic sensitivity tests. It is essential to define the specific species of Campylobacter in order to reach a proper diagnosis. In particular, it is important to differentiate catalase-positive species (*C. jejuni, C. coli*) from catalase-negative ones, as a finding of catalase-positive Campylobacters may be clinically important. For the detection of *C. jejuni* and *C. coli* antigens, a rapid, simple, and inexpensive ELISA diagnostic tool, with an efficiency comparable to that of bacterial cultivation, has been developed. Several PCR tests have also been developed for the detection of different Campylobacter species. Although PCR tests may be more sensitive, yield faster results, and detect a wide range of species, it is not clear if their higher sensitivities would actually help in the diagnose of Campylobacteriosis or add to potential false-positive diagnoses (Marks and Kather 2003; Chaban et al. 2009; Chaban et al. 2010; Granato et al. 2010; Kaakoush et al. 2015).

Unlike canine infections, the lower prevalence of colonialization with Campylobacters in humans means that Campylobacteriosis can be more easily diagnosed. Campylobacter morphology and dart motility can be detected in feces by darkfield or phase-contrast microscopy. Culture is the most widely used tool in the detection of thermophilic and non-thermophilic species. Testing specifically for the thermophilic *C. jejuni* and *C. coli* should be sufficient for a diagnosis of Campylobacterosis in humans. Test for the non-thermophilic *C. fetus*, and *C. upsaliensis,* and other species with a variable growth capacity at 42°C may not be so helpful.

Moreover, the type of culture media used, especially for the detection of *C. upsaliensis,* may have a major influence on the accuracy of results, since *C. upsaliensis* is vulnerable to selective medium antimicrobials. Due to the fastidious nature of Campylobacter, single negative cultivation does not preclude Campylobacteriosis. In addition to PCR technologies, other molecular approaches used in recognizing or detecting Campylobacter organisms include random amplified polymorphic DNA, whole-genome sequencing and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) (Moore et al. 2005; Davis and DiRita 2008; Silva et al. 2016; Schürch et al. 2018).

Therapeutic

Treatment of Campylobacter infections in dogs is often conservative. For one thing, Campylobacter infections in dogs are invariably self-limiting. In addition, Campylobacter spp. isolated from dogs have demonstrated resistance to the commonly used antimicrobials, which would otherwise damage the normal intestinal microflora. Thus, supportive therapy constitutes the primary line of treatment, with the antimicrobial treatment given only if required. Treatment seems to be more focused at controlling clinical signs rather than at the clearance of the culprit bacteria. In young animals with moderate infections, or in patients with high fever, bloody or severe diarrhea, and patients who experience chronic infections, antimicrobials should be prescribed early. The best first-line drug options are macrolides (e.g., erythromycin). Other medications are often reserved for cases with erythromycin resistance or for refractory conditions. These include tetracyclines, fluoroquinolones, and chloramphenicol (Marks et al. 2011; Cho et al.-2014; Rodrigues et al. 2015; Weese et al. 2015).

In human infections, management of Campylobacteriosis primarily involves the administration of fluid and electrolyte therapy. Antibiotics become necessary when symptoms persist. Effective antibiotic therapy should be initiated within three days of illness. Antibiotic drugs of choice for Campylobacteriosis treatment in humans include fluoroquinolones, aminoglycosides, tetracyclines, macrolides, beta-lactams, and erythromycin. However, erythromycin should be avoided in cases of extra-intestinal infections caused mainly by *C. fetus* because of the prevalence of resistance to this drug. Ciprofloxacin, vancomycin, and quinolones are other good alternatives (Gilbert et al. 2007; Bardon et al. 2009; Guarino et al. 2014; Bolton 2015; Bruzzese et al. 2018).

Pasteurellosis

Overview

Pasteurellosis is a bacterial disease caused by organisms of the genus Pasteurella. It afflicts animals and is often transmitted to humans through bites or scratch injuries. Over six Pasteurella species as well as multiple serotypes exist. *P. multocida* and *P. canis* are typical dog isolates. *P. multocida* is the primary cause of human illness, with *P. multocida subsp. multocida, P. canis, P. multocida subsp. septica, P. stomatis,* and *P. dagmatis* subtypes cause infection in humans (Oehler et al. 2009).

Etiology

Pasteurella multocida belongs to the family Pasteurellaceae. It is a gram-negative, 0.3–1.2 μm long, non-motile, facultatively anaerobic coccobacillus, often with bipolar staining. It is usually found in the oral cavity and intestinal tracts of a number of animal species. This organism is an opportunistic pathogen that causes infection both in livestock and humans. Studies have shown *P. multocida* to be a part of natural mammalian mouth microbiota with colonization rates ranging from 50 to 66%. Among dogs and humans, infection is commonly associated with intimate contact with a dog through

sniffing, licking, or bed-sharing Lefebvre et al. 2006; Guet-Revillet et al. 2013; Christenson et al. 2015).

Clinico-pathological features

Pasteurella infections, which can be primary or secondary in nature, are usually more progressive than most other bacterial infections. An infected dog may show a bite or scratch wound. Within 8–48 hours, soft tissue cellulitis, erythema, tenderness, swelling, focal abscesses with serosanguineous to purulent, malodorous, dark yellow discharges may develop around the site of the wound. More serious sequalae in the form of soft tissue infection, septicemia, tenosynovitis, septic arthritis, osteomyelitis, and meningitis may occur as a result of bacterial dissemination. Low-grade fever, typically associated with lymphadenopathy, may also be observed. The proliferation of Pasteurella species after viral infections induce an influx of inflammatory cells and cytokine mediators, resulting in upper and lower airways infection, including the typical Pasteurella fibrinopurulent pneumonia (Wiebe 2015).

The spectrum of human disease resulting from infection with *P. multocida* ranges from a mild, superficial affliction, with swelling, inflammation, and intense pain at the bite site a few hours after the exposure to more serious infections, both invasive and localized. The more serious infections may involve the oral cavity, respiratory tract, and/or soft tissue, leading to pharyngitis, sinusitis, meningitis, tracheobronchitis, pneumonia, empyema, and/or abscess formation (Bauerfeind and Krauss 2016).

Diagnosis

Since *P. multocida* is not a normal human inhabitant, a diagnosis of a *P. multocida* induced illness is comparatively uncomplicated. A sample taken from an infected site and examined under microscope reveals the presence of small gram-negative rods, often bipolar in shape. *P. multocida* grows best on media enriched with serum, blood, brain or heart infusion. Antigens are not commercially available as part of routine laboratory study kits, so antibodies cannot be detected in serum samples. Other diagnostic techniques, including PCR, capsule typing, and 16S rRNA gene sequencing, have also been developed for the detection of *Pasteurella multocida* infection in humans (Bauerfeind and Krauss 2016).

Therapeutics

Penicillins are the antibiotic of choice for the treatment of dogs with Pasteurella infections. Localized infections are typically treated with an oral single-agent beta-lactam. For deep or severe disseminated infections, therapy may involve parenteral administration of penicillin, cefoxitin, and a carbapenem for 10-14 days. For the management of tenosynovitis, septic arthritis, osteomyelitis, or meningitis, however, 4–6 weeks of intravenous therapy is typically required.

In humans, a lack of culture findings would suggest the use of antibiotics with broad-spectrum coverage against Staphylococci, Streptococci, and anaerobes, since infections with bites are mostly polymicrobial. Dog bite treatment typically requires penicillin as the antibiotic of choice, but most cases are more commonly treated with amoxicillin-clavulanate. Other alternatives include the use of second and third-generation cephalosporins (e.g., cefuroxime, cefpodoxime). Patients allergic to penicillin are usually treated with doxycycline or fluoroquinolones (Oehler et al. 2009;Wiebe 2015; Bauerfeind and Krauss 2016; Bennett et al. 2019).

Bordetella infection (kennel cough)

Overview

Bordetella infection, primarily caused by *Bordetella bronchiseptica*, is an opportunistic bacterial pathogen. Bordetella species, frequently involved in respiratory diseases in both animals and humans, are highly infectious and spread primarily by aerosolization among animals living under stressful conditions. In dogs, it is common in puppies with Canine Infectious Respiratory Disease Complex (CIRDC, “kennel cough”), causing rhinitis, tracheobronchitis, and pneumonia. It may affect humans who are immunosuppressed, leading to pneumonia and infections of the upper respiratory tract (Bhardwaj et al. 2013; Echeverri-Toro et al. 2015; Rampelotto et al. 2016).

Etiology

Bordetella is a motile, aerobic, gram-negative coccobacillus capable, colonizing the mucous membranes of the upper respiratory tract, causing specific damage to the respiratory epithelia of a large number of potential mammalian hosts. Such damage paves the way for super-infections with resident commensal bacteria, including Streptococcus spp., Mycoplasma spp., and Pasteurella spp. Such super-infections invariably worsen the prognosis and render decisions regarding the best antimicrobial treatment more complex. The most common cause of illness among Brodetella spp is *B. pertussis*, the leading causative agent of whopping cough in humans. The whopping cough may also result from *B. parapertussis* infection. A similar disease in dogs is caused by *B. bronchiseptica*, which can attack animals of all breeds and of all ages alongside humans. The incubation period of the infection ranges from 2 to 10 days. Shedding of the bacteria may continue for up to two months post-infection. The most common routes of transmission are direct contact with oral or nasal secretions or the inhalation of sneeze droplets contaminated with the infectious agent. Bordetella spp. can survive for a few hours in respiratory secretions, for 45 days in the soil, and for up to 24 weeks in lake water. Fomites or water sources contaminated with *B. bronchiseptica* may also serve as sources of infection (Parkhill et al. 2003; Weese and Fulford 2011; Wiebe 2015; Bauerfeind and Krauss 2016; Weese and Evason 2019).

Clinico-pathological features

Infection with Bordetella is typically starts with the bacteria adhering to the cilia of respiratory tract epithelium with the help of several virulence factors. Once attached, ciliostasis, epithelial destruction with mucociliary clearance results in failure of the system, promoting further colonization and persistence of bacteria. After colonization, toxins released from *B. bronchiseptica* are responsible for local and systemic inflammatory damage. Following the incubation period, infected dogs start coughing. Initially, the cough is dry and paroxysmal, eventually becoming productive with serous to mucopurulent nasal discharge, conjunctivitis, and fever. Focal areas of epithelial degeneration, necrosis, and congestion in the lamina propria, infiltrated with macrophages and lymphocytes, may be seen on microscopic examination. In addition, mucopurulent exudates often accumulate in the lumen of the airways, and "carpet-like" bacterial clusters between the cilia of tracheobronchial epithelium can be seen. Infection with fever, lethargy, and productive cough can progress to bronchopneumonia and contribute to tracheal collapse. In some cases, the alveoli become filled with neutrophils and macrophages, while fibrin and erythrocytes are predominate in the alveolar spaces in other cases.

In humans, although *B. bronchiseptica* infections remain clinically rare, a pertussis-like disease caused by this species of Bordetella has been reported among immunocompromised individuals. Upper and lower respiratory tract infections may develop, with affliction ranging from an asymptomatic carriage or the development of mild sinusitis to severe infections such as bronchitis or necrotizing pneumonia; although occurrence of such infections is rare (Woolfrey and Moody 1991; Mattoo and Cherry 2005; Oskouizadeh et al. 2011; Wernli et al. 2011;Taha-Abdelaziz et al. 2016).

Diagnosis

In cases of suspected *B. bronchiseptica* infections, a variety of samples may be used for bacteriological culturing or conducting a modern PCR analysis. These include pharyngeal swabs, bronchoalveolar wash fluids, or transtracheal washings. Specimens obtained from the lower respiratory tract are more dependable relative to nasal or oropharyngeal swabs, as *B. bronchiseptica* has been frequently isolated from upper respiratory tracts of clinically healthy dogs. Moreover, prior vaccination with one of the newer bacterial intranasal vaccines and low bacterial numbers could yield both false PCR negative and positive results. Consequently, differentiating between the presence of *B. bronchiseptica* as part of the normal microbiota or as a cause of disease, interpretating test results, and deciding whether or not to administer treatment can be quite challenging (Chalker et al. 2003; Schulz et al. 2014; Viitanen et al. 2015).

Therapeutics

In both dogs and other animal species with uncomplicated conditions, the antimicrobial treatment seems unnecessary. Complete and quick recovery should not always be anticipated, as viral co-infections are common. Antimicrobials can shorten the course of the disease. Where necessary, antibiotic treatment should be based on culture and sensitivity, as some strains have shown resistance to both penicillin and sulfa drugs. The most frequently used antibiotics for the treatment of *B. bronchiseptica* infections include amoxicillin/clavulanic acid and cephalexin. Aminoglycosides appear to be particularly useful against *B. bronchiseptica*. The administration of aerosolized gentamicin, intravenous fluids, good nutrition, and extra oxygen can help treatment of severe infections when animals fail to respond to parenteral medication (Ford 2006; Rath et al. 2008; Vieson et al. 2012).

Leptospirosis

Overview

Leptospirosis is one of most widespread zoonotic water-borne bacterial diseases. It is caused by bacteria of the genus Leptospira. To date, more than 260 different Leptospira serovars have been discovered. They often cause hepatorenal disease with occasional affliction of other body systems. Nearly all mammals and marsupials can become renal carriers and cause human infection. Of all animals typically kept as pets, leptospirosis is quite frequent among dogs, especially those who spend time in forests or swampy areas. Dogs are natural carrier hosts. They are susceptible to infection with the *L. canicola* serovar as well as various other serovars and shed these pathogens in urine for a period of a few weeks. Humans become accidental hosts via direct contact with infected animals, by being licked by an infected dog, by petting it, or through contaminated soil or water. The risk of infection rises among veterinarians, farmers, sewerage and slaughterhouse workers (Bharti et al. 2003; Leal-Castellanos et al. 2003; Ko et al. 2009; Adler and de la Peña Moctezuma 2010; Nelson and Couto 2014).

Etiology

Leptospira spp., are a complex group of highly mobile, obligate aerobic, long and thin bacteria. They are spiral-shaped with both ends bent in a hook-like fashion and measure about 6 to 20 × 0.1 µm. Leptospira spp. are gram-negative bacteria with particular affinity for the urogenital tract, especially the kidneys. Disease outbreaks occur within 3 months of rainy weather. Excreted leptospira can survive for months in a humid environment but die very quickly under dry conditions. Dogs can become infected by drinking, swimming and wading in contaminated water sources or when exposed to livestock or rat urine. The Leptospira can be transmitted through mucous membranes, cuts or abrasions, or by the inhalation of aerosolized infected water. The leptospiremic phase lasts approximately for 7 days, after which rapid multiplication, coagulopathy, and vasculitis within organs can result in kidney and/or liver disorders or lead to leptospiral

pulmonary hemorrhage syndrome (Kohn et al. 2010; Sykes et al. 2011; Weese and Fulford 2011; Weese and Evason 2019).

Clinico-pathological features

After seven days of incubation, leptospirosis manifestations in dogs usually differ based on the virulence and serovar type of the organism, prior health status of the host and the target organs infected. Leptospirosis can vary from subclinical form with mild, intermittent, unrecognized fever to acute hemolytic anemia, septicemia, and hepatorenal failure with uncontrollable vomiting and bloody diarrhea. Hemolytic anemia leads to icterus and a swollen yellowish liver. Microscopic findings typically include hepatic portal lymphocytic infiltration, splenic hemosiderosis and, occasionally, centrilobular hepatic cell necrosis (as a consequence of anemic anoxia). Subacute illness may either involve fever and jaundice or chronically mild and persistent nephritis. Microscopic examination of the kidneys reveals swelling of tubular epithelia caused by bile pigment and hemoglobin. Kidney-related symptoms such as polyuria/polydipsia and/or oliguria/anuria, vomiting, anorexia, and lethargy may extend from one month to one year after infection. The causative bacteria may remain in the kidney tubules causing chronic disease, evident microscopically in the renal parenchyma (most commonly in the cortex) in the form of greyish-white focal lesions with swollen, granular and vacuolated epithelium.
In humans, most patients are diagnosed on the basis of subclinical flu-like febrile disease after an incubation period of 7–12 days. In its initial febrile phase, which usually lasts for 4–7 days, fever, headache, myalgia, conjunctivitis, nausea, and vomiting are commonly noted. Jaundice and renal failure can start by the end of the first week in severe cases. Significant icterus with elevated bilirubin, usually accompanied by severe glomerulonephritis or interstitial nephritis, is typically detected by the third week (Levett 2001; Ellis, 2015; De Brito et al. 2018; Esteves et al. 2018).

Diagnosis

Canine leptospirosis can be diagnosed using various methods, including urine examination with darkfield microscopy, blood and urine microbiological analysis, renal tissue histopathology, serology, and PCR of the blood, urine, and tissue. Currently, microagglutination tests (MAT) are the gold standard for serologic diagnosis. A diagnosis of leptospirosis entails that, over three weeks, the titer to at least one serovar increases four-times in early phase or decreases four-times in late phase. Modern molecular techniques such as PCR and immunoblotting can, in contrast to culture, reduce diagnostic time because culture requires special media, specific conditions, and a period of up to 16 weeks to reach a diagnosis. Traditional PCR-testing remains less sensitive than serological testing, often producing numerous false-negative results. Recently, improved techniques, such as multi-locus sequence typing (MLST) that work by analyzing the bacterial genome or its specific regions for the typing of Leptospira strains, have been developed (Miller et al. 2011; Koizumi et al. 2015; Schuller et al. 2015; Miotto et al. 2018; Troìa et al. 2018).
For diagnostic purposes, human leptospirosis requires detailed and comprehensive case history, including a review of occupational and outdoor activities, animal contacts, and the occurrence of sudden or recurrent fever. During the first ten days of illness, different visualization methods, such as darkfield microscopy, light microscopy on the silver stain, and immunostaining preparations, can be used to visualize Leptospira in blood or urine smears. However, the sensitivity of visualization methods is limited, and several samples must be taken due to the intermittent nature of leptospiral shedding in the urine. Microbiological and serological techniques are typically used to confirm the diagnosis. The Microagglutination Test (MAT) and an Indirect Hemagglutination Test (IHA) are both designed for the diagnosis of human illness, as well as to detect recent dog infections. In contrast with MAT and ELISA, the IHA is specific and sensitive and is easy to perform (Vijayachari and Sehgal 2006; American Academy of Pediatrics and Pickering 2009).

Therapeutics

Canine Leptospirosis antimicrobial therapy is mandatory, irrespective of the severity of the disease, because of the associated public health risk. Shedding of viable Leptospira should end within 24-48 hours after the commencement of treatment. Leptospira spp. are highly susceptible to both penicillin G and doxycycline, which are essential to the mitigation of leptospiremia and the prevention of further organ damage. To these ends, they are most efficacious when given in early phase of the disease in both dogs and humans. Doxycycline is the drug of choice for the treatment of renal infection in animals with serious renal impairment but minimal hepatic dysfunction. Parenteral penicillin G or ampicillin can be used as alternatives, but both are less effective in removing organisms from the kidney.
In severe Leptospirosis patients, intravenous penicillin G, cephalosporin of third-generation, or erythromycin can be prescribed in severe cases. More recently, azithromycin, cefotaxime, ceftriaxone, and fluoroquinolones have been shown to have comparable potency to doxycycline against multiple serovars *in vitro*. Intense care unit admission is often vital for patients with icteric Leptospirosis because multiple organ involvement and decompensation can rapidly occur. Corticosteroids may help, but their use iscontroversial for the treatment of patients with renal impairment (Greene 2012; Jiménez et al. 2018; Wang et al. 2020).

Brucellosis

Overview

Brucellosis is an infectious disease of humans and animals caused by bacteria of the genus Brucella. In humans,

Brucellosis is mostly a foodborne disease, caused by *Brucella melitensis* through consuming unpasteurized milk or its products. In dogs, Brucellosis is most frequently a reproductive disease caused by *B. canis*, a zoonotic pathogen that is the major cause of diskospondylitis. *Brucella canis,* however, is the least frequent cause of human Brucellosis. Dogs can be infected by several Brucella species, including *B. abortus* and *B. melitensis*, and contribute to the spreading of these organisms in farms, potentially becoming a source of human infection (Weese and Fulford 2011; Bennett et al. 2019).

Etiology

B. canis is a small (1.0 to 1.5 µm), gram-negative, coccoid to short rod-shaped, non-motile, obligately parasite, with a moderate ability to survive outside the host. Differences in biochemical and antigenic reactions distinguish *B. canis* from other members of the genus Brucella. In domestic and wild canids, *B. canis* infects a susceptible canine host by penetrating the mucous membranes, especially those of the oral cavity, vagina, and conjunctiva. *B. canis* usually harbors in the lymph nodes of gastrointestinal tracts for extended periods and then transmitted primarily by ingestion or inhalation of aerosolized post-abortion material. Venereal transmission of Brucellosis has also been reported (Lucero et al. 2010; Atluri et al. 2011; Von Bargen et al. 2012; Galinska and Zagorski 2013).

Clinico-pathological features

Acute lymphadenitis is caused by intracellular proliferation of organisms within histiocytes and macrophages residing in the regional lymph nodes. Passage of the organisms into the blood stream leads to bacteremia. Organisms may then localize in male or female reproductive organs, the placenta, fetus, or udder. They may also localize in distant lymph nodes, the spleen, liver, joints, or bone, resulting in varied signs. In dogs, *B. canis* infection is characterized by prolonged bacteremia, reproductive failure, and infertility as a result of abnormal spermatogenesis in males and abnormal oogenesis in females. In pregnant bitches, *B. canis* colonizes in placental epithelial cells, inducing placentitis and abortion at late-term (weeks 7–9). Macroscopically, necrosis of the cotyledons appears dull and granular. The intercotyledonary chorion becomes edematous and full of brownish exudate. Many organisms in the chorionic epithelial cells are seen microscopically. Puppies infected *in utero* do not usually survive to weaning. In male dogs, *B. canis* causes orchitis, epididymitis and prostatitis. Complications such as lymphadenopathy, splenomegaly, discospondylitis, uveitis, meningitis, glomerular nephritis, and dermatitis can also occur periodically.

In humans, symptoms and signs of *B. canis* infection are, for the most part, unspecific and may vary from an undulant or persistent fever, splenomegaly, malaise, myalgia, headaches, and anorexia, to symptoms of more serious conditions such as endocarditis, osteomyelitis and septicemia (Wanke 2004; Lucero et al. 2005a, Hollett 2006; Olivera and Di-Lorenzo 2009; Lucero et al. 2010).

Diagnosis

Isolation of *B. canis* through culturing is the gold standard diagnostic test. Appropriate sites for sample-taking include the placenta, lymph nodes, prostate and spleen. Direct Köster stain microscopy guarantees rapid orientation, and blood cultures are typically effective within the first eight weeks after infection. The sensitivity of blood cultures is reduced with time, as bacteria are most frequently isolated following acute, not chronic, infections. Compared with blood culture, the diagnostic sensitivity of whole blood PCR is far superior. It stands at 100% in naturally infected dogs and is often used to confirm the species of the Brucella isolates. *B. canis*-specific serological testing may also be used, and a definitive diagnosis can be made from a positive result in combination with consistent clinical signs. *B. canis* total antibody screening is performed using a rapid slide agglutination test with 2-mercaptoethanol. A confirmatory indirect ELISA test should then be conducted in order to detect antigen-specific IgG or IgM antibodies (Lucero et al. 2005b; Sánchez-Jiménez et al. 2014).

Therapeutics

For Brucellosis, the general recommendation is to avoid medication and to euthanize truly infected animals because of the threat to both canine and human populations. Traditionally, tetracyclines were most commonly used in the treatment of infections. Monotherapy, however, has been associated with high relapse rates. Combination therapies that include rifampin or streptomycin appear to have the best efficacy. Neutering, along with enrofloxacin therapy for a minimum one-month period are needed because of the intracellular nature of causative organisms and the inability of some pharmaceutical products to penetrate certain areas of the canine body.

For humans, typical antibiotic therapy comprises oral doxycycline and rifampin for a period of 6 to 8 weeks, to which a one-to-two-week course of aminoglycosides is usually added. In children below eight years of age and pregnant mothers, where tetracyclines are contraindicated, a trimethoprim-sulfamethoxazole mixture and rifampin or an aminoglycoside should be prescribed (Hollett 2006; Wanke et al. 2006; Sykes 2013; Wiebe 2015).

Lyme borreliosis

Overview

Lyme disease (borreliosis) is a multisystemic, tick-vectored, zoonotic disease caused by the bacterium *Borrelia burgdorferi,* which is transmitted by Ixodes spp. It may be manifested with any of a large number of

dermatological, rheumatological, neurological and cardiac symptoms in both humans and dogs. Dogs have been identified as a competent reservoir of *B. burgdorferi* among pet animals, serving as carriers of infected ticks from wildlife hosts to human settings. Dogs have also been proposed as a 'sentinel animal' giving advanced warning of possible human exposure to infected ticks and helping detect emerging risk areas of Lyme disease, as dogs and humans mostly live in and share the same environment and visit the same outdoor areas (Duncan et al. 2004; Mead et al. 2011; Otranto et al. 2015; Steere et al. 2016; DeLong et al. 2019).

Etiology

The *Borrelia burgdorferi sensu lato* complex is a representing term for all genospecies of *B. burgdorferi* that cause Lyme disease. Borreliae are small, thin, elongated spiral-shaped, commonly spirochetal-structured bacteria, 0.2 µm to 30 µm in size. They cannot survive as free-living organisms in the environment and are transmitted between vertebrate reservoir hosts and tick vectors. The main reservoir hosts are small mammals. Dead-end hosts are sheep, dogs, and humans. Naive ticks that feed on infected dogs invariably become infected. These ticks would then infect other vertebrates, including humans. By introducing infected ticks into the human population, dogs raise the level of dog owners' exposure to *B. burgdorferi* (Fritz and Kjemtrup 2003; Greene 2012; Parry 2016).

Clinico-pathological features

Clinical Lyme disease is a less frequent consequence of an infection in dogs than in humans; over 95% of naturally infected dogs are asymptomatic. Others may show fever, lethargy, decreased appetite, lymphadenopathy, lameness, and/or a form of renal disorder. Shifting limb lameness is a common manifestation of Lyme disease in dogs. It results from joint swelling coupled with fibrin and neutrophils effusion, occurring in 9–28% of the seropositive dogs. In most cases, lameness starts in the limb nearest the tick bite spot, improves and resolves within two days then possibly remanifesting in the other limb. Skin biopsy samples typically show superficial perivascular lymphoplasmacytic infiltrates with mast cell accumulations. Lameness can progress to non-suppurative, predominantly lymphoplasmacytic inflammation, leading to chronic non-erosive polyarthritis if the infection remains undiagnosed and untreated. Lyme nephritis is an uncommon but extreme type of severe protein-losing nephropathy with renal failure. It is typically seen in less than 2% of seropositive dogs. Renal lesions typically include glomerulitis, diffuse tubular necrosis with regeneration, and interstitial inflammation. Dogs with Lyme nephritis can become dehydrated, suffer from edema, pleural effusion, ascites, elevated blood pressure, neurological, cardiac, and retinal problems (Krupka and Straubinger 2010; Greene 2012; Leschnik 2014).

Lyme disease may also cause serious complications in humans. A typical human infection starts with an expanding skin lesion termed erythema migrans. This so-called erythema migrans is a red spread out rash that may or may not show central clearing. It is often accompanied by signs of weakness, fever, headache, mild stiff neck, arthralgia, or myalgia, which, if untreated, can be followed by early disseminated infection, with the development of neurologic or cardiac abnormalities. Moreover, an untreated infection may progress to cause arthritis, peripheral neuropathy, or encephalopathy. These long-term sequelae can take place over variable periods, ranging from one week to a few years (Steere and Angelis 2006; Steere and Sikand 2003; Wormser 2006; Ray et al. 2013; Steere et al. 2016).

Diagnosis

In dogs, diagnosis of the Lyme disease is based on clinical symptoms, history of exposure, and positive antibody response. Recently developed methods of diagnosis, including the C6 peptide test for Lyme, as well as serology and PCR testing, could be performed. A complete blood picture may demonstrate mild to moderate thrombocytopenia and leukocytosis. Azotemia, moderate to pronounced hypoalbuminemia, metabolic acidosis, and electrolyte changes can be present in dogs with renal disease. Urinalysis may show isosthenuria, proteinuria, pyuria, and hematuria. In such cases, a renal biopsy would identify the affliction as an immune-complex disorder but would not confirm a case of Lyme nephritis (Wiebe 2015; Parry 2016).

Strategies for the diagnosis of Lyme disease in humans vary. With the exception of cases with pathognomonic erythema migrans, the diagnosis of Lyme Borreliosis usually requires confirmation by means of a microbiological, serological, or molecular diagnostic assay. *B. burgdorferi* can be cultured from erythema migrans skin lesions, but such testing is not consistently available. In early disseminated Lyme Borreliosis patients, *B. burgdorferi* are typically cultured from blood samples. In patients with early disseminated disease, clinical severity predicts the likelihood of hematogenous disease. Serological diagnosis can be difficult, especially in early cases, because IgM is typically not detectable within the first 1-2 weeks following infection and IgG does not often appear for 4-6 weeks. In addition, certain patients with solitary erythema migrans may never show seroconversion.

Serological testing is further limited by the fact that antibodies could last in the blood stream for many years post-infection and thus cannot be used to determine the effectiveness of antibiotic treatment. In recent years, commercial serological tests for the diagnosis of Borrelia infections have been developed. Among these is the ELISA-based TickPlex assay, which incorporates a new antigen from the round body/persister forms of Borrelia. This assay was shown to be useful at different Lyme Borreliosis stages, and the improved test can concurrently assess IgM and IgG antibodies of various bacterial and

viral tick-transmitted pathogens. *B. burgdorferi* DNA has been successfully detected by PCR in synovial fluid and less definitively in cerebro-spinal fluid (CSF). Due to lack of standardization, Lyme Borreliosis PCR testing is not considered part of routine clinical practice. There is a continuous interest in novel approaches to diagnostics, including Borrelial antigen identification, nucleic acid amplification, and genomic sequencing (Wormser et al., 2005; Li et al. 2011; Steere et al. 2016; Wormser et al. 2017; Branda et al. 2018; Trevisan et al. 2020).

Therapeutics

Management of canine cases of Lyme disease is multifaceted. Dogs require routine medication for cases of protein-losing nephropathy. Treatment of dogs also necessitates long-term administration of antimicrobials, such as cephalosporins, amoxicillin, doxycycline, or azithromycin. Immunosuppression therapy may also be required. If animals do not tolerate doxycycline, oral cefuroxime axetil is an effective alternative in early Lyme disease cases (Fritz and Kjemtrup 2003; Krupka and Straubinger 2010; Wiebe 2015).

The aim of antibiotic therapy for early human Lyme disease, whether local or disseminated, is to shorten the duration of erythema migrans and symptoms associated with them and to prevent the development of the late-stage disease. Efficient antibiotic treatment with oral doxycycline and amoxicillin is effective in most cases. Cefuroxime axetil is an option for those patients who cannot be treated with doxycycline or amoxicillin. Azithromycin, clarithromycin, and erythromycin can also be used, but may be less effective (Sanchez et al. 2016; Steere et al. 2016).

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FEED-BORNE *BACILLUS CEREUS*: AN EMERGING THREAT TO FOOD CHAIN RELATED HAZARD, SAFETY AND PATHOGENIC POTENTIALITY

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INTRODUCTION

Bacillus cereus is a Gram-positive, rod-shaped, motile (flagellated), aerobic or facultative anaerobic, spore and biofilm forming bacterium, commonly found in nature. It belongs to a group of genetically similar forms assigned to the genus *Bacillus*, consisting of several closely related species. This opportunistic pathogen is often isolated from food and gastrointestinal disorders, as well as from non-gastrointestinal infections. Furthermore, it leads to vomiting and diarrheal syndromes in both animals and humans, which are linked to quickly fatal systemic and local infections, notably in neonates and immunosuppressed hospitalized patients. The ability of these pathogens to sporulate, and production of lipases and thermostable proteases allows them to withstand the common cleaning procedures in the food industry, resulting in finished product defects and food poisoning outbreaks.

B. cereus has also been used as a probiotic in human medicine and livestock production, but due to low standards of safety evaluation, toxins production, transfer of toxins and antibiotic resistance gene (ARG) in humans via the food chain, it is potentially posing a new threat to food safety. Consequently, feed-borne *B. cereus* contamination worsens extreme diarrhea and malnutrition in poultry by causing gizzard erosion and ulceration (GEU) syndrome, as well as hemorrhagic inflammation in lungs and immunosuppression, when co-infected with other pathogens. Considering the pathogenic potential of the entire *B. cereus* group, it is critical to gain insight into their genomes through whole-genome sequencing and gene analysis. This chapter includes an overview of the historical data on possible risk factors and pathogenesis of feed-borne *B. cereus* from animal feed to the human food chain, along with their implications for the food industry, focusing on food safety risks, classical and molecular analysis, advanced diagnostic methods, and their diversity, sensitivity, and ability to discover toxic and nontoxic bacteria.

Background and Taxonomy

The word "bacillus" means a "small rod," while the Latin word "cereus" refers to "wax-like", mostly used interchangeably with any "aerobic endospore-forming bacterium" (AEFB), which was first isolated from air in a cowshed in 1887 by Frankland and Frankland and discovered in 1906 by Plazikowski in connection with food poisoning in Europe (Fritze and Pukall 2011; Haque et al. 2021). *B. cereus* was first linked to food poisoning in the 1950s, when outbreaks of vanilla sauce poisoning were reported in Norway (Eglezos and Dykes 2014). *B. cereus* spores can last for years, even surviving during cooking due to their resistance to extreme temperatures, their growth is optimal in the presence of oxygen, but it can also thrive in anaerobic conditions, or at very low or high temperatures (Lutpiatina 2020).

There are currently 376 species in the *Bacillus* genus, with the *B. subtilis* and *B. cereus* group being two of the most common, however, several gene structures and regulatory mechanisms vary between these two groups of bacteria (Yin et al. 2020). *B. amyloliquefaciens*, *B. atrophaeus*, *B. licheniformis*, *B. mojavensis*, *B. paralicheniformis*, *B. pumilus*, *B. subtilis*, *B. tequilensis*, *B. vallismortis*, and *B. velezensis* are all members of *B. subtilis* group, while *B. cereus* group includes *B. anthracis*, *B. cereus sensu stricto (s.s.)* (usually referred to as *B. cereus*), *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis* (Lindbäck and Granum 2019; Yin et al. 2020; Haque et al. 2021). Currently, *B. cereus* group has been divided into: i) genomospecies such as, *B. pseudomycoides*, *B. paramycoides*, *B. mosaicus*, *B. cereus s.s.*, *B. toyonensis*, *B. mycoides*, *B. cytotoxicus*, and *B. luti;* ii) putative genomospecies such as, *B. bingmayongensis*, *B. gaemokensis*, *B. manliponensis*, and *B. clarus;* iii) subspecies such as, *B. mosaicus* subsp. *anthracis*, *B. mosaicus* subsp. *Cereus;* iv) Biovars such as Biovar *Anthracis*, Biovar *Emeticus* and Biovar *Thuringiensis* (Carroll et al. 2020). Even though the *B. cereus* group is phylogenetically heterogeneous in general, single strains with highly similar 16S and 23S rRNA sequences, especially *B. cereus* or *B. cereus s.s.*, *B. thuringiensis*, *B. anthracis* and *B. toyonensis* isolates, can be considered as single species due to transfer of virulence factors through plasmids;these are also subsumed under '*B. cereus sensu lato*' (Ehling-Schulz and Messelhäusser 2012; Griffiths and Schraft 2017; Lindbäck and Granum 2019). *B. thuringiensis* produces δ-enterotoxin (BT toxin), which appears as a crystalline parasporal inclusion body and is insecticidal, making it a biopesticide; *B. anthracis* is the causative agent of anthrax in human and animals; spores of this organism may be used in bioterrorism; *B. toyonensis* is the current species designation for *B. cereus*

var. *toyoi* strain that has been used as a fattening farm animals feed additive under the brand name of Toyocerin (Pontieri 2016; Griffiths and Schraft 2017).

B. cereus s.s. has been increasingly recognized as an evolving foodborne pathogen, with enterotoxins capable of causing emetic or diarrheic gastroenteritis in recent years (Pontieri 2016). It may also lead to local skin and wound infections, ocular infections (panophthalmitis, endophthalmitis, and keratitis), fulminant liver failure, and pervasive disease in cancer patients, such as endocarditis, osteomyelitis, pneumonia, brain abscess, meningitis myelodysplasia and extreme bacteremia.

Characteristics of the organism, growth and reservoirs

B. cereus are ubiquitous bacteria that can be found in decaying organic matter, air, dust, fresh and marine water, rhizosphere, animal and plant materials, vegetables, fomites, invertebrates' guts, beddings, feed and feedstuffs, pasture, with their adhesive spores can tolerate adverse conditions, like average cooking temperature, heat, dehydration, radiation and other physical stresses (Kumari and Sarkar 2016; Ramarao et al. 2020). Bacillus cells range in size from 0.5 × 1.2 to 2.5 × 10 µm and contain oval or cylinder-shaped spores that do not disclose the sporangia clustered in individual or short chains and located centrally, subterminally, or terminally (Fig. 1). The hydrophobic structure of the spores and presence of protrusions (1-30 in number of 0.45-3.8 µm x 13.6 nm) on the exterior result in strong adhesion to food processing surfaces, such as stainless steel. *Bacillus* species bacteria quickly sporulate in most media after 1 to 3 days (Kumari and Sarkar 2016; Grutsch et al. 2018; Lindbäck and Grnum 2019). They grow best at temperatures between 28-40°C, while they can multiply at a temperature between 4-50°C. Thermophilic varieties, on the other hand, grow best at 65°C. A water activity (a_w) ≥ 0.91, a pH of 4.0-9.3 (optimal 7.0) and a NaCl concentration <10% are also required for their development. Under ideal conditions, growing time is between 12 and 27 minutes (Eglezos and Dykes 2014). *Bacillus* spp. colony morphology varies by species, but they all grow on common agar media, like nutrient agar (NA) or plate count agar (PCA), producing large colonies (3-8 mm in diameter) with a flat, greyish and 'ground-glass' appearance, sometimes with irregular borders. They metabolize organic substrates, like amino acids, organic acids, and sugars through aerobic/anaerobic respiration, or fermentation, depending on species and environment (Kumari and Sarkar 2016; Grutsch et al. 2018; Ramarao et al. 2020).

Mode of transmission and contamination

B. cereus is a common soil saprophyte that can be found in a variety of environmental habitats, as well as man-made settings, such as food manufacturing plants, food handling and processing facilities, transportation vehicles and hospital environments, where they may constitute reservoirs for the disease. Such habitats can provide a favorable environment for *Bacillus* spp. production, or may still harbor spores, which can quickly be transmitted to different raw foods, such as grain and cereals products, dried herbs, spices, eggs, milk and dairy products, fruit, vegetables, meat products, sauces, puddings, sprouts, rice and other carbohydrate-rich foods, as well as commercial RTE meals and products, which may become contaminated, resulting in transient colonization of the animal and human intestine (Fritze and Pukall 2011; Eglezos and Dykes 2014). *B. cereus* spores can become contaminant in the dairy sector when they come in contact with cows' udders during pasture or by feed or bedding material, then move into the raw milk. The spores may also withstand pasteurization, dehydration, γ radiation, and other physical stresses (Grutsch et al. 2018; Lindbäck and Grnum 2019). The ability of *B. cereus* to bind firmly to surfaces and form biofilms, which shield their cells and spores against the antimicrobial action of sanitizers, accounting for their survival in food processing environments (Grutsch et al. 2018). Intake of food or air or a wound in the body contaminates spores or vegetative cells (Ramarao et al. 2020).

Virulence factors and Pathogenicity

B. cereus is most commonly associated with food poisoning and other serious systemic and local infections, owing to the synergistic effects of a range of virulence factors that foster intestinal cell disruption and/or immune system tolerance in the host. Table 1 summarizes the substances formed by *B. cereus* during bacterial growth, primarily enterotoxins, hemolysins, phospholipases and emetic toxin.

Incidence of illness and outbreak data

The exact incidence of the *B. cereus* food-borne poisoning is mysterious for many reasons: a) it is widely underestimated because the symptoms of the disease are intermittent (<24h), slight, and self-limiting, so people do not seek treatment; b) most of the community is partially covered by resistance gained through chronic exposure; c) large numbers are needed to induce infection; d) symptoms are usually misdiagnosed with clostridial or *Staphylococcus aureus* intoxications (Griffiths and Schraft 2017). *B. cereus* appears to be responsible for 1.4-12% global food-borne illness outbreaks (Grutsch et al. 2018). Tables 2 and 3 summarize the outbreak and occurrence data for *B. cereus* poisoning over the last two decades.

Pathogenesis of the diseases

B. cereus and other members of the *B. cereus* group induce two forms of food poisoning: emetic syndrome and diarrheal illness. The emetic (vomiting) syndrome, which is similar to *Staphylococcus aureus* poisoning, is exacerbated by a ready toxin found in cooked rice and other cereal-based foods that resist high temperatures, trypsin, pepsin, and pH; whereas the diarrheal illness, which is similar to *Clostridium perfringens* poisoning,

Table 1: Virulence factors of *B. cereus*

Virulence factors	Properties	Encoded gene	Reference
Emetic toxins			Carlin and Nguyen-The (2013); Lindbäck and Granum (2015); Visiello et al. (2016); Ehling-Schulz et al. (2019); Haque et al. (2021)
Cereulide (Ces)	Thermo-stable, Cyclopeptide (1.2 kDa), hepatic and immune dysfunction, toxic in various mammalian cell lines, cerebral effects, bioaccumulation in vital organs and necrotic cell death	*ces*	
Enterotoxins			
Hemolysin BL (Hbl)	Thermo-labile, protein, 3 components (35, 36, & 45 kDa), pore formation, hemolysis, cytotoxicity, dermonecrotic and capillary permeability	*hblA, hblB, hblC, hblD*	
Nonhemolytic enterotoxin (Nhe)	Thermo-labile, protein, 3 components (39, 45, & 105 kDa), pore formation, intestinal fluid secretion, osmotic and Vero cell lysis, cell death	*nheA, nheB, nheC*	
Cytotoxin K (CytK)	Thermo-labile, single-cell protein (34 kDa), pore formation, hemolysis, cytotoxicity and necrosis	*cytK1, cytK2*	
Enterotoxin FM (entFM)/ CwpFM	Single-cell protein (45 kDa), cell wall peptidase, hemolysis, capillary permeability and cytotoxicity	*entFM/ cwpFM*	
Enterotoxin T (entT)	Single-cell protein (40/41 kDa), diarrheal toxigenicity, capillary permeability and cytotoxicity	*bceT*	
Hydrolytic enzymes			
Hemolysin I (HlyI)/ Cereolysin O	Thermo-labile, cholesterol-binding and thiol dependent hemolysin, pore formation	*Clo*	
Hemolysin II (HlyII)	Thermo-labile, cholesterol-independent, cytotoxicity, pore formation, apoptosis in macrophages (caspase-3,8 pathways)	*hlyII*	
Hemolysin III (HlyIII)	Hemolysis, transmembrane pore formation	*hly-III*	
Phospholipase C/ Cerolysin A	Degradation of neutrophils	*plC*	
Phosphatidylinositol specific phospholipases C (PI-PLC)	Destroying of protein harborage on plasma membranes	*piplC/ plcA*	
Phosphatidylcholine specific phospholipases C (PC-PLC)	A small, monomeric enzyme (28.5 kDa), general hydrolytic action, hemolysis, involved in substrate binding and necessary for enzymatic activity and protein formation	*pcplC/ plcB*	
Sphingomyelinase (SMase)/Cerolysin B	Hemolytic protein that binds to sphingomyelin on erythrocytes, hemolysis, decrease in phagocytosis, dodging macrophage in initial phases of infection	*sph*	
Cerolysin AB	2 components (PC-PLC+Smase) cytolysin, that function together to lyse human erythrocytes	*cerAB*	
Camelysin	A cell-bound metalloprotease, capability to cleave hemoglobin, albumin and casein in non-gastrointestinal infections	-	
Immune inhibitor A1 (InhA1)	A zinc metalloprotease, efficient escape from macrophages	*inhA*	
Bacillolysin	A metalloprotease	*nprA*	
Neutral metallopeptidases/ Neutral protease	Proteolytic activity	*Npr/ nprB*	
IlsA	Iron-regulated, leucine-rich surface protein, iron deprivation in the host	*ilsA*	
Collagenase	Degraded soluble and insoluble collagens, Azocoll, gelatin and bradykinin	*cola*	
Antibiotic resistance			
β-lactamase I	Class A β-lactamases and is an extracellular penicillinase with a serine in the active site	*bla1*	
β-lactamase II	Class B β-lactamase, is activated by binding Zn (II) and Co(II) ions	*bla2*	
β-lactamase III	Class A membrane-bound lipoprotein also having a secreted form	*Blm*	

is provoked by a complex enterotoxin throughout vegetative growth of *B. cereus* in the small intestine, mainly linked to proteinaceous foods (Eglezos and Dykes 2014; Haque et al. 2021). Table 4 shows the key characteristics of *B. cereus* poisoning found in food and feed. According to our earlier studies, feed-borne *B. cereus* caused GEU, as well as hemorrhagic inflammation in lungs of chicken. Co-infection with other pathogens, such as avian influenza virus (H9N2) and *Chlamydia psittaci* worsened acute diarrhea and led to the development of GEU and immunosuppression in birds. Importantly, Hbl and Cytk, enterotoxins of *B. cereus,* disrupt the koilin layer of the gizzard, causing long-term ulceration, necrosis, mucosal damage and diarrhea by damaging the digestive tract (Zhang et al. 2019; Zuo et al. 2020).

In a recent study, it was found that stomach ulceration caused by feed-borne *B. cereus* in conjunction with severe diarrhea, and co-infection with *Aspergillus fumigatus* alleviated gastric lesions and immunosuppression in weaned piglets (Li et al. 2020).

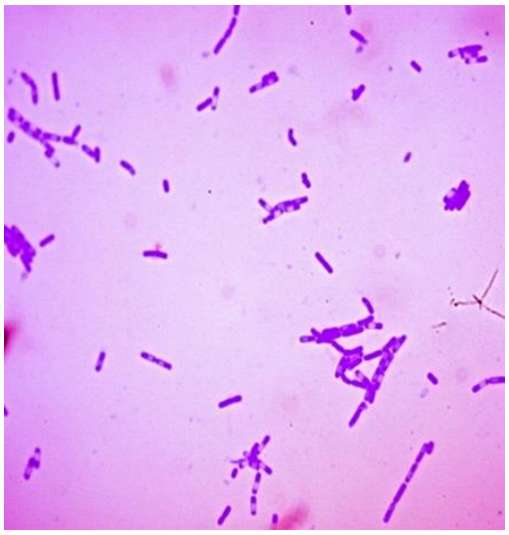

Fig. 1: *Bacillus cereus* on gram staining (Photo by Md Atiqul Haque). **Fig. 2:** *Bacillus cereus* on MYPA (Photo by Hongkun Quan).

Table 2: Food poisoning outbreaks due to *B. cereus* worldwide from 2000 to 2020

Year	Country /Region	Food	Affected persons/ consequences	Contamination level (cfu/g)	Attack rate (%)	Type of poisoning	Reference
2000	Italy	Cakes	173 people; N and D (watery), 23 patients hospitalized	10^2	n.a.	EPP	Osimani et al. (2018)
2003	Belgium	Pasta salad	Family outbreak; 5 children hospitalized; V, LF, 1 death	1.0×10^7 –1.0×10^8	n.a	EP, EPP	Dierick et al. (2005)
	Greece	No information	A 72-year-old woman, V, A, LF, death	n.a. n.a.	n.a	EP EP	Latsios et al. (2003)
1991-2005	Canada	Mainly Asian food, followed by raw food	39 outbreaks, V, A, N and D		32		McIntyre et al. (2008)
2005	Spain	Seafood cocktail and fried shrimp	100 people	3.8×10^4	95	EP, EPP EP, EPP	Osimani et al. (2018)
	Finland	Pasta and meat dish	2 persons; V and D	$1.3\text{-}1.8\times10^5$	n.a. n.a.		Pirhonen et al. (2005)
	Korea	Cooked and fried rice	37 persons; V, A and headache	n.a.	n.a.	EP	Kim et al. (2010)
2004-2006	Korea	Not specified	Sporadic food poisoning case	n.a.	n.a.	EP EP	Chon et al. (2012)
2006	Germany	Rice dish, cooked cauliflower	18 people (17 children, 1 adult), V, collapsed with hospitalization	1×10^4	n.a		Osimani et al. (2018)
	Italy	Ricotta cheese	57 persons	n.a.		EPP	
2007	Germany	Ready-to-eat rice pudding	46 people (43 children, 3 adults), V	n.a.	30	EP	
	Spain	Cooked tuna fish	5 persons, G	8.0×10^6	n.a.	EP	
1998-2008	USA	Rice, meat and poultry dishes	235 outbreak, 2050 people, 17 hospitalizations, D, A,V	n.a.	n.a.	EP, EPP	Haque et al. (2021)
2006-2008	India	Not specified	42 case, D	n.a. n.a.	n.a. n.a.	EPP	Banerjee et al. (2011)
2008	France	Pasta	A 15-year-old boy; V, A, LF	n.a.	n.a.	EP	Saleh et al. (2012)
	Belgium	Spaghetti meal (pasta)	A 20-year-old man; death	n.a. n.a.		EP	Naranjo et al. (2011)
	Oman	Hospital meal	58 people; D,V	n.a.	14.1	EPP	Al-Abri et al. (2011)
	Switzerland	Pasta	A 9-year-old girl; A, V, LF, shock	n.a. n.a.	n.a.	EP	Posfay-Barbe et al. (2008)
	Korea	Raw fish	8 persons, family outbreak	n.a.	n.a.	EP, EPP	Kim et al. (2009)
	Brazil	Fruits and vegetables	93 cases, N, V, D, A		n.a.	EP, EPP	Elias et al. (2018)
2009	Brazil	Fruits and vegetables	21 cases, 3 hospitalized, D, A		n.a.	EP, EPP	
2010	Korea	Lunch buffet	43 persons, D, A		20.3	EPP	Choi et al. 2011
	Germany	Lunch buffet	4 persons, acute V	2.8 x 10^4 CFU/g	n.a. n.a. n.a.	EP EP EP	Ehling-Schulz and Messelhäusser (2012)
	Japan	Fried rice	An 11-year-old boy; G, LF, acute E,	n.a. n.a.	n.a.		Ichikawa et al. (2010)

	Japan	Reheated fried rice	3 persons; V, acute E, 1 death				Shiota et al. (2010)
2011	Germany	Mixed lunch (pasta)	22 persons (20 children, 2 adult), 4 hospitalized, acute D	2.2 x 10^6 CFU/g		EPP	Ehling-Schulz and Messelhäusser (2012)
	Belgium	Rice-based dishes	8 people; 1 hospitalized	2.8×10^5 –2.4×10^7	88	EP	Osimani et al. (2018)
2008-2012	France	n.s.	39 people, 8 death	n.a. n.a.	n.a	EPP	
2012	Italy	Basmati rice	12 people, V, N, A, D		92	EP	
	UK	Dried haricot beans	200 people (182 children, 18 adults) V	2.0×10^6	63.2	EP	
	Belgium	Mashed rice-cucumber-chicory	20 children, V	> 1.5×10^7	90.9	EP	
2001-2013	Australia	Multiple foods	6 outbreaks, 114 cases, 1 emetic and 5 diarrheal; V, D	n.a.	n.a. n.a.	EP, EPP	May et al. (2016)
2003-2013	Brazil	Mainly cereals, sauce	346 people; 3 hospitalized, D, A, V	n.a.	n.a.	EPP	Lentz et al. (2018)
2007-2013	Germany	Multiple foods	Several affected people, V	≤1×10^2 –6.1×10^7		EP	Messelhausser et al. (2014)
2013	UK	Ready-to-eat meat pie	5 people	1.5×10^6 –1.0×10^8	n.a.	EP	McLauchlin et al. (2016)
	Australia	Buffet lunch	125 people, D, A	n.a.	n.a.	EPP	Sloan-Gardner et al. (2014)
	Austria	Mashed potatoes Dish	14 people, 3 hospitalized	2.1×10^5 –3.4×10^5	44.0	EP, EPP EP, EPP	Osimani et al. (2018)
		Pancake strip soup	14 people	1.0×10^2 –1.0×10^4	22.2	EP, EPP	
		Fruit salad	106 people	n.a.	29.3		
2007–2014	France	Starchy food and Vegetables	74 outbreaks, 911 cases, A,V, D	4.0×10^2 –1.0×10^9	n.a. n.a.	EP, EPP EP,EPP	
2014	China	Fermented black beans (douchi)	139 people, N, V, D	1.6×10^7 –2.3×10^7	n.a. n.a.	EP,EPP EP,EPP	Zhou et al. (2014)
	EMS	Mixed food	287 outbreaks, 3,073 cases, 257 hospitalized	n.a. n.a.	n.a. n.a.		EFSA and ECDC (2016)
2015	EMS	Mixed food	291 outbreaks, 3131 cases, 101 hospitalized	n.a. n.a.	n.a.		
	Norway	n.s.	4 outbreaks,17 cases		n.a.	n.a.	
	Argentina	Cooked chicken	A 39-year-old healthy woman, hospitalized, V, D (watery)	n.a. n.a.	n.a.	EP, EPP	Lopez et al. (2015)
	Germany	Rice meal	A 13-month-old boy; V, A, LF	n.a.	n.a. n.a.	EP	Tschiedel et al. (2015)
2016	France	Human milk	3 infant cases, hospitalized, sepsis, brain hemorrhage,2 death	n.a. n.a.	n.a.	EP, EPP	Rigourd et al .(2018)
	USA	Refried beans	179 people, V, D	n.a. n.a.		EP,EPP	Carroll et al. (2019)
2011-2017	Korea	n.s.	50 outbreak, 491 people	n.a.		n.a.	Kim and Kim (2021)
	USA	n.s.	69 outbreak,1389 people			n.a.	
2017	Indonesia	Sardines	22 people, V, D			EPP EPP	Depo et al. (2018)
	India	Sweetened curd	204 people, A,V		44.0		Sahu et al. (2021)
2018	EMS	n.s.	98 outbreak, 1539 people, 111 hospitalize, 1 death		n.a.	EP, EPP EP, EPP	Rodrigo et al. 2021
	Australia	Multi-course-dinner (Beef)	15 people, V, D	3.5×10^3 –1.9×10^4	37.0		Thirkell et al. (2019)
	China	School canteen food and drink	209 people, V, D, A, fatigue, dizziness, fever, headache	10 - 1.6 × 10^5	3.9-12.5	EPP	Chen et al. (2019)
2019	Germany	Buck wheat	A 57-year-old woman; massive V, D, esophageal perforation, Boerhaave syndrome	n.a. n.a.	n.a. n.a	EP	Dichtl et al. (2019)
2020	China (Taiwan)	Breast milk	A 1490-g female infant was hospitalized, A, V, tachycardia, hyperglycemia, and elevated C-reactive protein			n.a	Liao and Tsai (2020)

EMS= European member states; n.s.= Not specified; n.a.= Not available; N=Nausea; V= Vomiting; D=Diarrhea; A= Abdominal pain; LF=Liver failure; E= Encephalopathy, G=Gastroenteritis; EP= Emetic poisoning; EPP= Enteropathgenic poisoning

Bacillus spp. as probiotics/feed additives, food safety implication and antibiotic resistance

Strains of *Bacillus* species have long been used as probiotics in human, veterinary, aquaculture, plant and environmental applications. Probiotic strains are used in animal production, either directly as microbial feed additives, or as a source of other feed additives, especially enzymes (EFSA 2011; Cui et al. 2019). Spores of *Bacillus* strains are used in human, veterinary, and aquaculture applications due to their probiotics characteristics, and the bacteria can then spread in food after ingestion (Carlin and Nguyen-The 2013). *Bacillus*-based probiotics can have a beneficial impact on poultry production by strongly activating immune-related components, controlling pathogenic bacteria, modulating immune responses, fostering gut integrity, raising feed conversion rate (FCR), acting as a growth factor and improving disease resistance and health (Bilal et al. 2020; Arsene et al. 2021). *B. subtilis* is a common food supplement in animal industry, particularly in poultry and fish farming (Arsene et al. 2021). In the swine industry, it is used as a replacement for antibiotics to treat diarrhea in weaning piglets; *Bacillus* spp.-fermented (notably *B. subtilis, B. licheniformis, B. amyloliquefaciens*) feed additives have been found to reduce morbidity and mortality rates, ameliorate enteritis, have a beneficial impact on the lessening diarrheal incidence and increasing the growth efficiency of weaning piglets (Lin and Yu 2020; Arsene et al. 2021). However, some bacteria of *B. cereus* and other group may cause problems by producing different enterotoxins and emetic toxins (Table 5), and carrying ARG, which can be transmitted to humans via the food chain or the environment. In light of the data about the above noted probiotic candidates, especially those belonging to the *B. cereus* group, it appears that they have no toxic potential (Cui et al. 2019).

Antibiotics are a common way to control or prevent bacterial infections in farming, and the widespread use of antibiotic growth promoters (AGP) in animal feed has led to a rise in livestock production. However, inappropriate and abusive antibiotics use can spread antibiotic residues in animal-derived foods, such as milk, meat, and eggs, as well as the environment, might spread antibiotic resistance in animal microbial communities, with the possibility of ARG being transferred from animal to human microbiota (Mingmongkolchai and Panbangred 2018; Arsene et al. 2021). Resistance determinants for β-lactams (bla_{BCL-1}), chloramphenicol (cat_{Bcl}), aminoglycosides (*aadD2*), macrolides (*erm34*), tetracycline (*tetM* and *tetK*) and erythromycin (*ermD* and *ermK*) have been found in probiotic strains of *B. cereus, B. clausii, B. subtilis* and *B. licheniformis* (Mingmongkolchai and Panbangred 2018). Consequently, global public health authorities have raised concerns about AGP and their role in the increased multidrug-resistant bacteria, with adverse effects on consumer health (Mingmongkolchai and Panbangred 2018; Arsene et al. 2021). Since the use of antibiotics in animal feeds has been banned in several countries, an alternative approach that has proved useful is the application of probiotics in consideration of the safety evaluation of new probiotics. Fig. 5 depicts the main route of transmission and development of antibiotic resistance from the feed and food chain to humans.

Isolation and Identification

B. cereus can be isolated and identified from food and other samples, using a variety of methods. Table 6 displays the advantages and limitations of various such approaches.

Traditional Approaches

B. cereus isolation and enumeration from foods, environment and clinical settings are usually done with conventional selective plating media. Food authorities suggest two standard media for *B. cereus* identification: Mannitol-egg yolk-polymyxin agar (MYPA) and Polymyxin-egg yolk-mannitol-bromothymol blue (PEMBA) for their characteristic colonies, Pink color and Peacock blue color precipitation zones of egg yolk hydrolysis, respectively (Figs. 2 and 3). Finally, to distinguish hemolytic and nonhemolytic *B. cereus* strains, a hemolysis test is conducted on 5% sheep blood agar plates at 37°C, which produces dull gray and opaque colonies with a rough matted surface (Fig. 4) (Eglezos and Dykes 2014; Pontieri 2016; Griffiths and Schraft 2017; Ramarao et al. 2020; Haque et al. 2021). Several biochemical and microscopic tests are performed after bacterial isolation, including glucose, mannitol, xylose, arabinose, oxidase, motility, catalase, citrate utilization, casein hydrolysis, nitrate reduction, Voges-Proskauer (VP) reaction, l-tyrosine reduction, and growth in 0.001% lysozyme to validate and distinguish various phylogenetically close *Bacillus* spp. (Table 7). The miniaturized API 50CHB test package (bioMerieux), which evaluates the capacity to assimilate 49 carbohydrates, is a quick *Bacillus* identification method based on conventional biochemical tests. This system is believed to be capable of classifying possible emetic strains, but it does not distinguish *B. cereus* and *B. thuringiensis* (Eglezos and Dykes 2014; Griffiths and Schraft 2017).

Molecular Methods

Molecular approaches for confirming *Bacillus* spp. identification include a variety of techniques, which are summarized in Fig 6. Genes encoding major enterotoxins (nhe, hbl, cytK, entFM, bceT, hlyII) and emetic toxin (cesA, cesB) at various levels of production are much more relevant with respect to species determination (Table 8) in *B. cereus* toxin gene profiling by PCR detection protocols, particularly in outbreak situations. Furthermore, diagnostics should focus more on determining toxin or virulence genes, as well as toxin output quantification (Pontieri 2016; Ramarao et al. 2020). PFGE is one of the most effective fingerprint typing methods for *B. cereus* outbreak in the epidemiological investigation, because it splits large pieces of genomic DNA and allows for precise

Table 3: Incidence of *B. cereus* food poisoning in different foods worldwide from 2000 to 2020

Year	Country/Region	Food	Incidence (%)	Reference
1998-2000	Germany	Mass catering food	60	Ehling-Schulz and Messelhäusser (2012)
	France	n.s.	4-5	Tewari and Abdullah (2015)
2000	Norway	n.s.	32	Haque et al. (2021)
2001-2002	Turkey	Meat and meat products	22.4	Tewari and Abdullah (2015)
2003	Czech Republic	Dairy products	31.0	Schlegelova et al. (2003)
		Meat products	28.0	
2004	USA	Retail chicken products	45.0	Smith et al. (2004)
1991-2005	China (Taiwan)	n.s	11.2	Raddadi et al. (2010)
2005	Spain	Seafood cocktail and fried shrimp	5.0	Hernandoa et al. (2007)
	India	Milk and milk products	53.8	Tewari and Abdullah (2015)
	Chile	Dried milk products	45.9	Kumari and Sarkar (2016)
2006	Netherland	n.s.	5.4	Haque et al. (2021)
	China	Pasteurized full-fat milk	71.0	Kumari and Sarkar (2016)
2006-2007	Australia	Chilled raw diced chicken	2.4	Haque et al. (2021))
	USA	Raw rice	46.6	Ankolekar et al. (2009)
2007	EU	Not specified	17.1	Tewari and Abdullah (2015)
	Belgium	Commercial food products	56.3	Samapundo et al. (2011)
1998-2008	USA	Rice, meat and poultry dishes	19.0	Haque et al. (2021)
2001-2008	Korea	Raw fish	3.7	Chon et al. (2012)
2006-2008	India	n.s.	3.5	Banerjee et al. (2011)
	Korea	Cooked rice	37.5	Chang et al. (2011)
2008	Turkey	Cheese	12.0	Kumari and Sarkar (2016)
	Austria	Ice cream	62.7	Heydarzadeh et al. (2020)
2007-2009	Korea	n.s.	1.5	Gwack et al. (2010)
2008-2009	Germany	Marinated pork products	21.0	Haque et al. (2021)
	Jordan	Various foods	23.3	Batchoun et al. (2011)
	Korea	Brown rice and glutinous rice	37.0	Lutpiatina (2020)
	Turkey	Milk and meat products, Boza	66.0	Güven and Mutlu 2009
2009	India	Traditional food	46.0	Tewari and Abdullah (2015)
	Egypt	Raw milk	30.0	Kumari and Sarkar (2016)
2009-2010	USA	Raw commingled silo milk	8.9	Liu et al. (2020)
2010	Scotland	Cheese	32.0	Heydarzadeh et al. (2020)
2011	Belgium	Cooked rice	18.5	Delbrassinne et al. (2012)
2010-2012	Korea	Fermented soybean products	67.9	Kim et al. (2015)
	Brazil	Milk and dairy products	24.2	Heydarzadeh et al. (2020)
2012	Mexico	Vegetables	57.0	Flores-Urban et al. (2014)
	India	Meat and meat products	30.8	Tewari et al. (2012)
		Raw milk	11.0	
2003-2013	Brazil	Cereals or sauces	3.1	Haque et al. (2021)
2007-2013	Germany	Multiple foods	10.0	Messelhausser et al. (2014)
2012-2013	Singapore	Sushi	5.1	Yap et al. (2019)
2013	Iran	Infant food	42.0	Lutpiatina (2020)
2007-2014	EU	n.s.	27.6	Haque et al. (2021)
2008-2014	Brazil	Fruits and Vegetables	6.6	Elias et al. (2018)
2013-2014	China	Raw milk	9.8	Cui et al. (2016)
	Iran	Powdered milk infant formula	67.2	Dallal et al. (2017)
	Iran	Beef Burger	31.2	Soleimani et al. (2017)
	EU	Not specified	5.5	Food safety authority of Ireland (2016)
2014	India	Various dairy products	32.0	Kumari and Sarkar (2016)
	Cambodia	Fermented vegetables	31.0	Chrun et al. (2017)
	Malaysia	Formula milk	41.7	Lesley et al. (2017)
		UHT milk	30.0	
	China	Infant formula	3.5	Zhang et al. (2017)
2013-2015		Rice flour	1.0	
	Nigeria	Retailed foods	36.8	Adesetan et al. (2019)
2014-2015	Canada	Pasteurized Fluid Milk	5.5	Saleh-Lakha et al. (2017)
	Italy	Dairy products	26.8	Haque et al. (2021)
	Egypt	Meat products	43.7	Mohamed and Ghanyem (2015)
2015	Ghana	Raw milk	46.6	Heydarzadeh et al. (2020)
	Nigeria	Milk-based infant food	3.0	Ranjbar and Shahreza (2017)
	Korea	RTE vegetables	48.0	Chon et al. (2015)
	Iran	Raw and cooked meat	14.5	Zeighami et al. (2020)
2002-2016	Italy	Ricotta cheese	15.9	Scatassa et al. (2018)
		Retail aquatic products	25.4	Zhang et al. (2020)

		Pasteurized milk	27.0	Liu et al. (2020)
2011-2016	China	Meat and meat products	26.3	Kong et al. (2021)
		Vegetables	50.0	Yu et al. (2019)
		RTE foods	35.0	Yu et al. (2020)
	China	Goat milk powder infant formula	36.1	Liu et al. (2018)
2016	Brazil	Pasteurized Milk	28.6	Chaves et al. (2017)
	Mexico	Artisan cheeses	29.4	Adame-Gomez et al. (2020)
2007-2017	Poland	Commercial food products	38.8	Berthold-Pluta et al. (2019)
2011-2017	Korea	n.s.	6.5	Kim and Kim (2021)
	USA	n.s.	3.5	
		Boiled milk	50.0	
2017	Egypt	Pasteurized milk	15.0	Abou Zeid and Yassin (2017)
		UHT milk	15.0	
		Beef products	26.0	Shawish and Tarabees (2017)
	Turkey	Milk and cheese	10.4	Yibar et al. (2017)
2017-2018	Malaysia	Ready-to eat cooked rice	34.0	Navaneethan and Esah (2020)
	China	Raw milk	16.0	Liu et al. (2020)
	Switzerland	PIF	78.0	Heini et al. (2018)
2018	China	School canteen food and drink	4.1	Chen et al. (2019)
	EU	n.s.	1.9	Rodrigo et al. (2021)
	Thailand	Mixed food stuffs	21.0	Sornchuer and Tiengtip (2021)
2018-2019	China	Dairy products	10.8	Liu et al. (2020)
	Iran	Dairy products	10.6	Heydarzadeh et al. (2020)
	Egypt	Buffalo milk	12.9	Abouelhag et al. (2021)
2019	Italy	Fried rice meals	7.8	Tirlonia et al. (2019)
	Indonesia	Cooked rice (yellow rice)	21.0	
2019-2020	Iraq	Soft cheese	67.0	Al-Jobory and Abdulaal (2020)
	Egypt	Various RTE food	5.0-10.0	Enan et al. (2020)
		Milk powder	6.9	Abdeen et al. (2020)
	Egypt	Ras-cheese	8.5	
2020		Meat and Chicken Products	21.5	Gharib et al. (2020)
	Pakistan	Different milk	20.0	Rafique et al. (2020)
	Malaysia	UHT chocolate milk	24.3	Ubong et al. (2020)
	China	Rice/ noodles	50.0	Lutpiatina (2020)
	Colombia	Powdered food	11.0	Sanchez-Chica et al. (2020)

n.s.= Not specified; RTE= Ready to eat; PIF=Powdered infant formula; UHT= Ultra heat treatment.

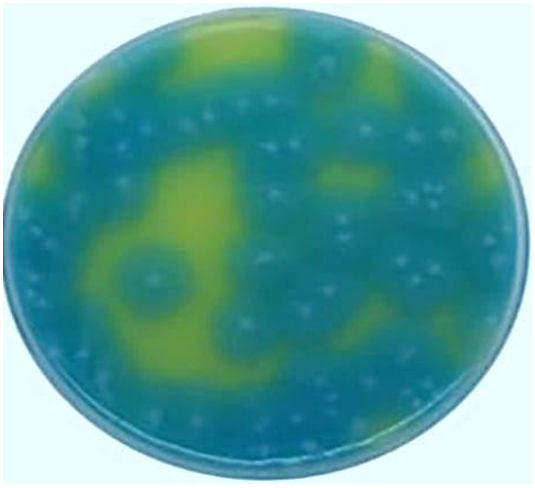

Fig 3: *Bacillus cereus* on PYMBA (Photo by Md Atiqul Haque).

Fig. 4: *Bacillus cereus* on Blood agar (Photo by Hongkun Quan).

resolution of minor variations in genomic sequences for bacterial group studies. The RAPD-PCR method is a reliable and widely used for molecular typing of diverse *Bacillus* spp; it uses specific primers to randomly amplify segments of target DNA. It can be used to distinguish emetic strains from other *B. cereus* strains and is thus commonly shown in the laboratory as a screening process. MLST is considered the "gold standard" for typing of *B. cereus* group strains, showing the sequences of many basic or housekeeping genes clustered across the chromosomes, occurring in three major *Bacillus* spp. clades.

For certain applications, AFLP may be preferable to classify various *B. cereus* strains into different phylogenetic classes (Pontieri 2016; Griffiths and Schraft 2017; Grutsch et al. 2018).

Spectrometry

MALDI-TOF-MS has been widely adopted and applied in clinical microbiology for routine pathogen detection at the species level. The mass/charge ratio of microbial proteins ionized from intact cells collected from pure culture is graphed as a peak in mass spectrometry results. The mass spectral profile is defined by comparing it to a reference database. MALDI-TOF–MS was used to detect enterotoxins (CytK1 and Nhe) produced by pathogenic strains and was found to be an effective risk assessment technique in routine pathogen detection for determining the presence of *B. cereus* strains in food-borne outbreaks (Pontieri 2016).

Biosensors

Biosensors have proven to be effective in detecting food-borne pathogens, such as *B. cereus*. Several biosensors-based techniques for detecting *B. cereus* have been developed and published so far. DNA-based biosensors, in particular, have shown a great success because they allow for the selective identification of different *B. cereus* strains. As an alternative to DNA probes, mono or poly-clonal antibodies targeting *B. cereus* cells can be used as identification elements in biosensors (Ramarao et al. 2020).

A biosensor incorporates rabbit polyclonal anti-*B. cereus* antibodies that have been shown to have high sensitivity, detecting *B. cereus* at concentrations as low as 10^1 CFU/ml, and rapidity with a detection period of just 6 minutes (Raddadi et al. 2010).

Detection and Quantification of toxins

Detection and Quantification of Cereulide (*Ces*)

For the detection and quantification of cereulide (Ces) toxin in various food matrixes, a variety of assays are now available. Generally, cell culture-based assays, using various cell lines (HEp-2, Hep-G2, CaCo-2, HeLA cells), and sperm-based assays in which the biological effects of Ces can be assessed by inhibition of mitochondrial function, cellular vacuolization and loss of motility of boar spermatozoa, have been used. These assays, however, are not so precise, since other mitochondrial toxins are also susceptible to them and impair sperm motility (Raddadi et al. 2010; Cui et al. 2019). Instrumental methods, such as high-performance liquid chromatography (HPLC), HPLC linked to ion trap mass spectrometry (HPLC-MS), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) are used to detect Ces toxin (Cui et al. 2019). A new reversed - phase chromatography (RPC) was developed recently to identify and quantitatively measure Ces toxin existence, using ultra performance liquid chromatography-mass spectrometry (UPLC–MS/MS) (Kalbhenn et al. 2021).

Detection and Quantification of Enterotoxins (Nhe, Hbl, and CytK)

Various tests, such as the vascular permeability reaction (VPR), the ligated rabbit ileal loop, and cell cytotoxicity assays, are currently available and widely used for the detection of diarrheal toxins (Raddadi et al. 2010). VPR

Table 4: Main features of food-borne diseases associated with *B. cereus*

Properties	Diarrheal syndrome	Emetic syndrome	Reference
Type	Toxico-infection	Intoxication	Raddadi et al. (2010), Lindbäck and Granum (2019), Kumari and Sarkar (2016), Griffiths and Schraft (2017), Lindbäck and Granum (2019)
Mode of action	Receptor unknown, however Hbl, Nhe and Cytk specific receptors are suggested; causes hemolysis, cytolysis, demonecrosis and vascular permeability activity	Binds to serotonin 5-HT3 receptors; causes emesis by action on vagus afferent, inhibition of mitochondrial fatty acid oxidation and T (NK) cells	
Regulatory gene	PlcR	SpooA and AbrB	
Infective dose	10^5–10^7 cells (total) or cfu/g	10^5–10^8 cells (per g/ml substrate) or 0.02–1.83 µ g/kg of body weight	
Toxin production	In the small intestine of the host	Preformed in foods	
Toxin involve	Cereulide	Hbl, Nhe and CytK	
Nature of toxin	Protein(s)	Cyclic peptide	
Heat stability	Labile, inactivated 56°C, 5 min	Extremely stable 121°C, 90 min	
pH stability	Unstable < 4 and > 11	Stable 2- 11	
Requirements for illness	Vegetative cells of spore production in food to an infectious dose, consumption of which leads to infection and formation of toxins inside of a host	Cereulide production in food at high concentration outside of host resulting illness due to consumption of pre-formed toxin	
Incubation time	8–16 h (occasionally >24 h)	0.5–6 h	
Duration of illness	12–24 h (occasionally >24 h)	6–24 h	
Symptoms	Abdominal pain, watery diarrhea (occasionally bloody type), sometimes with nausea and lethality	Nausea, vomiting, malaise (sometimes followed by diarrhea), in some cases fatal liver failure	
Foods commonly implicated	Proteinaceous foods: meat products, fish, poultry, soups, vegetables, puddings, sauces and stews, milk and milk products	Farinaceous/Starch-rich foods; fried and cooked rice, pasta, potatoes, bread, pastries and noodles	
Assays available for detection	Hep-2 cell bioassay, rapid sperm bioassay, HPLC-MS and PCR-based assays	BCET-RPLA, Tecra BDE-VIA kit, and Duopath Cereus Enterotoxins test assay	

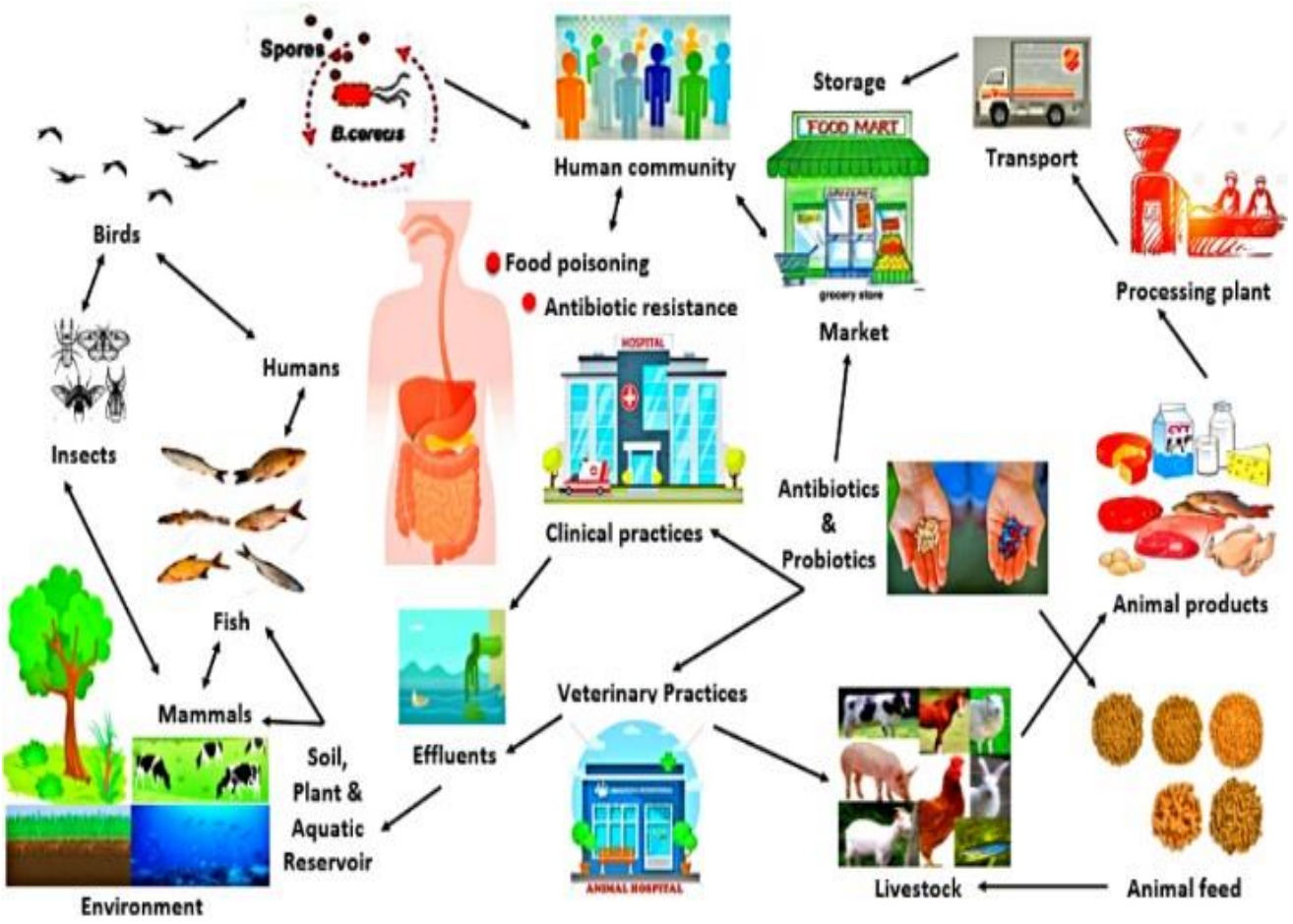

Fig 5: Flowchart showing the presence of antibiotic residues arising from the use of probiotic and antibiotic in animal feed (Arsene et al. 2021; Haque et al. 2021; Hassan et al. 2021).

Table 5: *Bacillus* spp. implicated in food-borne infections and the related toxins

Species	Toxins	Features	References
B. thuringiensis	Enterotoxins	Heat-labile, cytotoxicity, risk of food-borne intoxication implicated	Griffiths (2010); EFSA (2011); Delbrassinne and Mahillon (2016); Mingmongkolchai and Panbangred (2018); Haque et al. (2021);
B. weihenstaphanensis	Cereulide	Heat-stable, risk of food-borne intoxication implicated	
B. subtilis	Amylolysin, fengycin	Surfactin-like components, heat-stable, inhibition of boar sperm motility, cytotoxicity, implicated in food-borne gastroenteritis	
B. licheniformis	Lichenysins	Heat-stable lipopeptide, inhibition of boar sperm motility, implicated in food-borne gastroenteritis, fatal cases reported (dairy and infant food), also involved in local and systemic infections	
B. pumilus	Pumilacidins	Complex lipopeptides, heat-stable, inhibition of boar sperm motility, implicated in food-borne poisoning, also involved in local and systemic infections	
B. fusiformis	Cytotoxins	Lipopeptides; heat-stable, cytotoxicity	
B. mojavensis	Amylolysin, fengycin	Surfactin-like components, heat-stable, cytotoxicity, inhibition of boar spermatozoa motility, implicated in food-borne poisoning	
B. amyloliquefaciens	Amylosin	Heat-stable lipopeptide, connected with food poisoning	
B. firmus	Cereulide-like toxins	Lipopeptides; heat-stable, inhibition of boar sperm motility	
B. simplex	Cereulide-like toxins	Lipopeptides; heat-stable, inhibition of boar sperm motility	
B. circulans	Cytotoxins	Implicated in food-borne poisoning, also involved in local and systemic infections	
B. lentus	Cytotoxins	Toxin production observed, but no food poisoning case reported yet	
B. megaterium	Cereulide-like toxins	Lipopeptides, implicated in food-borne poisoning	

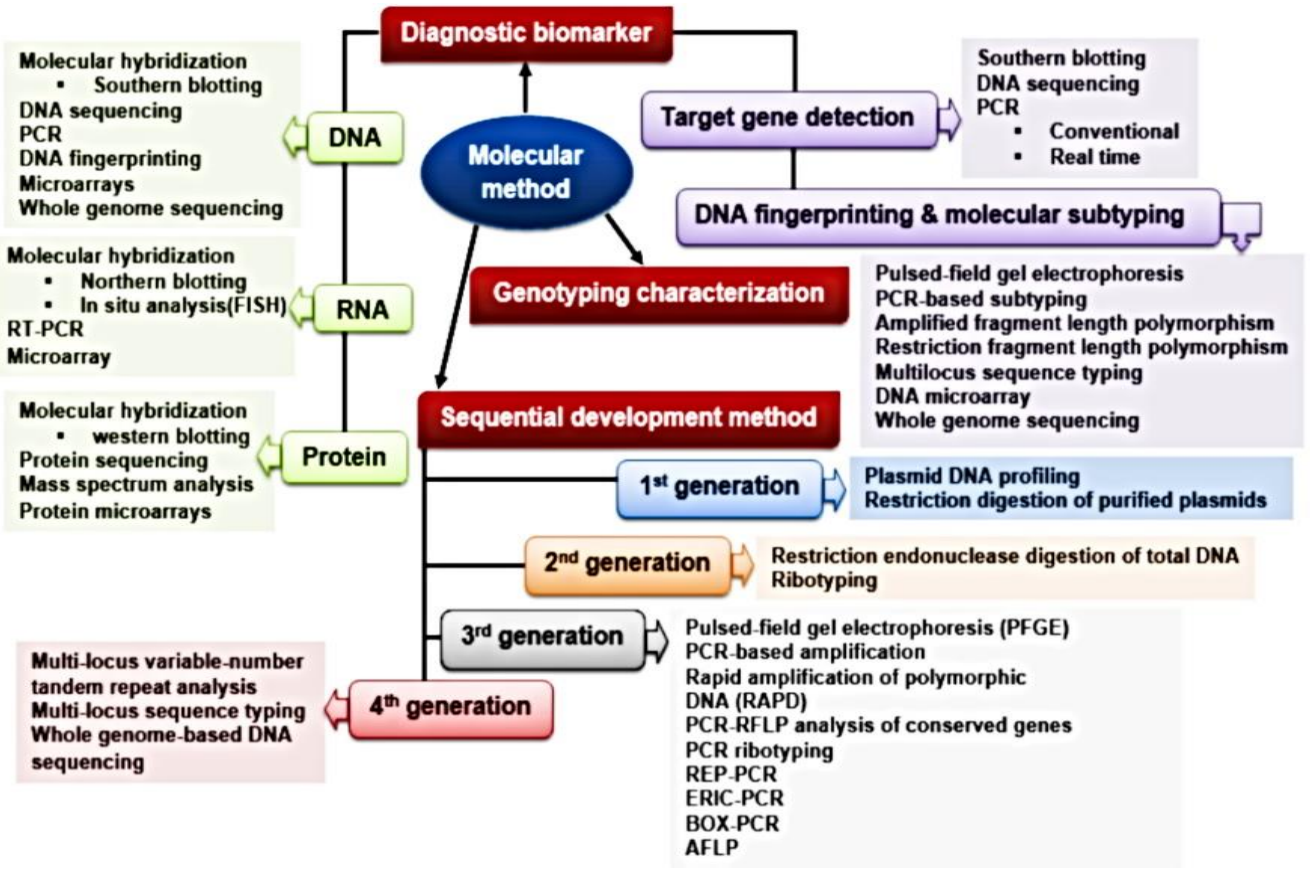

Fig. 6: Molecular methods for the isolation and identification of bacteria (Raddadi et al. 2010; Quinn et al. 2016).

Table 6: Advantages and disadvantages of different diagnostic methods

Methods		Advantages	Disadvantages	References
Traditional method	Culture medium-based method and microscopic observation with Biochemical assay	Simple, cheap and easy handling Able to recognize a single bacterial strain Identification of viable cells Optimal toward suitable media For phenotypic drug susceptibility testing Inexpensive equipment	Time-consuming, laborious and resource demand Poor sensitivity and specificity Not possible to detect injured or VBNC cells Can lead to misidentification Risk of contamination Require qualified personnel	Raddadi et al. (2010); Pontieri (2016); Ehling-Schulz et al. (2011);
Immunological method	ELISA, RPLA and immunofluorescence assay	Precise and reproducible results Inexpensive equipment Onsite application	Relatively low sensitivity and selectivity/ specificity Immunodeficient host may not be able to respond	Abbasian et al. (2018); Grutsch et al. (2018); Bao et al. (2020);
Molecular method	PCR	Highly accurate Relatively high sensitivity and specificity	Not real-time Not able to distinguish dead and alive cells Not for qualification Sophisticated, expensive equipment and costly Sometimes amplification errors or false-negative results Require enrichment step in case of a lower number of pathogens Gel electrophoresis is laborious, time-consuming and low-resolution False-positive results due to laboratory contamination.	Kim and Kim (2020); Mishra et al. (2020); Ramarao et al. (2020); Vidic et al. (2020); Kalbhenn et al. (2021)
	Real-time PCR	Highly accurate Quantitative Strong sensitivity and specificity Very precise and robust Easy and fast data processing	Not real-time Not able to distinguish dead and alive cells Require enrichment step in case of a lower number of contaminants Risk of contamination with genomic DNA Possibility of false-positive results	

	Multiplex PCR	Detection of multiple pathogens or toxins simultaneously Small quantities of DNA extracted Economic and time saving	Intrusion by the existence of polymorphism Possibility of false-negative results Only qualitative, not for quantification Further confirmation test is required
	Nested PCR	High sensitivity and specificity Decreases non-specific target sequence amplification	Costly and time-consuming Risk of cross-contamination
	ERIC- PCR	Highly reliable Easy and first Inexpensive	Low discrimination
	RAPD	Very simple and fast Inexpensive	Extremely laboratory-based Requires carefully designed laboratory protocols for reproduction
	AFLP	Strongly distinguishable Sensitive and specific High resolution and sampling power Reproducibility Genetic heterogeneity detection	Costly More reaction phage and reagents
	RFLP	Strong discrimination and typification Clarity	Lower sensitivity for specific mutant Laborious and time-consuming
	MLST	Rapid tool for large population analysis Repeatable and indisputable Fully automated analysis	Expensive Requires intensive sequencing efforts
	NGS	Highly accurate Quantitative Detection of multiple pathogens in one sample	Expensive Not real-time Required bioinformatics analysis
	PFGE	Stability, Reproducibility More discriminatory	Time-consuming Expensive Labor intensive
	LMAP	Highly accurate High specificity and sensitivity Rapid, simple and efficient Without expensive equipment and complicated thermo-cycling	Strict primer design principle Possibility of false-positive results
	CAMP	Highly accurate Relatively more rapid, specific and sensitive Easy, reliable and simpler primer design	Possibility of false-positive results
	Hybridization	High sensitivity and specificity Low cost Fast detection	Costly and time-consuming Focused on DNA assay
	DNA microarray	High efficiency Multiple genes analysis	Confounding first time users Findings are not reproducible
Spectrometry and chromatographic method	MALDI-TOF-MS	Highly accurate Fast, easy and reliable Cost-effectiveness	Pure cultures are needed Quantification error
	LC-MS	Highly accurate	Expensive MS equipment required
	SIDA–MS/MS	Highly accurate	Sophisticated and expensive high-end MS equipment required
	RPC	Fast, easy and robust Cost-effective	Require trained personnel
Biosensors	Gold nanoparticle (AuNPs) aggregation	Onsite application Easy, rapid and detection by the naked eye	Costly and relatively low affinity Not easy to apply in solid or liquid-solid matrix

VBNC=Viable but not-culturable; PCR=Polymerase chain reaction; ERIC-PCR=Enterobacterial Repetitive Intergenic Consensus-PCR; ELISA=Enzyme-linked immunosorbent assay; RPLA=Reverse passive latex agglutination; LMAP=Loop-mediated isothermal amplification; CAMP=Competitive annealing mediated isothermal amplification; MLST=Multilocus sequence typing; NGS=Next generation sequencing; MALDI-TOF-MS=Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; LC-MS=Liquid chromatography coupled to mass spectrometry; SIDA–MS/MS=Stable isotope dilution assay tandem mass spectrometry; RPC=Reversed-phase chromatography; RAPD=Random amplified polymorphic DNA; AFLP=Amplified fragment length polymorphism; RFLP=Restriction fragment length polymorphisms; PFGE= Pulsed-field gel electrophoresis.

Table 7: Phenotypic criteria to differentiate members of the *B. cereus* group

Features	*B. cereus*	*B. anthracis*	*B. thuringiensis*	*B. mycoides*	*B. pseudomycoides*[a]	*B. weihenstepanensis*[b]	*B. cytoticus*[c]	*B. toyonesis*[d]	References
Gram stain	+	+	+	+	+	+	+	+	Fritze and Pukall (2011); Jimenez et al. (2013); Eglezos and Dykes (2014); Pontieri (2016); Lindbäck and granum (2019); Ramarao et al. (2020); Haque et al. (2021)
Colony morphology	White	White	White/gray	Rhizoid	Rhizoid	White	White	white	
Hemolyis	+	-	+	(+)	(+)	+	+	+	
Motility	±	-	±	-	-	+	+	+	
Susceptibility to penicillin	-	+	-	-	-	-	-	-	
Parasporal crystal	-	-	+	-	-	-	-	-	
Growth temperature range (°C)	10-45	>10-<50	10-45	15-40	10-40	5-37	20-50	10-45	
Lysis by gamma phage	-	+	-	-	-	-	-	-	
Catalase	+	+	+	+	+	+	+	+	
Citrate utilization	+	-	+	-	-	-	-	+	
Lecithinase activity (Egg yolk reaction)	±	(+)	±	±	(+)	+	(+)	+	
Acid from mannitol	-	-	-	-	-	-	-	-	
Glucose anaerobic utilization	+	+	+	+	+	+	+	+	
Reduction of nitrate	±	+	+	+	+	+	+	+	
VP reaction	+	+	+	+	+	+	(+)	+	
Tyrosine decomposition	+	(+)	+	(+)	+	+	+	+	
Resistance to lysozyme	+	+	+	+	+	+	+	+	
Anaerobic growth	+	+	+	+	(+)	-	(+)	+	
Starch	+	+	+	+	+	+	-	+	
Indole	-	-	-	-	-	-	-	-	

[a]Differentiated from *B. mycoides* based on fatty acid composition and 16S RNA sequence; [b]Differentiated from *B. cereus* based on growth at <7°C and not at 43°C; it can be identified rapidly using rRNA gene- or cspA (cold shock protein A gene)-targeted PCR; [c]Differentiated from *B. cereus* by maximum growth at 50°C and minimum growth at 20°C, by the absence of starch hydrolysis, and by the absence of growth on synthetic media without tryptophan; [d]distinguished from other *B. cereus* group members by pairwise calculation of the average nucleotide identity; +, Positive; -, negative; (+), weakly positive; ±, usually positive but occasionally may be negative; VP, Voges-Proskaurer

involves injecting 0.1 ml of cell-free culture supernatant intra-dermally into 2.5–3.0 kg rabbits. After 3 hours, a 4 ml intravenous injection of 2% Evans blue dye solution is given. After 1 hour the perpendicular diameters of the light and dark blue areas, as well as any necrosis, are measured. The ligated rabbit ileal loop assay involves injecting bacterial culture supernatant into a 5 cm ileal loop of female New Zealand White rabbits. If the amount of fluid retention to loop tube diameter is >0.5, the test is positive. In cytotoxicity assays, filtered supernatant is added to a cell line, and the treatment effects on the cells are assessed. The diarrheal toxins can affect a variety of cell lines, such as Vero (monkey kidney), Chinese hamster ovary (CHO), HeLa S3, Human Embryonic Lung (HEL) and McCoy cell lines (Raddadi et al. 2010).

Commercial kits/immunoassays

Enterotoxins can be detected by using different commercial immunological assays. The presence of the L2 portion of HBL and NheA is measured by the BCET-RPLA Toxin Detection kit (Oxoid, UK) and Tecra BDE-VIA kit (Tecra Diagnostics, Australia), respectively. The Duopath Cereus Enterotoxins (Merck KGaA Chemicals, Germany) test assay simultaneously detects both HBL and Nhe (Raddadi et al 2010; Lindbäck and Granum 2015).

Control and prevention

B. cereus and its latent spores are ubiquitously present in nature, so they can easily contaminate various types of food and degrade the organoleptic properties of food (especially eggs, meat and milk based products), affecting their market quality. This is a serious public health issue, as well as a significant economic risk for the food industry. However, due to the absence of any legislation for the systematic screening of food items for pathogen contamination, limits on the quantity of *B. cereus* cells in foodstuffs have been set in various nations and regions, based on standard recommendations (Table 9). The majority of *B. cereus* of food-borne outbreaks have been linked to bacterial concentrations >10^5 CFU/g of food material, while some instances are linked to number as low as 10^3 CFU/g. Furthermore, determination of a safe limit is difficult, as the pathogenicity is not only assessed by the quantity of bacterial cells. Regulation will focus on *B. cereus* group food safety, with a maximum tolerable limit (MTL) of 10^3 cfu/g in dairy products for the general population, 10^2cfu/g in infant formula, 10^3 cfu/g in RTE meat and 10^5 cfu/g in egg products. Food processors should guarantee that *B. cereus* counts of 10^3-10^5/g are not reached (or surpassed) at the point of consumption under normal storage and handling settings, which must also be

Table 8: Diagnostic marker genes for *Bacillus* spp.

Species	Target gene by PCR			References
	Gene		Prevalence (%)	
B. cereus	*ces*	*cesA*	1.5-32.8	Raddadi et al. (2010); EFSA (2011); Haque et al. (2021)
		cesB		
	hbl	*hblA*	29–92	
		hblB		
		hblC		
		hblD		
	nhe	*nheA*	85– 100	
		nheB		
		nheC		
	cytK	*cytK-1*	40–89	
		cytK-2		
	entFM		84– 100	
	bceT		12–75	
	hlyII		19–56	
B. cereus group	*gyrB*		-	
	groEL		-	
B. subtilis group	*gyrase A*		-	
	gyrase B		-	
B. weihenstephanensis	*cspA (heat shock protein)*		-	
B. cytoticus	*cytK-1*		-	

Table 9: Maximum tolerable limit (MTL) of *B. cereus* contamination in different foodstuffs

Country/authority	Food items	MTL (cfu/g)	Reference
EU	DIF	50	Chon et al. (2015); McLauchlin et al. (2016); EFSA (2016); Osimani et al. (2018); Ramarao et al. 2020; Haque et al. (2021)
CAC, FAO, WHO	IF	10^2	
FSANZ, Korea	RTE food	10^2 - $<10^3$	
Ireland	RTE food	10^3 - $<10^4$	
UK	RTE food, Dried herbs and spices	10^3 - $<10^4$	
HPAUK, CFSHK	RTE food	10^3 - $<10^5$	
FDA	Dairy products, cheese and cheese products	$<10^4$	
	DIF and DDF intended for infants <6 months of age	10^2	
France	Cooked ham and salami, CFP, cooked meat-based products, RTE meals, sauces, cold starters, salads containing raw vegetables and cheese, fish- or meat-based starters, cooked starters, cured meats served hot or cold, RTE cooked pastries	$<10^2$	
	Starch-rich food	10^5	
Philippine	Frozen entrees containing rice or corn flour as main ingredient, Tofu, CBF for infants	10^2	
Italy	Pasta or rice salads, cheese meals, pizza, bread, cooked products and cold served foods, pastries and biscuits, fish-based products, honey, cereals, RTE vegetables, cooked ham and salami, cheese made from pasteurized milk, gastronomic products, fresh pastry, egg-based pasta, RTE dishes	$<10^2$	
	Raw vegetables, spices, herbs, sandwiches, salads containing uncooked ingredients	$<10^3$	
Spain	Teas and derivates, herbs and spices	$<10^3$	
Germany	Herbs and spices, tofu	$<10^3$	
	CBP, sandwiches, sprouts, RTE hot products	$<10^2$	
Portugal	Sashimi	$<10^2$	
Croatia	Puddings, heat-treated dairy desserts and related products	$<5\times10^2$	
	RTE dried foods for infants	<10	

EU=European Union, CAC= Codex Alimentarius Commission, FAO= Food and Agriculture Organization of the United Nations, WHO= World Health Organization, FSANZ= Food Standards Australia New Zealand, UK=United Kingdom, HPAUK=Health Protection Agency United Kingdom, CFSHK=Centre for Food Safety, Hong Kong, FDA=Food and Drug Administration, RTE=Ready to eat, DIF=Dried infant formulae, IF= Infant formulae, DDF=Dried dietary foods, CPF=Cooked food products, CBF=Cereal base foods, CBP=Cereal based products.

applied to rehydrated foods reconstituted with hot water before intake (Blackburn and McClure, 2009; Zhang et al. 2020; Ramarao et al. 2020; Yu et al. 2020; Haque et al. 2021). While *B. cereus* is present in many foods, its vegetative form is inhibited by most cooking methods, it still challenges with spores survival and later outgrowth remains in damp protein-based foods and rice.

Cooked foods should either be kept at a temperature above 60°C or quickly cooled and refrigerated below 4°C to prevent the growth of *B. cereus* spores (Eglezos and Dykes 2014). *Bacillus* strains also have the potential as promising probiotics to enhance human and animal health by consuming large amounts of live cells directly. Probiotic *Bacillus* spp. may possess toxicity and transmit

ARGs between probiotics and opportunistic or pathogenic bacteria in GITs. Toxicity testing is a primary safety concern for probiotics candidates that are to be consumed by humans and livestock, thus the absence of *B. cereus* toxin and susceptibility to antibiotics in *Bacillus* spp. intended for use as feed additives must be thoroughly investigated. To reach a consensus on the phenotypic and genotypic characteristics of targeted *Bacillus* spp. and their correlation with those having generally recognized as safe (GRAS) status, the entire genome should be sequenced and analyzed to look for genes that are responsible for the production of enterotoxins and the emetic toxin.

The necessity of strain-level identification, on the other hand, is essential for detecting and removing any causative relationship between probiotics and strains obtained from immune-compromised hosts. As a result, it is critical to remember that clinical studies of these regimens should include a large proportion of the target population, including persons with poor immunity. Therefore, more work is needed to be done in terms of monitoring virulence factors, toxins and antibiotic resistance determinants in probiotic *Bacillus* spp. (Elshaghabee et al., 2017; Mingmongkolchai and Panbangred 2018; Cui et al. 2019; Deng et al. 2021). In addition, widespread antibiotic use can result in the development of antibiotic-resistant bacterial strains, with the potential for resistance genes to be passed on to other pathogenic and nonpathogenic bacteria, as well as the human food chain. So, it is critical to use legal antimicrobials in food-produing animals by registered health experts, maintaining withdrawal periods, limiting antimicrobials products as feed additives, to ensure proper sewage treatment of human and veterinary hospital effluents, and prohibiting the use of poultry offal, litters and livestock waste in aquaculture (Hassan et al. 2021). Antimicrobial resistance profiles and other virulence factors of *Bacillus* spp. have recently been evaluated, using next generation sequencing. This method could change probiotic exploration, because it can detect other probiotic characteristics, such as bacteriocin production, adhesion-ability, and signaling pathways at the genome level, in addition to safety hazards (Ramlucken et al. 2020). Organic acids (acetic, butyric, citric, formic, lactic, propionic, malic, and sorbic acids) and their salts (sodium acetate, sodium butyrate, sodium citrate, sodium formate, sodium lactate, and sodium propionate) have also been used as acidifiers in animal feeds to improve gut health and performance, as well as weight gain, survival, and FCR. Acidifiers have a similar effect to antibiotics in that they significantly regulate gut bacterial populations and boost immune response. Acidifiers coated salts are now commercially available for usage in food animals, particularly pigs and poultry. Combining organic acids with other antimicrobial substances, such as phytochemicals or permeabilizers, in an effort to use possible synergy to more efficiently combat pathogenic bacteria, fungi or mold in feed prophylactic measure, is a new emerging strategy to modulate gut microflora and reduce pathogens in the gut (Pearlin et al. 2020). Fermented feed ingredients (soybean and corn) in herd and poultry diets, as well as soybean food for human consumption, may contain *B. cereus* vehicles that can be regulated using uniform fermentation principles, such as the structure and composition of the testing products, the basic culture technique, fermentation criteria, post-fermentation methods and the utilization of bacterial peptides, bacteriocins and other antimicrobials (Haque et al. 2021). Since the emergence of antibiotic-resistant bacteria, phage endolysins, especially LysB4EAD-LysSA11, hybrid endolysin have piqued interest as a promising alternative to antibacterial agents for the simultaneous control of multiple bacteria, including *B. cereus*. Furthermore, this strategy would allow for the development of multifunctional and highly specific antimicrobials, thereby reducing the prevalence of multidrug-resistant bacteria (Son et al. 2020). Besides this, natural antibacterial agents, such as Makino, Asteraceae, Roselle, Rosemary, clove, thyme and others, may be possible candidates for the production of new strategies to combat the spread of *B. cereus* in the food and feed industry (Haque et al. 2021).

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ANIMAL AND PUBLIC HEALTH SIGNIFICANCE OF CHLAMYDIOSIS

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INTRODUCTION

Chlamydiaceae family has only one genus known as Chlamydia, which has eleven member species. Amongst those, *Chlamydia abortus (C. abortus)* occupies an important place due to its ability to induce abortion in sheep and goats and the risk of zoonosis (Sachse et al. 2015). The disease in sheep is known as Ovine Chlamydiosis, Ovine Enzootic Abortion (OEA) or Enzootic Abortion of Ewes (EAE). *C. abortus* is a Gram-negative bacterium with the characteristic of obligate intracellular relationship (Seth-Smith et al. 2017). Chlamydiosis causes acute placentitis and abortion in advance pregnancy, particularly in the last 2 to 3 weeks of gestation. There may be stillbirth or birth of weak lamb(s) if abortion does not occur (Aitken and Longbottom 2007). There are no specific symptoms or clinical expressions of the disease. Behavioral changes and vulvar discharge may be seen before abortion in some cases. The prominent sign of this disease is the expulsion of dead or weak lambs, peculiarly 2–3 weeks before expected lambing. The lambs usually look mature and normal but, in some cases, there may be 'pot-bellied' lambs due to subcutaneous edema. Moreover, in some cases, lamb fleece may also be covered with exudate, which is creamy pink, brown in appearance (Maley et al. 2009). However, sometimes lambs are born to live but prematurely, and are enough weak to survive beyond 24 hours. In this disease, the vaginal discharge and placenta are highly infectious, with placenta having dirty pinkish and reddish-yellow-colored exudates on its surface. Metritis, especially in goats, may also be found in this disease due to retention of placenta, which mainly is due to secondary bacterial infection. Abortion before 2-3 weeks of expected lambing could be the first detecting sign (Selim 2016). The environment is contaminated by uterine discharge and fetal fluids and the organisms are shed in the infected placenta.

Transmission of Chlamydiosis occurs through ingestion and inhalation of organisms from the contaminated material and environment (Navarro et al. 2004). The exact pathogenesis is still unknown. However, it is suggested that the organisms colonize in the trophoblast cells of the fetal cotyledons and spread the infection to the inter-cotyledon area of the chorion to produce necrotic placentitis and edema. This causes characteristic thickening of the placental membranes and cotyledons which impairs the exchange of nutrients and oxygen between fetus and mother (Buxton et al. 2002). Moreover, there are disturbances in the blood progesterone levels that lead to abortion (Soomro et al. 2015). The organisms may reside in lymphoid tissue in the latent or silent form in non-pregnant animals until the onset of pregnancy. The pathological changes in the placenta start to develop after 90 days of gestation but the infection remains subclinical (Essig and Longbottom 2015).

The products of abortions are sources of contamination for the environment and susceptible animals, as well as humans. The organisms can be found in large numbers in the vaginal fluids, placenta, and fleece of dead lambs. The live lambs may be carriers and a risk factor for naive sheep and goats (Caspe et al. 2020). The animals with abortion in their last pregnancy due to *C. abortus* may shed this infectious bacterium amid both the subsequent periovulation period and at the time of their next lambing, while this risk is minimal as proved by the latest molecular studies. The risk of venereal transmission by males is very low and has relatively no role in the spread of the disease. Although vertical transmission in ewes is possible but horizontal transmission has great havoc for susceptible animals. An aborting ewe can infect other pregnant ewes, however, the animals infected after 110–120 days of gestation will normally complete their gestation length.

Several Chlamydial species that belong to the genus Chlamydia and family *Chlamydiaceae* can cause various infectious diseases in humans, other mammals, and birds. Different species of genus Chlamydia can cause respiratory illness and reproductive problems in men (Joseph et al. 2015). In animals, they can cause abortion, keratoconjunctivitis, infertility, and respiratory disease (Girjes et al. 1988). As the diagnosis of the disease is not confirmatory, precautions should be taken to minimize the risk of infection for the ewes and humans.

The serological diagnosis in the past was mainly based on CFT, but it is less sensitive and less specific due to cross-reaction with other gram-negative bacteria and other Chlamydia species like *Chlamydia pecorum* having LPS antigens. At present, more sensitive and more specific serological ELISA-based test has been developed which uses major outer membrane protein (MOMP) and polymorphic outer membrane protein (POMP).

Table 1: Prevalence of *Chlamydia abortus* with different diagnostic techniques

Country	Prevalence percentage			The method used for diagnosis	Reference
	Tested	Positive	%		
Bosnia and Herzegovina	178	77	43.3	ELISA	Krkalić et al. (2016)
Ireland	201	42	20.9%	ELISA	O'Donovan and Forsythe (2015)
Iraq	124	12	11.1%	ELISA	Fahad and Salman (2017)
Jordan	25	13	52%	IHC and PCR	Hailat et al. (2018)
Saudi Arabia	399	30	7%	ELISA	Aljumaah and Hussein (2012)
Turkey	71	7	9.8%	PCR	Kalender et al. (2013)
Iran	364	20	5.71%	ELISA	Borujeni et al. (2019)
Mexico	246	12	4.9%	ELISA	Campos-Hernández et al. (2014)
	801	118	14.73% (Himachal Pradesh)		
	1221	60	4.91% (Andhra Pradesh)		
India	24	3	12.5% (Jammu and Kashmir)	AGPT	Chahota et al. (2015)
Belgium	958	38	4.05%	ELISA	Yin et al. (2014)
Egypt	675	93	13.7%	ELISA	Selim et al. (2021)
China	1732	323	18.65%	IHA	Qin et al. (2014)
Saxony (Germany)	1714	259	15.1%	ELISA	Runge et al. (2012)
Italy	27	3	11.1%	PCR	Greco et al. (2005)
Algeria	144	51	35.4%	ELISA	Merdja et al. (2015)
Costa Rica	359	19	5.29	ELISA	Villagra-Blanco et al. (2015)
Switzerland	235	10	4.2%	PCR	Borel et al. (2006)
Taiwan	112	37	33.3%	PCR	Wang et al. (2001)

Table 2: Human infections of *Chlamydia abortus*

Serial No.	Gender infected	Pregnancy duration	Symptoms	Reference	Country
1.	Female 22	24th week	Fever, headache, heartburn, shivering, sweating, vomiting, abortion	(Roberts et al. 1967)	UK
2.	Female 28	28th week	Fever, influenza-like illness, shock, renal failure, DIC, stillbirth	(Wong et al. 1985)	UK
3.	Female 25	Not available	Fever, dry cough, fatigue, malaise, Pneumonia	(Hyde and Benirschke 1997)	USA
4.	Female 20	26th week	Sepsis, respiratory distress, Preterm fetal loss	(Kampinga et al. 2000)	Netherlands
5.	Female 39	Non-pregnant	Lower abdominal pain, fatigue, intermittent fever, irregular menstrual cycle, hypochromic anemia, previous miscarriages	(Walder et al. 2003)	Austria
6.	Female 29	25th week	Abdominal pain, headache, dry cough, malaise, fever, renal and hepatic dysfunction, leukocytopenia, thrombocytopenia, and Increase C-reactive, Stillbirth	(Meijer et al. 2004)	Netherlands
7.	Female 32	16th week	High fever, Septicemia, dyspnea, Pneumonia, increase C-reactive, thrombocytopenia, fetal death	(Walder et al. 2005)	Italy
8.	Female	31st week	Fever, septic shock, respiratory distress, multiple organ failure, premature delivery	(Janssen et al. 2006)	Netherlands
9.	Male 47	NA	Respiratory distress, atypical pneumonia	(Ortega et al. 2016)	Spain
10.	Female 27	23rd week	Headache, cough, fever, in utero fetal death	(Pichon et al. 2020)	France

Prevalence

Chlamydia abortus is prevalent worldwide and is a major cause of abortion in sheep in many countries. Prevalence of *Chlamydia abortus* in different geographic regions of the world is shown in Fig. 1 and Table 1.

Transmission

Its unique biphasic developmental cycle has morphologically and functionally two different chlamydial forms; 1) an Elementary body that attaches to the eukaryotic host cell and initiates infection; 2) a Reticulate body. After getting entry into the host cell, the elementary body converts into the reticulate body, which is non-infectious but metabolically active. Reticulate bodies start to multiply by asexual binary fission in non-fusogenic vacuoles, which are also named as inclusions (Marschall et al. 2020). These elementary bodies keep growing until inclusions fill the cytoplasm of the host cell. Within 24-48 hrs, elementary bodies start to re-convert into reticulate bodies, which are then released from the host cell and may cause infection in nearby cells (Longbottom et al. 2019).

Aborted ewes are the main source of Chlamydial transmission to other animals (Pellerin et al. 2019). The product of abortion (uterine discharge, fetus, placental membranes, fetal fluids) are risk factors for the contamination of the environment. Such an environment is highly infectious for the naive ewes (Li et al. 2018). So, the new animals are at greater risk in such an environment. The organism may survive in the environment for several days under favorable climate (Gitsels et al. 2020).

The overwhelming courses of transmission can vary between Chlamydial species, infection disorders, and hosts. *C. abortus* is regularly transmitted to other creatures through aborted material; it can also spread

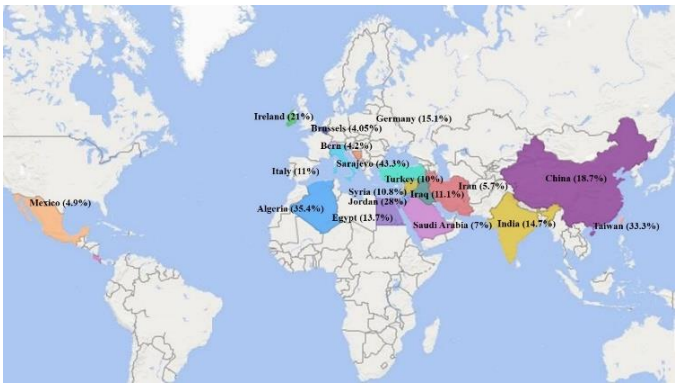

Fig. 1: GIS mapping of the prevalence of Chlamydiosis in different countries. infected materials (Longbottom and Coulter 2003; Sillis and Longbottom 2011). As most of the reported cases of C. *abortus* in humans have been through direct or indirect contact with animals, especially those with a history of abortion due to Chlamydial infection, women in pastoralist families must take precautionary measures while approaching the animals, especially during lambing season (Meijer et al. 2004: Essig and Longbottom 2015). The infection in humans is mostly associated with pneumonia, pulmonary edema, and placentitis. Histologically, inter-villositis and the presence of Chlamydial inclusion bodies in the placental trophoblasts are the most characteristic findings (Essig and Longbottom 2015). A review of clinical symptoms observed in most of the reported cases is presented in Table 2.

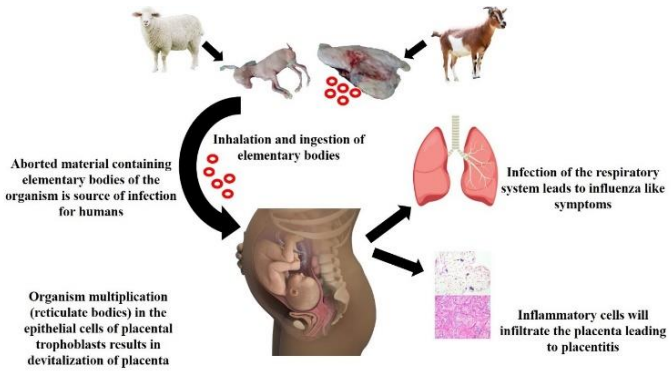

Fig. 2: Transmission of *Chlamydia abortus* from animals to humans.

through other secretions and excretions like feces, etc. Pregnant ruminants can shed a huge number of C. *abortus* within the placenta and vaginal liquids during normal and abnormal births. Shedding of a pathogen in vaginal fluid starts within two weeks before abortion of fetus especially in goats and may continue (often discontinuously) for many weeks afterward. Sheep and goats can be carriers of C. *abortus*, which cause persistent infection in sheep for at least 2-3 years. A few studies have reported that this organism can also be shed in small quantities at the time of estrus and during subsequent pregnancies of animals.

Aborted fetal membranes and fluids are the main sources of spreading infection. Transfer of this pathogen to susceptible ewes occurs after consumption of contaminated feed and water with aborted fluids and tissues (Arif et al. 2020). After ingestion, this infective agent can cause abortion within about 60 to 90 days and sometimes ewes that become infected late in the gestation period may give birth to weak lambs (Sargison et al. 2015). Once the aborted ewes recover, they show no signs of sickness, unless the secondary infection occurs.

The weak-born lambs should be isolated from the healthy herd, as they are also a source of infection for the susceptible animals. The infection becomes latent in healthy sheep and goats, without causing any illness until the next pregnancy and leads to abortion in the last month.

Aborting sheep can also be an infectious risk for the other pregnant sheep in the same lambing season, while the ewes which get infected in the last 1-2 months of pregnancy usually go on to normal delivery (Laroucau et al. 2018).

Pathogenesis

Chlamydia abortus is recognized as the main cause of abortion in the late-term pregnancy of sheep and goats. The other symptoms of *Chlamydia abortus* infection in sheep are conjunctivitis and other health pathologies (Singh et al. 2017). The organism gets into the body mainly through inhalation and rarely through ingestion or abrasions. It spreads to other body organs through blood or lymph. Initially, the organism affects the tonsils and nearby lymph nodes, causing inflammation of these organs, and resulting in the necrosis of cells. It shows biphasic development in the host cell (Essig and Longbottom 2015). It enters the host cell in the form of an elementary body (EB) and is converted to a reticulate body (RB), which is replicating and metabolically active but noninfectious stage and resides intra-cellularly as cytoplasmic inclusions bonded by lipid membranes. The cell cytoplasm becomes filled by these replicating vacuoles and RB re-condenses to EBs (Wheelhouse et al. 2012). The cell bursts, releasing the pathogens to infect the other cells (Wheelhouse et al. 2012).

The *Chlamydia abortus* presents a specific type of proteins named polymorphic membrane proteins (Pmps), also known as autotransporters (ATs), which are strongly immunogenic and cause the release of inflammatory mediators from cells. These proteins are of great importance in comprehending the pathogenesis and virulence of the organism (Wheelhouse et al. 2012). The fact that the infection with *Chlamydia abortus* before pregnancy or in early pregnancy does not show any clinical signs and remains latent can be correlated with the activation of the immune system of the host due to the high immunogenicity of these specific proteins. The infection during pregnancy becomes apparent due to the suppression of the immune mechanism of the body (Longbottom et al. 2013).

The clinical manifestation of Chlamydial infection is mainly characterized by placentitis, which leads to various complications such as septicemia, stillbirth, and abortion in advanced stages of pregnancy (Pan et al. 2017). As the ewes get pregnant, the latent infection reappears and triggers subclinical Chlamydiosis (Essig and Longbottom 2015). This leads to the infection and inflammation in the placental trophoblasts of chorionic

villi. The growth and pathogenesis of *C. abortus* are not obvious till day 90 of the pregnancy. The infection increases following the advancement of the pregnancy. The infection in the placenta may be correlated to the formation of hematoma in chorionic villi of the placenta (Essig and Longbottom 2015). The cause of these hematomas is the release of blood from capillaries in the hilus of ovine placentomes. The organism gets access to the trophoblastic epithelial cells through this route where they proliferate, and cytoplasmic inclusion bodies are produced. Inflammation and edema are produced in the placentomes and inter-cotyledonary tissues when the infection spreads to the surrounding tissues of the placentomes. The cotyledons and placental membranes appear reddened and thickened in response to the infection (Essig and Longbottom 2015; Caspe et al. 2020).

The exact mechanism of the abortion by *Chlamydia abortus* is not fully understood, however, one of the possible causes can be the devastation of the chorionic epithelium and placental membranes, leading to the mutilation of the function of placentomes and impairing the transport of oxygen and nutrients during the maternal-fetal exchange. The other possible underlying cause is the decrease in the release of progesterone in the infected dam. Progesterone is the main pregnancy hormone in the pregnant ewe and is mainly produced from epithelial cells of the chorionic villi. The progesterone release is decreased in response to the damage of these cells. The local production of PGE_2 and estradiol is increased (Caspe et al. 2020; Essig and Longbottom 2015). The inflammatory mediators, such as tumor necrosis factor and interleukin, are produced from the trophoblastic cells, causing the inflammation. The interaction of these hormones and mediators leads to early induction of parturition, resulting in premature birth or abortion. The organism also affects the liver, lungs, and brain of the fetus, impairing the function of these organs (Essig and Longbottom 2015).

Lesions

Grossly, the placental tissue shows purulent to necrotic placentitis. There is edema and hemorrhages of the placenta along with the presence of necrotic foci and purulent exudate in the cotyledons (Borel et al., 2018). Dissemination of infection to fetal tissues results in necrotic and inflammatory lesions in multiple fetal organs.

Histo-pathological examination shows the necro-suppurative placentitis with the infiltration of inflammatory cells, including neutrophils, monocytes, and macrophages (Livingstone et al. 2017). There is evidence of vasculitis and thrombosis in the inter-cotyledonary membranes. The mononuclear cells also invade and can be seen in the inflammatory exudate and affected arteries and arterioles (Essig and Longbottom 2015).

Diagnosis

There is no specific or characteristic sign of this disease, as the infection remains undetected. There may be vulvar discharge and behavioral changes just before the abortion, but this is not a specific sign (Villagra-Blanco et al. 2015). In some cases, there may be thickened cotyledonary membranes with reddish appearance due to edema having viscous creamy exudate, while in some cases inflamed and necrotic placenta can also be observed. These signs may be confused with other abortion-causing pathogens; therefore, laboratory confirmation is required for the exact diagnosis of *Chlamydia abortus* (Livingstone et al. 2017). The tumor necrosis factor can play a major role in the progression of abortion (Buxton et al. 2002).

The PCR and ZN staining can't detect infectious agents, as the excretion of the pathogens till the onset of abortion and maternal antibodies to *C. abortus* rapidly increase to develop the protective immunity. There are distinctive strategies for the clinical diagnosis of *C. abortus*. Serological detection is done by immunological techniques, like complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) (Rekiki et al. 2006).

Several methods and tests have been developed for the laboratory diagnosis of *C. abortus;* these are based on serum evaluation of aborted animals, examination of tissues taken from the aborted fetuses and other abortion products. Isolation and culture of the organism are highly reliable method, but it requires proper facilities and skillful expertise (Opota et al. 2015). Complement fixation test (CFT) is widely used for the serological diagnosis of *Chlamydia abortus,* but it tends to show higher sensitivity for the other gram-negative organisms like *C. pecorum*. Therefore, results can be confusing with other gram-negative organisms due to their low specificity (McCauley et al. 2007). Tissue samples from the fetus and placenta can be used for the isolation and identification of *C. abortus,* using Giemsa and immunohistochemical staining and specific monoclonal antibodies (Sargison et al. 2015). ELISA-based serological tests are now being used for the diagnosis of *Chlamydia abortus*. Blood samples from animals having a history of abortion are collected for ELISA-based serological assays. These assays detect the anti-chlamydial antibodies in serum. These tests use specific antigen proteins like MOMP (major outer membrane protein) and POMP (polymorphic outer membrane protein) for the identification of *C. abortus* (Livingstone et al. 2005) and to minimize the chances of cross-reactivity.

DNA of *Chlamydia abortus* can be detected through PCR by using vaginal swabs, aborted fetuses, placental tissues, and other fetal tissues. The organisms are present in large numbers in the abortion material and vaginal exudates. Vaginal swabs have some advantages to be used for PCR. Inoculation of organisms in chicken eggs is also used to identify the Chlamydia DNA (Kalender et al. 2013).

Polymerase chain reaction (PCR) is another new reliable method for the identification of *C. abortus* (Campos-Hernández et al. 2014). Recently, real-time PCR has become the technique of choice for many diagnostic laboratories due to its rapidity, sensitivity, and ease of standardization. This method is rapid, as culturing of

organisms is not essential for diagnosis (Santoro et al. 2019). PCR detects the Chlamydial DNA by targeting different components of the genome. Outer membrane proteins (ompA), including omp1, omp2, and amplification of pleomorphic genes enables the detection of Chlamydia through PCR. Moreover, detection of *Chlamydia abortus* has been achieved through other components, like genes encoding 16S RNA, helicase, and 16S-rRNA. There may be some problems due to which results may be false negative or isolation cannot be achieved if the samples are taken during the pregnancy or vaginal swabs are taken after many days. At the time of abortion, sampling gives remarkable results for isolation and identification the organism. PCR-mediated recognition of *C. abortus* may not relate to fruitful isolation and culture of the bacteria from tissue samples at all times (Kalender et al. 2013).

Zoonosis

Human infections of *C. abortus* may be acquired from contaminated items of premature birth, parturition or carelessly processing laboratory cultures of the organism. Women involved in handling the livestock, especially the aborted animals, are at high risk of acquiring the infection. Besides causing generalized septicemia, the organism is capable of inducing stillbirth or abortion in pregnant women (Pospischil et al. 2002). Approximately 94% cases of animal acquired Chlamydiosis result in fetal loss and 6.3% in maternal death. Clinical signs vary from the acute febrile condition, respiratory distress, fatigue, malaise, liver, and kidney function failure to shock, disseminated intravascular coagulopathy, and death (Katsura et al. 2020). Some authors have reported "influenza-like illness", terminating in fetal death, in pregnant females. The Chlamydial organism reaches the placenta, and multiplies in the epithelial cells of the trophoblasts, which damages the placental vitality. The level of C-reactive protein is raised, along with a decrease in platelet count in the infected females (Pichon et al. 2020). In non-pregnant females, the infection is associated with the development of "pelvic inflammatory disease" (Walder et al. 2003). A recent review reported that out of 23 cases of gestational Chlamydiosis, 20 were acquired from sheep and goats (Katsura et al. 2020). A case of atypical pneumonia in a veterinary researcher has been associated with C. *abortus* (Ortega et al. 2016). Therefore, biorisk management should be adopted during the handling of culture and potentially

Prevention and Control

Management of flock

It is an important aspect to control this disease, as it causes great zoonotic and economical losses. It spreads from animal to animal; horizontal transmission occurs in a herd and also from animal to humans by direct contact with aborted fetal material. For the prevention of *C. abortus*, one should take strict measures like isolation of seropositive animals from the rest of the herd/flock. Animals with recent abortion history should be kept isolated until their uterine discharge is completely dried up (approximately in 7-10 days). The disposal of infected dead fetal membranes or bedding should be properly buried or incinerated out. The infected place or bunkers should be properly disinfected and thoroughly cleaned (Robertson et al. 2018).

The veterinarian or flock manager should use his protective equipment during handling of infected animals and materials. Health workers should not use the same PPE to handle other animals, until proper disinfection of PPE is done to reduce the risk of spreading the disease in healthy animals, as the infected animals may shed organisms (Zezekalo et al. 2020). Purchasing new animals should be from a reliable source. The introduction of newly bought animals in the flock should be done after serological testing from a trusted laboratory.

Antibiotic treatment

Seropositive animals should be isolated from rest of the herd and provided supportive treatment, such as multivitamins or minerals, to reduce the severity of the infection. Antimicrobial susceptibility tests have shown that macrolides and tetracycline (20 mg/kg) can be used for treatment (Barhoom 2015). The mode of action of anti-chlamydial antibiotics is through inhibition of the Chlamydial protein synthesis by binding to the 30S ribosomal subunit (Bommana and Polkinghorne 2019). Treatment should be given on the first onset of clinical signs (if any sign appears). Single-dose of long-acting antibiotics minimizes severity of the disease. But such antibiotic therapy does not recover the animal completely or reverse any pathological changes that had occurred. It has also been reported that routine oral administration of tetracycline (400-500 mg) fortnightly can reduce the shedding of Chlamydia in the lambing season. This also reduces degree of contamination of the environment and farms. The best way of controlling the disease is the combined use of antibiotics, vaccination, and proper herd management (Longbottom et al. 2013).

Vaccination

The control of *C. abortus* infection has been focused on the vaccination protocol to minimize the abortion rate and excretion of the organism. For *C. abortus,* both attenuated and live vaccines, having origin from different countries, are available in the market. Commercially available live attenuated vaccine, which has strain *C. abortus* (temperature-sensitive), shows good results (O'Neill et al. 2019). But it is compulsory to maintain the cold chain of vaccines for best performance against infection. It is also observed that vaccines may induce the disease and abortion (Caspe et al. 2020). The vaccine should be administered 4 weeks before mating and antibiotics should not be administered with the living vaccine. The inactivated vaccine, prepared on embryonated eggs or cell culture, is also available. Now-a-

days, scientists are focusing on preparing vaccines with different strains that would be easy to handle, cheap and safe to administer in animals.

A vaccination trial of a commercially available, inactivated vaccine at different doses revealed that there was no significant difference between the control group and the vaccinated groups after the administration. The vaccine showed favorable effects on the birth weight and weight gain in lambs during the first 30 days of their life (García-Seco et al. 2016).

Live attenuated vaccine of Chlamydial strain 1B has been seen to induce abortion in the ewes, as the same strain was isolated from the aborted fetuses and other abortion material. This study showed that 1B strain may not be properly attenuated and has a risk for induction of abortion. There should be the repetition of live vaccine after every 2-3 years and the administration of inactivated vaccine should be done annually. Now-a-days, research on the vaccine is focused to develop more effective, cheap, stable and safe vaccines, which may not cause disease in animals and have a good ability to produce sterile protective immunity.

Disinfection

Cleaning and sterilization, together with individual cleanliness (e.g., hand washing, cleaning/disinfection of footwear) are important in preventing the spread of fomites. Aborting animals with Chlamydiosis should be separated because they are the risk of infecting others. Abortion or birth products from infected animals should be removed, and the area should be cleaned and disinfected. Most strains of Chlamydia are susceptible to many available disinfectants. Chlamydia is inactivated in the presence of sodium hypochlorite, glutaraldehyde, 70% ethanol, peracetic acid, and also in the presence of Quaternary ammonia. It is resistant to some alkalis and acids. Like some bacteria, application of moist heat having a temperature of 121C° for 15 minutes and dry heat at 160-170C° for one hour can cease the biological activity of Chlamydia.

Concluding remarks: The global distribution of Chlamydial bacteria infections and its sharing at the animal-human-environment interface suggest an urgent need for interdisciplinary approach, such as One Health, to control this neglected disease. Various pathogenic species of Chlamydia are associated with zoonotic transmission to humans and adverse public health outcomes or losses. There is a dire need to circumvent drug resistance in Chlamydia and the development of protective vaccines for animals and humans. Awareness, containment, and community education would be indispensable to mitigate occupational risk and dissemination of Chlamydial pathogens at the animal-human nexus.

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STAPHYLOCOCCUS AUREUS: COMMENSAL TO MUTATING PUBLIC HEALTH PATHOGEN

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INTRODUCTION

The word "*Staphylococcus*" is taken from two Greek words i.e., "staphyle" meaning a bunch of grapes and "kokkos" meaning berry, which indicates the microscopic appearance of organism following Gram staining. The word *Staphylococcus aureus* (*S. aureus*) means "Golden Cluster Seed" that's why named as "golden staph". *S. aureus* is coccus in shape, Gram-positive, non-spore forming, non-motile, opportunistic, catalase positive, coagulase positive, and oxidase negative bacterium. It gives different color colonies on different agar media, such as yellow on mannitol salt agar, greyish to greyish white or golden colonies on blood agar, and pink color colonies on chromogenic agar. Under microscopy, *S. aureus* appears as round, in the form of bunches, that shows the multiplication of *S. aureus* in different planes. This bacterium is categorized as an important pathogen that causes mild to life-threatening diseases. Its commensal and opportunistic capabilities make it able to colonize at different sites of animals, and humans. The *S. aureus* is a common inhabitant of skin, mucosa, GIT (gastrointestinal tract), urinary tract, and especially the respiratory tract in anterior nares (Cuny et al. 2010). It produces many kinds of different substances like proteins, enzymes, toxins etc. The proteins produced by *S. aureus* include protein A and fibronectin binding protein that help the bacterium to adhere and colonize on cell surfaces. The enzymes include coagulase, catalase, lipases, nucleases, proteases, collagenases, and beta-lactamase, while the toxins include exotoxin, endotoxins, enterotoxins, alpha, beta, gamma hemolysin and PVL (Panton-Valentine leukocidin). These all substances enhance the ability of *S. aureus* to infect healthy persons, which may lead to necrotizing and hemorrhagic fatal pneumonia (Gillet et al. 2002).

The infectivity of *S. aureus* has been aggravated by increasing resistance to antibiotics, and methicillin-resistant *S. aureus* (MRSA); it is usually encoded by a gene called *mecA* that encodes the penicillin-binding protein 2a (PBP 2a) that is linked with increased mortality and morbidity compared to methicillin-sensitive *S. aureus* (MSSA) (Katayama et al. 2000). Firstly, MRSA was mainly linked with hospital or health-care settings and its acquisition-related with known risk factors (Chambers 2001). But recently, it is propagated into the community known as Community-Associated MRSA (CA-MRSA) and has been concerned in reports of threatening infections in salubrious persons. These reports of infection in humans and companion animals have exhibited the animal potential to act as a source for the spread of MRSA (Cefai et al. 1994). Increasing interest about MRSA in the community has recommended for surveillance, including carriage rates in healthy cats, dogs and also in humans. Almost 25% of humans contain *S. aureus* in the nasal cavity, which acts as significant source for infection (Noskin et al. 2005). The pathogen holds zoonotic and humanotic transmission of MRSA from humans to animals and animals to humans that puts the community at a great risk. It causes light to severe life-threatening infections in humans and animals. An investigation in the USA showed the huge loss due to HA-MRSA, approximately seven million admissions in the hospital were due to *S. aureus* infections. The annual loss due to these infections is estimated to be $2.7 million, which is a huge loss that puts the country to an economic burden of somewhat $9.5 billion with 12,000 mortalities annually (Noskin et al. 2005). MRSA is exceptionally predominant at medical centers around the world. However, Higher MRSA prevalence (>50%) was observed at medical centers of North America, South America, Asia, Sri Lanka, South Korea, Vietnam, Taiwan, Thailand and Hong Kong. Conversely, lower number of reports are observed in India (22.6%) and Philippines (38.1%) (Song et al. 2011).

Staphylococcus aureus to methicillin-resistant *Staphylococcus aureus*

The bacterium derived from the puss was named as "*Staphylococcus aureus*" in 1881. In 19th century, a strong wave of mortality, reaching 90% of deaths from *S. aureus* infected people, remained prevalent until availability of penicillin, discovered in 1928 by Sir. Alexander Fleming.

Sooner, this bacterium developed resistance against penicillin, using beta-lactamase enzyme that hydrolyses beta-lactam ring of penicillin and makes the drug ineffective. A long way after this resistance, there was discovery of methicillin in 1950s, an antibiotic equally effective against *S. aureus*. To the dismay, the drug no longer remained effective due to strong resistance developed by the bacteria. The resistance was so strong that new strain has to be named as methicillin-resistant *Staphylococcus aureus* (MRSA). The resistance was due to penicillin-binding protein 2a (PBP 2a), and was equally resistant against all beta-lactam antimicrobials. Going into molecular analysis, it was found that resistance was due to *mec A,* which is present on large mobile genetic element called as Staphylococcal cassette mec (SCC mec) (Vengust et al. 2006). In 1961 in an experiment, 18 out of 50 Staphylococci were regarded as Celbenin (Methicillin) resistant Staphylococci. These isolates were found to have ability to retain both hemolytic and coagulase activities. Not to this only, the isolates even in the absence of methicillin, kept on retaining typical culture characteristics and resistance patterns. In initial years of emergence of MRSA, only three isolates out of 5444 of tested *S. aureus* could be identified (Barrett et al. 1968) due to complication in identification of MRSA because methicillin resistance in *S. aureus* varied in many isolates. Therefore, heterogeneous strains mainly consist of comparatively sensitive cells and extremely resistant cells that show phenotypical susceptibility to methicillin. However, phenotypic resistance expression can be increased by adding sodium chloride (NaCl) or sucrose to culture medium in the presence of β-lactam antibiotics (Datta et al. 2011).

Pathogenesis of *Staphylococcus aureus*

S. aureus is a commensal, as well as pathogenic, organism. It is normally present in the anterior nares of humans and animals. Some other sites of its colonization may include the axillae, groin, and gastrointestinal tract. Colonization increases chances for bacterial infection when host defense is broken, either due to shaving, aspiration, insertion of a catheter or surgery (Wertheim et al. 2005). Virulence factors for *S. aureus* may include numerous surface proteins, called "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), which are responsible for attack on host tissues by binding with collagen, fibronectin, and fibrinogen, and produce endovascular, bone, joint, and prosthetic-device infections (Menzies 2003). *S. aureus* can make biofilms (slime) on host and prosthetic surfaces, permitting it to stick on them by evasion of host immune system and antimicrobials. *S. aureus* can also make small-colony variants (SCVs), leading to tenacious and recurring infection. Chief protection of *S. aureus* is by making an anti-phagocytic microcapsule (type 5 or 8). The zwitterionic capsule can also make abscess. The MSCRAMM protein A can inhibit opsonization by binding with Fc portion of immunoglobulin (Gordon and Lowy 2008). *S. aureus* is responsible for neutrophil extravasation and chemotaxis to the site of infection due to secretion of chemotaxis inhibitory protein or extracellular adherence protein. During infection, *S. aureus* infection can metastasize to other sites by producing several enzymes like proteases, lipases, and elastases (Fig. 1). Septic shock develops in case of *S. aureus* due to its ability to activate immune system and coagulation pathways by peptidoglycan, lipoteichoic acid, and toxins production. In addition to this, some *S. aureus* strains also produce super antigens, responsible for toxicosis, like food poisoning and toxic shock syndrome (Dinges et al. 2000).

Pathogenesis of HA-MRSA

Methicillin-resistant *S. aureus* (MRSA) is a major pathogen in comparison to methicillin-sensitive *S. aureus* due to higher chances of disease occurrence and death rate. However, particular mechanism of pathogenicity is not known. Though, it is considered that protein related b-lactam antibiotic resistance, penicillin-binding protein 2A (encoded by *mecA* gene), directly causes immunopathology during MRSA infection. PBP2A is responsible for poor peptidoglycan cross-linking, which causes increased degradation and detection by phagocytes, and it leads to vigorous IL-1b production. Peptidoglycan separated from b-lactam confronted MRSA powerfully stimulates the NLRP3 inflammasome in macrophages, however these effects disappear due to peptidoglycan solubilization (Turner et al. 2019). Transmuted MRSA containing decreased peptidoglycan cross-links produce high IL-1b levels *in vitro* and cause severe diseases *in vivo*. Treatment of MRSA skin infection by b-lactam aggravates IL-1 related immunopathology. So, antibiotic provoked appearance of *mecA* during MRSA skin infection is responsible for immunopathology due to change in peptidoglycan structure (Madzgalla et al. 2016).

Pathogenesis of CA-MRSA

Virulence of CA-MRSA strains is increased due to increased fitness, enhanced evasion of the host immune system, and exclusive toxin production by *S. aureus*. Some researchers have suggested that PVL protein present in *S. aureus* has leukocyte lytic and dermonecrotic activity, leading to CA-MRSA infection (Chini et al. 2006). However, other studies proposed that the linkage of PVL with higher *S. aureus* virulence is multifaceted, so it needs additional research. Additionally, recent studies have revealed that phenol-soluble modulins are up-regulated in CA-MRSA strains, in comparison to the level in HA-MRSA strains; so, it damages neutrophils and causes inflammation in mouse and bacteremia models. Additionally, enterotoxins may also play important role in these infections (Wang et al. 2007; Lee et al. 2018).

Pathogenesis of LA-MRSA

Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) is a comparatively new classification in veterinary medicine. Initially, it was caused by a single

clonal complex CC398, but now it is caused by many varieties of clonal complexes. Most common clonal complex is CC398 in Europe, while CC9 is common in Asia. LA-MRSA contains SCCmec cassettes limited mostly to SCCmec IVa and SCCmec V, but non-typeable cassettes and SCCmec type XI, containing *mecC*, also have been found. Livestock associated MRSA (LA-MRSA) was first identified in 2005 due to CC398 (Voss et al. 2005). Numerous studies have shown that CC398 MSSA of human origin misplaced human related factors such as Panton Valentine Leukocidin (PVL)-associated phages, toxic shock syndrome toxin I and exfoliative toxins (Ballhausen et al. 2017) and developed antibiotic resistance genes e.g., *mecA* and *tetM* related to livestock. Moreover, it is reported that chances of LA-MRSA infection are higher in individuals who are in contact with livestock (Goerge et al. 2017). Nonetheless, colonization depends upon frequency, strength and period of animal contact, as livestock are supposed to be momentarily colonized (Bangerter et al. 2016). Though CC398 is the chief MRSA strain separated from livestock, some clonal complexes, and sequence types other than CC398, have also been found in livestock and animal products. Amount of staphylococcal protein A gene (spa) types within CC398 is presently increasing (Peeters et al. 2015). Moreover, other *S. aureus* strains of animals have developed methicillin resistance. Now-a-days, methicillin resistance is being reported more commonly in pet animals compared to livestock, and resistance is also increasing. Though, the epidemiology in pets is totally altered and is restricted to some Staphylococcus pseudo intermedius and human related clones in addition to methicillin-susceptible *S. aureus* (MSSA) of CC398 (Gómez-Sanz et al. 2013; Lee et al. 2018).

Microbiological and Molecular Techniques for diagnosis of MRSA

MRSA is detected by using conservative approaches involving oxacillin disc diffusion, MIC and oxacillin screen agar methods. But in recent times, the Clinical and Laboratory Standards Institute (CLSI) suggested the use of cefoxitin disc diffusion technique for MRSA identification. Cefoxitin is a cephamycin type antibiotic that acts as an inducer of the PBP2a-encoding *mecA* gene (Velasco et al. 2005). Other method to identify MRSA is the latex agglutination assay, which is specific monoclonal Abs against PBP2a antigen. Additionally, CHROM agar is a new method to detect MRSA by using chromogenic medium (Diederen et al. 2005).

Agar plate methods

Cefoxitin and Oxacillin Disc Diffusion Test

S. aureus suspension equal to 0.5 McFarland standards was made for all isolates and tested with cefoxitin (30 µg) and oxacillin (1 µg) disc, on Muller Hinton agar. Incubation was done at 35°C for 24 hours. Zones of inhibition were then measured and compared with guidelines of CLSI. CLSI approves use of cefoxitin rather than oxacillin in disk diffusion method to monitor resistance against methicillin for *S. aureus*. Cefoxitin disk diffusion test gives better results with greater sensitivity as compare to oxacillin. Cefoxitin disk diffusion test has 97.3% sensitivity and 100% specificity, in contrast to the oxacillin disk. This higher sensitivity of cefoxitin is due to its higher ability to activate *mecA* gene to express PBP 2 compared to oxacillin (Broekema et al. 2009).

Test with Oxacillin Resistant Screening Agar (ORSA)

The 0.5 McFarland standards *S. aureus* suspension is inoculated on ORSA medium at 35°C for 48 hours incubation. ORSA comprises oxacillin (2 µl), 5.5% NaCl to prevent non-*staphylococcal* growth and aniline blue dye to identify mannitol fermentation by *S. aureus*. Development of blue colonies specifies the existence of MRSA. The ORSA is used to identify MRSA in laboratories, because it has ability to recognize mannitol fermenting bacteria. To confirm better sensitivity of MRSA, an enrichment broth is required and incubation time of 48h is given to primary culture on ORSA. Although ORSA is inexpensive and can be easily performed, but its chief disadvantage is delay in getting results. So, cefoxitin can be a better substitute indicator to detect MRSA. However, an additional E-test along with cefoxitin disc diffusion can be used to identify *S. aureus* strains, which show 20-22 mm inhibition zone diameter (Panda et al. 2016).

MIC by E-Test

Oxacillin MICs is examined by E-test on Muller Hinton agar with 2% NaCl. Incubation of plates is done at 35°C for 24 hours. The MIC value >4 µg/ml is deliberated as MRSA (CLSI 2012). E-test for MIC gives better results in comparison to other tests, because it is easy to perform as disc diffusion test and gives quite accurate results in specific test conditions under the support of PCR for *mec-A* gene (Ercis et al. 2008).

Genomic analyses

S. aureus genome was first sequenced by Kuroda et al. (2001) and until now eighteen genomes have been sequenced. Numerous other incomplete sequences have been kept in gene bank. Examination of this tremendous measure of information indicates that the genome structure has three chief segments, a spine of core genes, Mobile Genetic Elements (MGEs), and extensive discrete parts of DNA that encode activation ability, appearance of continuous exchange and (less normally) recombination. The *mecA* gene is placed on a versatile hereditary component, named staphylococcal cassette chromosome mec (SCCmec) embedded in the *Staphylococcus* chromosome up stream to the orf X (Ali et al. 2018). Four contrastingly composed SCC-mec components have been described. But, three kinds of SCC-mec components are ordinarily found in HA-MRSA strains, namely type I, type

II, and type III, present in various countries (Katayama et al. 2003). Chromogenic agar is the best medium to identify 92.9% MRSA isolates. PCR result was found to be positive in all isolates showing resistance to cefoxitin discs. Generally used marked genes to confirm *S. aureus* are *femA*, *orfX*, *Sa442*, and the *nuc* quality genes. The *femA* gene of *S. aureus* has a few areas of similarity with CoNS, and requires much greater attention during primer development (Kobayashi et al. 1994). *Sa442*-particular PCR has appeared to be valuable after DNA extraction from positive blood culture isolates, yet few disease-causing *S. aureus* strains may not have this particular locus. Other valuable targets incorporate staphylococcal chromosomal cassette (SCCmec)- related loci form the stable DNA nuclease gene (nuc).

The 16S rRNA amplification of gene sequencing (479 bp) is the most ordinarily used strategy for distinguishing the bacteria, including Staphylococci. Transferring binding protein (Tbp), having size of 42-kDa, encodes gene situated inside the cell wall of the *Staphylococcus* and also contains an enzyme known as Glyceraldehyde 3-Phosphate Dehydrogenase (housekeeping gene) (Ghebremedhin et al. 2008). PCR identification of the nuc gene (270 bp) is also recommended for the identification of *S. aureus*. Different mechanisms of resistance in MRSA include presence of resistant genes TcaR, TcaA, TcaB, TetR, TetM, PBP2a (*mecA*), or secretion of enzymes like DNA gyrase (A), DNA gyrase (B), Topoisomerase IV (A), Topoisomerase IV (B) and Beta-lactamase repressor); these are responsible for the use of efflux pump mechanism (Li et al. 2014). In short, typical phenotype of MRSA is due to the existence of mecA encoding penicillin-binding protein (PBP2a), having less efficiency for b-lactams. The *mecA* is entrenched in a large heterologous chromosomal cassette (SCCmec) element. Several MRSA strains bring upstream to the mecA gene (mecI-mecR1) coding for a repressor and an inducer of the *mecA* expression, correspondingly, as shown in Fig. 2 (Oliveira et al. 2011).

Proteomic analyses

Proteomics is defined as the study of structure and function of proteins in living organisms to understand the multifaceted nature of the organism. A few studies have used this method to clarify the efficacy of natural product as antibiotic agents (Khairon et al. 2016). Regulation system associated with the multidrug protection, pathogenesis and transmission of MRSA is basic system for the improvement of new antimicrobial agents developed for the treat MRSA infections and to monitor their anti-microbial activity for the anticipation of this superbug. The Bla and mec frameworks predict a basic region that protects from β-lactam drugs in MRSA. The regulation mechanism associated with the outflow of methicillin protection has been uncovered (Wilke et al. 2004). As mobile genetic element (MGE), MRSA contains the Staphylococcal cassette chromosome mec (SCC mec) region that develops the multidrug resistance. Resistant from beta-lactam anti-toxins, *S. aureus* is fundamental because of 1-lactamase, which is a chemical activated by β-lactam anti-toxins. The 1-lactamase gene (*blaZ*) is kept on a plasmid with two firmly connected loci firmly under the regulation of P-lactamase generation. After the confirmation of blaZ gene, two new genes have been recognized and sequenced named as blaI and blaR1. In spite of the fact that the elements of the blaI and blaRl genes have not been built up, their structural parts have been supposed similar to genes that control 1-lactamase generation in *Bacillus licheniformis*. In that framework, the chromosomally found structural genes blaI and blaRi are likewise encoded upstream of the 3-lactamase genes (blaP). *S. aureus* has also been equipped with *mecA* gene that encodes one kind of trans-peptidase, called penicillin binding protein 2a (PBP-2a). PBP2a opposes hindrance to β-lactam group of anti-microbials.

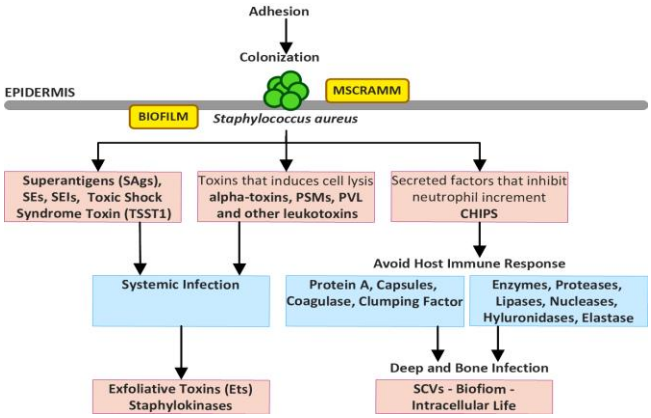

Fig. 1: Pathogenesis of *Staphylococcus aureus*.

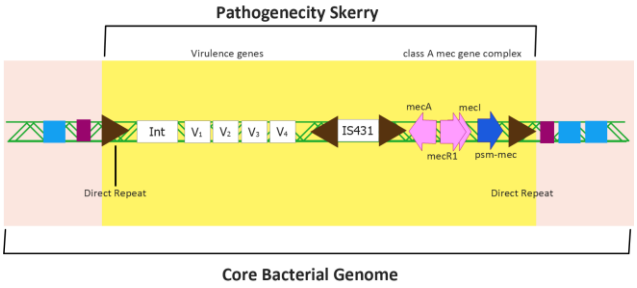

Fig. 2: Genome structure of MRSA.

Therapeutics and preventive measures

S. aureus is the leading cause of mortality in patients having developed MRSA infections. Almost 50% of the population may face invasive infections that lead to bacteremia and may cause death within 90 days (Nickerson et al. 2009). However, the incidence rate of MRSA is declining as a result of prevention strategies and use of a new combination of antibiotics available in the market. The reason behind the high mortality rate may be the lack of proper treatment at the initial stages of infections (Simor et al. 2016). Moreover, various virulence factors are associated with the mortality e.g., accessory gene regulator (agr) of Group I is an intrinsic virulence factor that is detected among MRSA isolates. In recent years, vancomycin has been considered as a drug of choice after methicillin resistance for aggressive MRSA

infections. But now the trend has been changed after the introduction of various new antibiotics in the market. Furthermore, researchers now suggest the treatment shift toward the combined therapy for MRSA infections.

Bloodstream infections and their management with combination therapy

A condition known to be caused by MRSA, called bacteremia, is more severe as compared to that caused by MSSA infection, and a long period of bacteremia will result with a more serious outcome. A study conducted in Australia has highlighted that 17% of MRSA cultures are found to be resistant to cephalosporin and ceftaroline. Many types of new agents are licensed in the market but they are not showing results superior to vancomycin (Abbott et al. 2015). A combination therapy recommended by the Spanish Society of Clinical Microbiology and Infectious Diseases includes a glycopeptide daptomycin along with B-lactam antibiotics against the MRSA infections (Gudiol et al. 2015). The phenomenon of action of daptomycin is also very important to know; it crosses the plasma membrane like calcium influx and potassium efflux, leading to apoptosis. Daptomycin reduces the expression of *mecA* gene by blocking both fem and aux factors. Daptomycin has an ability of reducing the attachment of PBP-2a to its peptidoglycan moieties before the synthesis of peptidoglycan in its early stages in the presence of oxacillin (Fig. 3). The benefit of using the daptomycin with B-lactam antibiotics is to enhance the binding of daptomycin (Dhand et al. 2011). During the previous decades, vancomycin has been considered as the most effective drug for treating the severe infections caused by MRSA. Collecting confirmation of aggregate resistance, unattainable pharmacokinetic/pharmacodynamic (PK/PD) targets and lesser consequences encounter the appropriateness of the primary place of vancomycin. The glycopeptides are used extensively for the treatment of VISA (vancomycin intermediate *S. aureus*) and hetero resistant VISA (hVISA). So, resistance also started developing against them, reducing the sensitivity to glycopeptides that resulted in the development of VRSA (vancomycin resistant *S. aureus*). Such isolates are more sensitive to other class of antibiotics, especially β-lactam antibiotics, even in the presence of *mecA* gene. This 'seesaw effect' describes sensitization of MRSA isolates to anti-staphylococcal β-lactam antibiotics by using higher minimum inhibitory concentrations (MICs) (Fig. 4) to vancomycin and daptomycin. The promising combined effects of vancomycin and b-lactam antibiotics are quite encouraging (Werth et al. 2013).

Fifth generation cephalosporin's role against MRSA infections

Out of five generations of cephalosporin, fifth generation is the most active against MRSA infections. Among the cephalosporin, the Ceftaroline and Ceftobiprole are the most commonly available drugs used at clinics. Ceftaroline is the approved drug against community-acquired pneumonia and acute bacterial skin and skin structure infections (Purrello et al. 2016). In 2006-2007, multicenter clinical trials were conducted among the hospitalized patients infected with community acquired pneumonia (CAP) to compare the ceftobiprole with ceftriaxone and linezolid. From 2008-2009, a multicenter FOCUS-1 trial was conducted by a scientist to check the efficacy of Ceftaroline against CAP. Almost 168 sites were selected across the world. Clinical curative rates of Ceftaroline were found to be high 86.6 and 83.3% in CE (clinical evaluable) population and mITT (modified intention-to-treat) population, respectively, while cure rates of Ceftriaxone were 78.2 and 77.7% in CE and mITT, respectively (File et al. 2011).

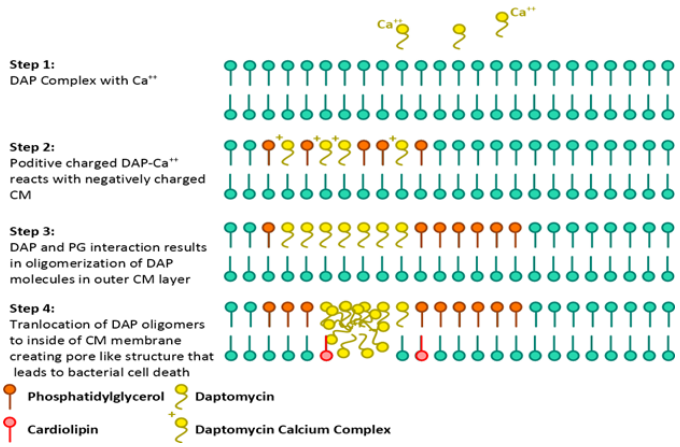

Fig. 1: Mode of action of Daptomycin against MRSA.

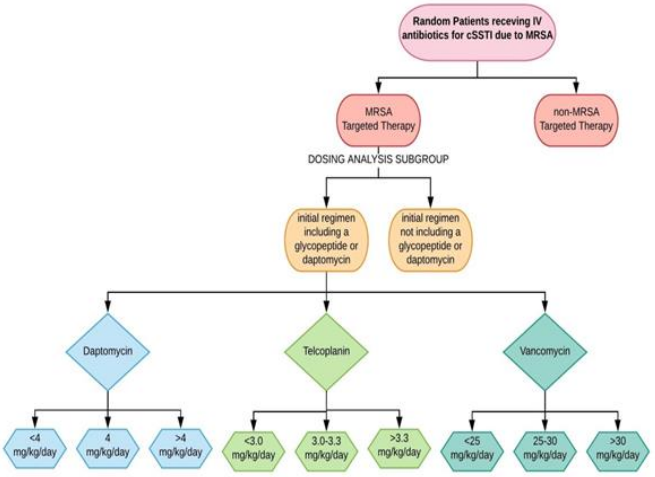

Fig. 4: Minimum inhibitory concentration (MIC) of various antibiotics against MRSA.

Novel antimicrobial agents for the intervention of MRSA infections

Several licensed and new agents have shown efficacy against MRSA infections, though defining their exact use needs further investigations. In this section, some of the newer antimicrobials active against MRSA have been discussed.

Oxazolidinones

Oxazolidinone is a new group of antibiotics, which are effective against a variety of Gram-positive bacteria, including methicillin-resistant and vancomycin-resistant Staphylococci. Oxazolidinone binds to P-site of 50S ribosome subunit and inhibits protein synthesis. Its activity is not affected by resistance to other inhibitors of protein synthesis though development of oxazolidinone resistance with 23S rRNA. Its high infiltration and accumulation in the tissue including bone, lungs, hematoma and cerebrospinal fluid permit its use for surgical infections (Bozdogan and Appelbaum 2004). Among the oxazolidinones, a new drug with the name of tedizolid is licensed for the treatment of skin and soft tissues infections for a standard course of 6 days. Tedizolid is a more effective drug and gives more advantages as compared to Linezolid. Among various benefits of tedizolid, the salient one is its efficacy against those isolates which are resistant to chloramphenicol-florfenicol resistant (cfr) methyltransferase gene (Flanagan et al. 2015). Cadazolid is a new oxazolidinone agent and it is quite effective against *Clostridium difficile* (Gerding et al. 2016), while radezolid is effective against the *S. aureus* isolates that are resistant to linezolid (Lemaire et al. 2010).

Tetracycline

Tetracycline antibiotics are inhibitors of protein synthesis. They inhibit translation initiation by binding to the 30S-ribosomal subunit and inhibit the binding of aminoacyl-tRNA to the translational mRNA complex. Several studies have shown that Tetracycline can bind to 16S and 23S rRNA (Chukwudi 2016). A new synthetic fluorocycline drug, Eravacycline, is effective against both Gram negative and Gram-positive pathogens including MRSA. Ribosomal hydrolysis and efflux pumps resistance is due to a mechanism called fluorination. Eravacycline is two to four times more active drug than tigecycline for Gram-positive organisms (Zhanel et al. 2016). Among aminomethylcyclines, omadacycline is more effective against MRSA infections, in addition to CAP and ABSSSI (Pfaller et al. 2017).

Fluoroquinolones

Quinolones are one of the most widely used antibacterial drugs in the world for the treatment of various bacterial infections in humans and animals. Structurally, these drugs contain a quinoline ring system due to which these are called quinolones. Quinolones and fluoroquinolones inhibit the replication of bacteria by blocking their path of DNA replication. Quinolones act by converting the target, gyrase and topoisomerase IV into toxic enzymes that break down the bacterial chromosome (Aldred et al. 2014). An investigational fluoroquinolone drug, delafloxacin, is active against Gram-negative and Gram-positive organisms. Due to its specific electro chemical properties includes uncharged at acidic pH and anion at physiological pH. A study was conducted in USA in 2011 to monitor the efficacy of Delafloxacin in comparison with vancomycin and Linezolid. The highest cure rates were observed with delafloxacin, followed by linezolid, while vancomycin showed the lowest cure rates (Kingsley et al. 2016).

Lipoglycopeptides

Lipoglycopeptides are a class of antibiotics with lipophilic side chains attached to glycopeptides that exhibit concentration-dependent bactericidal activity. They inhibit cell wall synthesis and disrupt the barrier function of the bacterial cell membrane. So, glycopeptide core binds to the terminal acyl-d-alanyl-d. The alanine chain of the cell wall has high affinity through hydrogen binding and interaction with hydrophobic filling. This prevents polymerization and crosslinking of the precursors of the cell wall (Damodaran and Madhan 2011). Among the lipoglycopeptides, three new agents are licensed and available in the market. Dalbavancin is a lipoglycopeptide licensed by FDA in 2014 and also by EMA (European medicine agency) after one year in 2015 for the treatment of ABSSSI (Bambeke 2015). Dalbavancin is semisynthetic lipoglycopeptide with long half-life derived from an actinomycete "*Nonomuria*" (Chen et al. 2007). Because of its long half-life of 10 days, it shows long activity for 7 days against MRSA with a single dose of 500 mg. Dalbavancin is especially used for the treatment of outpatient having complicated infections (Juul et al. 2016). Dalbavancin was also compared with vancomycin in a multicentre trial in 2011-2012 for ABSSSI. Excellent results were seen by dalbavancin with fewer adverse effects as compared to vancomycin (Boucher et al. 2014). Oritavancin is a second lipoglycopeptide approved by FDA and EMA in 2014 and 2015 respectively. It is a long-acting lipoglycopeptide for the treatment of ABSSSI (Takahashi and Igarashi 2018). It is dual in nature that suppresses transglycosylase and transpeptidase. This ability enhances its bactericidal property, and it shows the broad-spectrum activity against Gram-positive organisms including VRSA, VISA and vancomycin-resistant enterococci (VRE) (Bambeke 2014). Telavancin showed very effective response against HAP (hospital acquired pneumonia) caused by Gram-positive pathogens including MRSA (Sandrock and Shorr 2015).

Clinical management of MRSA by antibiotic combination

For clinical management of MRSA, many new combinations of drugs are being used, for example vancomycin and daptomycin are considered effective in bacteremia, while in hospital-acquired pneumonia (HAP) vancomycin or linezolid show more effective results. In case of acute bacterial skin and skin structure infections (ABSSSIs), any of both combinations can be used to treat MRSA. Other antimicrobial agents, such as doxycycline, clindamycin and trimethoprim/ sulfamethoxazole, are also effective in cases of ABSSSIs, depending upon the severity of infection (Liu et al. 2011). The use of these

agents also has adverse effects or drawbacks, for example, vancomycin is available only for parenteral use, minimum inhibitory concentration (MIC) creep, difficulties in the achievement of curative levels and emergence of vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA) and heteroresistant VISA (hVISA). The drawbacks associated with daptomycin are that it is not indicated in case of pneumonia, and is available only for parenteral use. Myelosuppressive, bacteriostatic and significant drug interaction type of drawbacks are associated with linezolid (Dhand et al. 2011).

Future drifts in the control of MRSA Infections

An act was passed in 2012 by US Congress with the name "US FDA Safety and Innovation Act" to facilitate the pharmaceutical industry by providing incentives about generating new antibiotics for the market. This act also provides fast-tracked approval of antibiotics with additional patent protection of five years for qualified products (Tillotson and Tillotson 2015). After the approval of this act, many antibiotics such as Oritavancin and dalbavancin, belonging to group lipoglycopeptides, and tedizolid belonging to group oxazolidinones, were approved by FDA. However, after their approval from FDA, several clinical trials were required, which are still in progress to explore the advantages of combination therapy of antibiotics for their clinical use.

Drug Modulation of MRSA strains

Outstanding amongst other techniques to control bacterial resistance and expand the life of existing antibiotics, is to connect them with modulators of drug resistance. For example, numerous β-lactam antibiotics mixed with potassium clavulanate proved to be best against MRSA. Coumarins involve a class of characteristic phenolic compounds described by solitary benzene intertwined to a α-pyrone ring. They are emerging with great organic potential, as exhibited in a few examinations; these are compounds with antifungal and antibacterial properties, and modulators of anti-toxin resistance (Bazzaz et al. 2010). The mending properties of some restorative plants against irresistible infections are outstanding and recorded through the human development. The dynamic auxiliary metabolites created from plants are for the most part responsible for these remedial properties. Various examinations have also shown the antibacterial properties of numerous plants extracts against MRSA. Although not all plants discovered dynamic against MRSA are enrolled in this group, however it is expected that the value of therapeutic plants as an elective hotspot for antibacterial specialists against MRSA would be demonstrated. Strangely, some restorative plants, when joined with a few anti-microbial agents, could upgrade the ability of the anti-toxins against MRSA pathogens (Babra et al. 2013). This upgrade of the anti-microbial action can be ascribed to Phyto-mixtures, which may obstruct the efflux pumps of microscopic organisms and enable the anti-microbial to cooperate and wreck the bacterial cell. This procedure is called "Synergistic multi target impact" (Coutinho et al. 2009). Numerous plants indicated synergistic impacts on safe pathogens. A combined effect of ethanol removed from *Turnera ulmifolia* leaves with antibiotics includes gentamicin and kanamycin that may improve anti-toxin activity against MRSA strains. The oil extracted from grapefruit was found to be effective against MRSA as potential efflux pump modulator (Abulrob et al. 2004). In a fascinating investigation, a Korean customary natural plan, known as Sami Hyanglyum-Hwan comprises of four herbs (Arecae semen, Coptidis rhizome, Aucklandiae radix and Rhei rhizome), has restabilized the adequacy of the anti-microbial ciprofloxacin after it demonstrated no impact when tried alone against some MRSA strains (Choi et al. 2015).

Antibacterial activity of non-steroidal anti-inflammatory drugs (NSAIDs)

Numerous studies have reported that non-steroidal anti-inflammatory drugs (NSAIDs) show antimicrobial properties but the mechanism of action is not clear. It has been reported that ibuprofen, diclofenac and aspirin show antimicrobial action against certain Gram-positive bacteria at 5 mg/mL, except mefenamic acid. Because Gram-negative bacteria have lipopolysaccharide layer which restricts the diffusion of most drugs due to its hydrophilic nature, so just aspirin has efficacy against Gram-negative bacteria. Conversely, Gram-posative bacteria lack this lipopolysaccharide layer, hence allowing easy infiltration of the antimicrobial agents into the cells (Zhong et al. 2015). NSAIDs show antimicrobial activity at much less concentration compared to normal therapeutic dose used for inflammation, pain or fever. In contrast to diclofenac, both aspirin and ibuprofen expressed bacteriostatic and bactericidal ability against tested MRSA, so they can be used as antibiotic adjuvant to treat MRSA infections (Chan et al. 2017).

Treatment of MRSA by combination of antibiotics and NSAIDs

NSAIDs, along with antibiotics, are used to treat community-acquired MRSA. Cefuroxime and chloramphenicol alone are used to treat MRSA, but these drugs do not show effective results. Since aspirin and ibuprofen show bacteriostatic and bactericidal effects against the MRSA strains, so the collective effects of both NSAIDs with cefuroxime and chloramphenicol were examined. It was observed that Ibuprofen/aspirin in combination with chloramphenicol/cefuroxime might act on various target sites of the bacteria, and ultimately produce either an additive or a synergistic effect. Practical implication of antibiotic-adjuvant method is seen in Augmentin (amoxicillin/clavulanate potassium), in which a β-lactam is combined with β-lactamase (resistance enzyme inhibitor) and it explains the use of amoxicillin to treat infections of β-lactam resistant bacteria (Yin et al.

2014). This NSAID–antibiotic combination can be prepared to treat MDR bacterial infections.

Vaccine development against MRSA

The ongoing reports by both the World Health Organization and Centers for Disease Control have featured the issue confronting us because of antimicrobial resistance with methicillin sensitive *S. aureus* (MRSA). MRSA contaminations have expanded levels of mortality, doctor's facility stays, septic shock and ensuing diseases. The contamination by this pathogen has risen to greater than 94,000 cases, with 18,000 deaths every year in the United States. It also causes billions of dollars losses in the United States and in many other countries (Klevens 2007). Given its significance, the improvement of an immunization protocol and development of new antimicrobials to control *S. aureus* is highly important. At present, there is no well-established antibody against MRSA. Efforts in the past have depended on single antigen arrangements, while current endeavors weighted towards different antigens. An immunization ought to be planned in the light of lethal factors communicated in various periods of disease, so that it can effectively fight against an expansive range of disorders induced by the microorganism (Broughan et al. 2011). *S. aureus* has distinctive sorts of destructive virulence factors; so, endeavors to build up a compelling immunization against this pathogen have been basically unsuccessful. In order to produce viable antibodies against various *S. aureus* strains, more than one antigen ought to be chosen. Also, to upgrade the host resistant reactions, the immunization must be joined by a suitable adjuvant.

In such manner, three antigenic determinants, including clustering factor A (ClfA), alpha-enolase (Eno1), and iron surface determinant B (IsdB), were assessed by accessible bioinformatics instruments for outlining an effective multi-epitope subunit immunization for the enlistment of safe reactions against Staphylococcal contaminations. Eno1 is a cell divider and multiple functional protein that is limited on the outer covering of multiple prokaryotic and eukaryotic cells. This protein also holds the ability to reside in the cytoplasm of a cell. It is available in all tested strains of *S. aureus* and has an exceedingly saved succession. This protein also helps in the adhesion process and plays a significant role in the spreading of this pathogen. ClfA is also a cell divider secured protein, which is present on the surface of *S. aureus* and helps its adhesion to host fibrinogen γ-chain. Past examinations have demonstrated that ClfA assumes a significant part in the acceptance of Staphylococcal diseases (Garcı-Laura and Foster 2009). Thus, this harmful factor, as an immunization segment, gives potential focus to the enlistment of a hearty dynamic and aloof insusceptible reaction to *S. aureus*. IsdB, the third antigenic determinant, is also a protein that helps in anchoring to the cell surface. It is uncovered on the exterior of cell. This protein is saved among different strains of *S. aureus*, and is communicated just under restricting iron shape. Immunoglobulins speak to the primary immunotherapy approach utilized as a part of people. For Staphylococcal infections, diverse arrangements, generally polyclonal, focusing on amassing factor or capsular polysaccharide, have been tried with clashing outcomes regardless of persuading animal models, particularly in pneumonia (Liu et al. 2011). These days, immunoglobulins are infrequently utilized for *S. aureus,* except for some particular signs where a few specialists will think about them. Isolation of *S. aureus* lethal factors is a gigantic test for immunization advancement. Without dependable biomarkers for *S. aureus* and surrogate insurance, trial disappointment can't be profoundly examined. Since *S. aureus* infections are conceivably heterogeneous, contrasted with Pneumococcus in pneumonia, it is additionally hard to develop an immunization agent focusing on a particular infection (Proctor 2012).

Conclusion

MRSA is not restricted to humans and animals but now it is transferred from humans to animals, animals to humans and from environment to both humans and animals. So, it has become a threat to one health and needs immediate attention. MRSA new strains are emerging continuously through the development of resistance to antibiotics and causing light to severe infections in both humans and animals. So, it is recommended to use a combination of drugs and alternative medicines to avoid this resistance. World health authorities have also suggested the need for effective control strategies to control MRSA infections by limiting the excessive use of antibiotics and by urging the health workers to prescribe the drugs in combination after correct diagnosis.

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PATHOLOGY AND PUBLIC HEALTH SIGNIFICANCE OF *SALMONELLA*

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INTRODUCTION

Genus Salmonella comprises of bacteria that are gram negative, rod shaped and belong to the family *Enterobacteriaceae* (Su and Chiu 2007; Arshad et al. 2008).) Organisms of this genus are non-spore forming, intracellular, ubiquitous, catalase positive, oxidase negative, and facultative anaerobes (Jantsch et al. 2011; Cox and Pavic 2014).) This genus has two species i.e. *Salmonella (S) enterica* and *S. bongori* (Park et al. 2017). Six subspecies also emerged out from *S. enterica* that include over 2600 serotypes or serovars (Issenhuth-Jeanjean et al. 2014) which are distinguishable by certain serological and biochemical tests (Fig. 1).

These subspecies include, *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica* and *houtenae* (Vohra et al. 2018). Approximately 99% of Salmonella infections result from these species (Tekintaş et al. 2018). The most common serovars reported globally are S. *typhimurium* and *S. enteritidis*, both are commonly found in human and animal cases (Archambault and Petrov 2006; Hendriksen et al. 2011; Manning et al. 2015).

Salmonella infection may manifest either as a self-limiting diarrhea/gastroenteritis, which is related to non-typhoidal Salmonella (NTS) or as typhoidal fever that involves septicemia and chronic diarrhea, and can be life threatening (Barrow and Methner 2013; Cox and Pavic 2014). The first form (NTS) is usually linked with inflammatory diarrhea, vomiting, nausea and abdominal pain (Smith et al. 2016). The severity of infection, like many other infectious diseases, depends upon host immune status, genetic makeup, the specie involved, serovar and dose of inoculation of the pathogen.

Public Health Significance

Public health issues and the capability for foodborne zoonotic spread have made Salmonella the focus of various international, national, and regional surveillance platforms (Pal et al. 2020). Salmonellosis is a major and economically important public health issue. Globally, an estimation indicates 33 million cases, and 0.5 million deaths associated with typhoid fever (Sandala et al. 2020), while NTS cause 93 million illnesses with 0.155 million deaths each year (Majowicz et al. 2010).

Salmonellosis lies among the most abundant food borne zoonotic diseases, and the disease can be spread to an extensive variety of hosts, including humans, animals and birds (Kaufmann et al. 2001; Jafari et al. 2007; Younus et al. 2011). It can also infect all species of animals including livestock, poultry, pigs, reptiles, amphibians, dogs and cats (Stear 2005). Children under 5 years of age (Su and Chiu 2007), elders and immune-compromised patients are most susceptible. Symptoms of the disease begin to appear 6–7 hours after infection with Salmonella and illness lasts for 2-7 days (Eikmeier et al. 2018). Intake of contaminated food related to animal origin, including meat, unpasteurized milk, raw or under cooked eggs or egg products, contaminated vegetables and fruit generally cause infections in humans (Lambertini et al. 2016). However, other food products, like contaminated green vegetables, can also cause transmission of disease (Cocciolo et al. 2020; Nguyen et al. 2021).

Consumption of poultry products, including eggs and meat, remains one of the leading causes of food borne infections of Salmonella in human population. Salmonella may enter poultry flocks through vertical and horizontal transmissions. The horizontal methods include direct transmission between flocks, contaminated feeds, and biological vectors like birds, insects, and rodents (Cocciolo et al. 2020), ultimately affecting human population. Epidemiological studies show that poultry flocks are infected directly by the environment of poultry farm (Dagnew et al. 2020).

According to US Food and Drug Administration, 2 to 4 million cases of Salmonellosis in humans occur every year only in US (Gerner-Smidt et al. 2006; Hassan et al. 2018). Salmonella causes wide range of diseases with enteric and typhoid fever, food poisoning, diarrhea and gastro-enteritis. Many serotypes of Salmonella do not have host specificity and cause disease in animals and humans. Salmonella has the capability to modify according to the climate and it can develop resistance against routine elimination practices of sanitation, chemical treatments and antibacterial drugs. *S. enterica* serovar Enteritidis is the most common serotype of Salmonella isolated from cases of food borne gastroenteritis throughout the world (Dantas et al. 2018; Das et al. 2019). *S. enteritidis* is responsible for outbreaks of human food borne salmonellosis and is generally associated with the consumption of poultry products. Inactivated *S. enteritidis* cell vaccine is one of the available methods to control *S. enteritidis* in breeders and laying hens, however, results in terms of efficacy vary (Crouch et al. 2020). Salmonella is an important zoonotic pathogen and its prevalence in the chicken meat and eggs poses a

continuous challenge to public health. *Salmonella enteritidis* has been the major cause of the food borne Salmonellosis pandemic in humans over the last 20 years (Raspoet et al. 2011), during which contaminated chicken eggs were the most important vehicle of the infection. Human Salmonellosis infections are usually acquired via the food chain as a result of the ability of Salmonella serovars to colonize and persist within the gastrointestinal tract of their hosts.

Antimicrobial Resistance

Antibiotics have consistently been viewed as one of the great revelations of the 20th century. The expansion in the use of antibiotics in emergency clinics, networks and the climate are increasing the antimicrobial resistance. The misuse of microorganisms has resulted in the massive economical and financial losses, and enhanced the overall burden of diseases. Antimicrobial resistance of pathogenic microorganisms is a test related with high morbidity and mortality (Johansson et al. 2021).

Antibiotics may be needed in high-risk groups, such as young children, the aged persons, and those with compromised immunity. With respect to the drugs, ampicillin, chloramphenicol, and trimethoprim–sulfamethoxazole can be utilized for the treatment of Salmonellosis. However, resistance to these drugs has increased significantly in recent years. Fluoroquinolones have been recommended for the treatment of Salmonella infections for adults, while third generation cephalosporin are the drugs of choice to treat very young patients or when fluoroquinolone resistance is present (Muhammad et al. 2015).

Resistance against the most common antimicrobials used in humans and animal production systems is a major concern in Nigeria, where Multi Drug Resistant (MDR) Salmonella strains are among the most common causes of bacteremia in children (Bogomazova et al. 2020). Salmonella serotypes with reduced sensitivity to fluoroquinolones in humans have been documented (Eguale et al. 2017). A previous study showed that the observed fingerprints had the same RAPD patterns, suggesting that grocers may have been infected from animal sources because the samples were taken from drug sites (Smith et al. 2011).

A major public health concern around the globe is the rapid emergence of antibiotic resistance in all types of bacterial species, especially in Salmonella. Use of antibiotics, even for very necessary purposes, is curbed when this resistance mechanism is developed. In addition to this, the transfer of this resistance potential to next generation makes this situation worse. The unjustified and unchecked use of antibiotics in feed animals favors the development of antibiotic resistance mechanism (Younus et al. 2009). A transferable plasmid is proven to be a source of resistance in many genetic analyses. Recent studies have shown that some serotype-specific virulence plasmids, through recombination with resistance plasmids, form hybrid plasmids or acquire gene cassettes consisting of different resistance genes. Such evolutionary events give virulent strain the advantage of surviving in an adverse pharmacological environment. With respect to serious consequences of drug-resistant Salmonella species, more targeted use of antibiotics in human medicine and the animal industry is needed (Lima et al. 2019). Continuous monitoring of antibiotic resistance and the use of antimicrobial agents in food animals is also essential.

Resistance of Salmonella to Different Classes of Antimicrobial Agents

S. *enterica* carries antimicrobial resistance genes against some drugs. For example, out of forty tetracycline resistant genes, five have been reported in Salmonella isolates. These include TetA (A, B, C, D and G). An efflux protein, comprising twelve trans-membrane parts, is being encoded by these genes that carries doxycycline, tetracycline, chlortetracycline and oxytetracycline. Minocycline can also be distributed by TetA(B). The five TetA genes are linked with different components, like Tn1721, Tn10 and Salmonella Genomic Island 1 or 2. Tn10 is prevalent among various Salmonella serovars. TetA (G) gene has solely been detected as an element of SGI1 or 2-allied with multi resistance gene clusters. In certain conditions, the same isolate was found in more than one TetA gene (Michael et al. 2005). Another group of β-lactam antibiotics includes penicillins and cephalosporins. β-lactamase enzymes deactivate the antibiotics, leading to the drug resistance. The β-lactamases form a distinct cluster of enzymes coded by significant repertory of genes (Michael et al. 2005).

Species of Zoonotic Importance

One of the major serotypes of S. *typhimurium* contaminates numerous animal species around the world, including livestock and poultry (Rao et al. 2020). The sort and seriousness of S. *typhimurium* disease differ through different species. Salmonella disease in calves prompts fever and looseness of the bowels, with high mortality if no anti-toxin treatment is given (Birhanu et al. 2018). S. *typhimurium* contamination in day-old chicks additionally prompts enteric and other fundamental illnesses, with a high death rate (Nascimento et al. 2019), while disease in more seasoned chicks prompts asymptomatic colonization of dung with relentless discharge of living organisms in defecation (Deng et al. 2020).

S. *enterica* subspecies enteric serotype *typhimurium* (S. typhimurium) is perceived as a human microorganism and represents a sanitation hazard around the world. It can taint a wide range of hosts, including poultry, pigs, sheep and steers. S. *typhimurium* is one of the five important serotypes from food delivering creatures and is a main source of food borne contaminations in various countries (Rao et al. 2020).

S. *enterica* have two serovars viz S. *typhi* and S. *paratyphi*, and these two serovars are capable of producing enteric fever in affected individuals (Monte et al. 2019). Both

serovars of S. *enterica* are pathogenic for humans and these are transmitted through oral and fecal routes associated with consumption of unhygienic water and foodstuff. Previous published data reveals many difficulties to observe the burden of this zoonotic disease in different locations, areas and countries, but all have high rate of death due to typhoid caused by these serovars. These Salmonella serovars are present in many countries, causing typhoid fever in Asia and Africa. Reports from some countries also show that its incidence has decreased in these countries.

Salmonella typhi is causing human infections in developing states (Wong et al. 2016). Primary source of transmission is through consuming unhygienic and contaminated food items and drinking of water. On the other hand, domestic birds, such as pigeons, can transmit *Salmonella typhi* infection to humans (Kaczorek-Łukowska et al. 2020). In a live bird market of Lahore Pakistan, domestic fowls and many fancy birds are sold and the contamination and spread of the disease is poorly controlled. As the international trade markets are the major source of Salmonella, blood contaminated feeds and poor sanitization may lead to its major spread.

Salmonella in Pakistan

Poultry sector is the second largest industry of economic importance in Pakistan. It plays a major role in the national gross domestic product (Hussain et al. 2015). In Pakistan, the total population of poultry birds is approximately 1105.91 million. Salmonella infections are comparatively more common in poultry and have great public health importance due to consumption of poultry products that may be contaminated with these pathogens (Vandeplas et al. 2010). Rearing of backyard poultry is an emerging interest of people around the country (Ali et al. 2018; Achakzai et al. 2020; Ahmed et al. 2021). This trend might possibly increase Salmonella outbreaks linked to these birds. For Salmonella, commercial chicken flocks reared for meat and eggs are usually monitored in Pakistan (Soomro et al. 2011; Shahzad et al. 2012; Uddin et al. 2018; Wajid et al. 2018), but no quantitative epidemiological investigations have been carried out to calculate prevalence of various Salmonella species in backyard chickens. Also, there is inadequate data available to assess the associated risk factors with this infection (Khan et al. 2012). Studies in other developing countries, like Iran (Khaltabadi et al. 2019), India (Balakrishnan et al. 2018) and Paraguay (Huarcaya Ramírez 2020) have suggested great load of Salmonella in chickens and other species.

Health authorities in Pakistan have gathered reports which provide a threat about an ongoing outbreak of extensively drug resistant (XDR) typhoid fever caused by Salmonella. It started to emerge in the Hyderabad district of Sindh province during November 2016 (Khan 2019; Qamar et al. 2020b). An increasing trend of typhoid fever cases caused by antimicrobial resistant strains of S. *enterica* serovar typhi (*S. typhi*) poses a notable public health concern in the region.

From January to December 2018, numerous samples from suspected cases of Salmonellosis were examined and *Salmonella typhi* was found to be prevalent in Karachi; moreover, it was an impending threat of extensive drug resistance (Hussain et al. 2019). It has been established through a number of studies that antimicrobial resistance to ciprofloxacin by both *Salmonella typhi* and *Salmonella paratyphi* has been found among the populations in the country (Table 1).

Taxonomic Classification

These bacteria are motile in nature and are mainly concerned with the animals (Ashton et al. 2016; Ryan et al. 2017). This genus has only species named as *Salmonella bongori* and *S. enterica*. The subspecies of S. *enterica* is mostly detected from the infections of humans and animals (Löfström et al. 2015; Ullah et al. 2017; Chiller 2019; Haley et al. 2019). The genus Salmonella nearly contains 2,600 serotypes, which are formed on the basis of their flagellar (H) and somatic (O) antigens. S. *typhimurium* and S. *enteritidis* are the most common serotypes related to the humans, but other serotypes can also cause infection.Among these serotypes, *S. newport*, *S. virchow* and *S. infantis* are most important ones (Fandiño and Verjan 2019).

Typhoid fever in humans is caused by the serotypes *S. paratyphi* and *S. typhi,* which are the most severe form of Salmonella infection, but most common infections are related to the non-typhoid infections, which normally cause gastroenteritis. It is characterized by symptoms like diarrhea, abdominal cramps, emesis and nausea, which normally last for one to seven days. In healthy adult individuals, the symptoms of the infection are less severe and only cause less than 1% mortality due to septicemia (Katz et al. 2019). In weak individuals, a dose range from 10-100 cells can initiate infection. The chances of infection are also higher when the food having high fat, like cheese and chocolate, is contaminated.

Clinical Aspects

Salmonella infection may manifest either as a self-limiting diarrhea/gastroenteritis that is related to non-typhoidal Salmonella (NTS), or as typhoidal fever that involves septicemia and chronic diarrhea and can be life threatening (Barrow and Methner 2013; Cox and Pavic 2014). The first form, NTS, is usually linked with inflammatory diarrhea, vomiting, nausea and abdominal pain (Smith et al. 2016). The severity of infection, like many other infectious diseases, depends upon host immune status, genetic makeup, the specie involved, serovar and dose of inoculation of the pathogen. In typhoidal fever, the microorganisms can spread to the circulatory system, attacking the lymphoid tissues of the intestinal tract.

On the off chance that the circulatory system is engaged with the disease, at that point it can affect any organ, including the bones, gallbladder, liver and meninges, yet its odds of event is under 5 %. About 12-72 hours is the

Table 1: Distribution of reported XDR typhoid fever cases in Sindh province, Pakistan [1 November 2016 through 9 December 2018] Source: WHO (2021)

Year	Districts in Sindh province			Total
	Karachi	Hyderabad	Other districts	
2016	0	11	0	11
2017	175	488	67	730
2018	3483	906	144	4533
Total	3658	1405	211	5274

Fig. 1: A 3D view of Salmonella serotype typhi (Source, CDC).

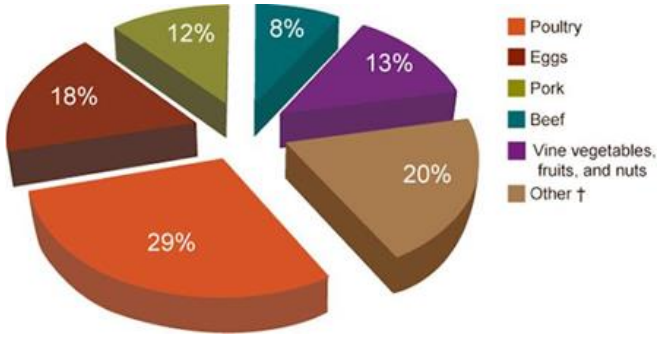

Fig. 2: Diffèrent sources of Salmonella transmissions in human population (Source : CDC website)

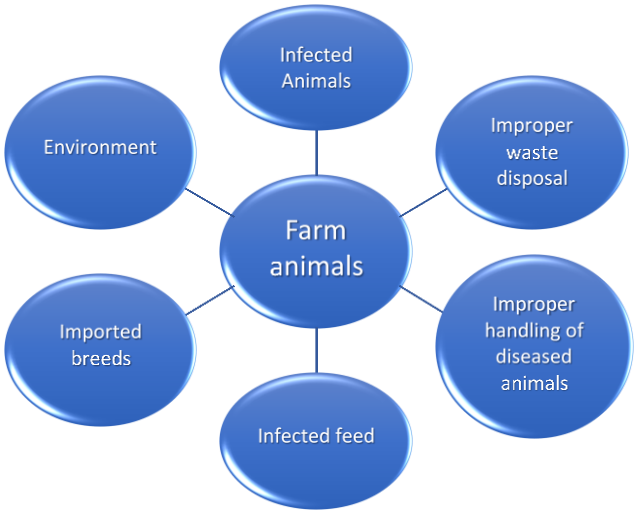

Fig. 3: Schematic illustration of Salmonella spp. indicating the source of infection and transmission modes.

brooding time frame for the Salmonellosis contaminations (Khan et al. 2019). The high hazard bunches for creating disease are kids under five years of age, aged persons and the individuals who are immunocompromised. The seriousness of contamination, in the same way as for other irresistible illnesses, relies on insusceptible status, hereditary cosmetics, the specie in question, serovar and status of vaccination against the pathogen (Asmar and Abdel-Haq 2016).

Although salmonellosis is seen in most domestic animals, pregnant and lactating young mammals and birds are found to be most susceptible (Wisittipanit et al. 2020). Cattle can be chronically infected, and therefore, may serve as carriers within the herd without showing any clinical signs. It has been reported that a single carrier cow can shed one billion Salmonellae per day through the feces (Gast and Porter Jr 2020). Abortion is the most common clinical consequence associated with *Salmonella dublin* (Sánchez-Miguel et al. 2018). Salmonellosis in cattle may cause watery or bloody diarrhea. The other symptoms include fever, depression, anorexia, dehydration, and endotoxemia. In rare cases, the infected cattle may suffer from respiratory disease and abortion, which is associated with high mortality rates (Hadimli et al. 2017).

Sources and Transmission

In poultry, Salmonella infections are transmitted horizontally, as well as vertically. They can also spread by contamination of the environment like soil, quilt, boxes used for nests, eggs, drinkers and waterers. Mechanical transmission occurs through the insects, rodents, wild birds, equipment, vehicles, clothing, unwashed hands, etc. (Chousalkar and Gole 2016). The infected animals, especially poultry and pigs, often do not show signs of disease (Nirmala et al. 2018) and are therefore, a major cause of disseminating the infection to other herd members and possibly to other humans and animals. According to CDC, poultry is the most common single food source associated with Salmonellosis in humans (Fig. 2).

According to Hale et al. (2012), about eleven percent (11%) Salmonella infections reported in humans are principally arise from animal handling. At the farm level, infection may be acquired by failure of adaptation of proper biosecurity measures. Introduction of new infected animals in the herd, unhygienic water and feed sources and improper handling of diseased animals may lead to transmission of Salmonella infection (Fig. 3).

Diagnostic Approaches

Conventionally, Salmonella spp. can be isolated first through non-selective pre-enrichment phase and after that selective enrichment is done, which is then coated on selective agars and alleged colonies are examined serologically and biochemically (Lee et al. 2015). Many regulatory agencies of USDA, like International Organization for Standardization, Association of Official

Analytical Chemists (AOAC), Food Safety and Inspection Service and Food and Drug Administration (FDA) have standardized many methods for the enrichment of Salmonella. Currently, the international standard operation (ISO) horizontal method validates buffered peptone water to be used as pre-enrichment media and after that Rappaport–Vassiliadis (soya base) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) selective enrichments are used (ISO 2007). Many other regulatory agencies also suggest standard methods for the isolation of Salmonella, which are fundamentally similar to ISO 6579:2002.

Instant Salmonella detection methods include molecular cloning and recombinant DNA technology. These quick methods allow the recognition of Salmonella spp. in samples and provide reliable results within a few hours to a day (Ferretti et al. 2001; Alakomi and Saarela 2009). New selective media, modified or improved conventional procedures, immunology-based and nucleic acid-based assays are commercially available rapid methods for the detection of Salmonella (Iqbal et al. 2000; Alakomi and Saarela 2009; Eijkelkamp et al. 2009). ELISA and PCR are more sensitive and specific as compared to these conventional methods (Lee et al. 2015). ELISA and PCR, after undergoing enrichment, can detect the concentration of Salmonella at the sensitivity level of 104–105 ml^{-1} and 104 ml^{-1}, respectively. Circumstantial microflora, presence of non-culturable cells, sample medium and inhibitory constituents (like adipose tissue, polysaccharides, proteins, hefty metals, organic composites and antibiotics) affect the sensitivity and specificity of these procedures (Naravaneni and Jamil 2005; Mozola 2006; Alakomi and Saarela 2009).

With the introduction of molecular techniques, the diagnosis of bacterial species like Salmonella has been revolutionized. The diverse serotypes of Salmonella can be serotyped accurately, using these state-of-the-art molecular tools.

Recent Outbreaks around the Globe

Outbreaks of typhoid fever have been controlled to some extent in developed countries, but illness caused by NTS still prevails both in developed and developing countries across the globe. Most of the outbreaks in humans are associated with consumption of contaminated food from animal origin. In Germany, a study conducted by Robert Koch institute has shown incidence of different Salmonella serovars during 2004-2010. According to WHO, there was an outbreak of Extensively Drug-Resistant (XDR) typhoid fever reported from Sindh province, Pakistan, which caused a total of 5707 cases (Chatham-Stephens et al. 2019).

A total of 53 Salmonella outbreaks related with live poultry have been recorded in United States between 1990 and 2014. These outbreaks caused around 2630 illnesses, 387 hospitalizations, and 5 deaths (Basler et al. 2016). Between September-October 2005, three different outbreaks of Salmonellosis were reported from Kerala, India poultry farms (Rajagopal and Mini 2013).

In New Hampshire, an outbreak of S. *typhimurium* in humans was reported in 2013. The outbreak was related to chicken jerky pet treats (Cavallo et al. 2015).

On October 17, 2018, a multistate outbreak of Salmonella *infantis,* related to under cooked chicken products, was reported to the Center for Disease Control and Prevention (CDC). In that outbreak, ninety-two cases were reported from twenty-nine states of United States. Twenty-one people were hospitalized, and no deaths were reported. Monitoring of the outbreak was done by the United States Department of Agriculture (USDA).

In July 19, 2017, different commercial, scientific, college and university coaching laboratories of Microbiology showed outbreak of S. *typhimurium,* involving the different states. From 16 states of US, 24 individuals were reported with infection. Hospitalized number of individuals was 6, and no mortality was recorded (CDC, 2018).

On October 6, 2016 CDC and the US Department of Agriculture studied 8 outbreaks of human Salmonella disease related to exposure with live poultry in backyard congregates. From 48 states, 8 outbreaks were reported, infecting 895 individuals with Salmonella strains (Control and Prevention 2014).

Worldwide Surveillance of Salmonella

This is critically important that detection methods should be consistent and integrated for the surveillance of Salmonella to improve the food safety standards (Ghafir et al. 2005). It is vital to run proper surveillance for the whole food chain by checking the feed and ingredients of feed for contamination of Salmonella (Alakomi and Saarela 2009; Mead et al. 2010). To efficiently avert and control the Salmonella infections, regulation, standardization, and worldwide surveillance programs are also compulsory. Information about the sampling technique, their storage and other important steps in the analysis of Salmonella is present in the Nordic Committee of Food Analysis (Validation 2002; Feldsine et al. 2003; ISO 2007; Löfström et al. 2010). In the form of EU Zoonosis Monitoring Directive, Regulation Number 2160/2003, and FDA Food Code regulations and guidelines are present in many countries (Commission 2003a&b; Food and Adminstration 2008). To decrease pathogens of Salmonella in the different stages of feed and its production, these guidelines and regulations provide the required information and control methods. They also help to develop the regulatory policies and the food safety rules (Kadykalo et al. 2018; Firestone 2020).

Pathogenesis

Salmonella displays momentous properties when it attacks non-phagocytic human host cells, adequately instigating its phagocytosis to enter the host cell. The phenomenal hereditary qualities behind this cunning procedure can be found in the Salmonella pathogenicity islands (SPI), in the quality bunches in the huge locale of chromosomal DNA and in the coding structures

associated with the intrusion cycle (Lenchenko et al. 2019). At the point when microscopic organisms enter the stomach related lot from defiled water or food, they will in general attack the epithelial cells that line the intestinal divider.

The seriousness of Salmonella diseases in people relies upon the serotype of the organism and the soundness of the human host. Youngsters under 5 yearsof age, the aged and immunocompromised patients are more susceptible to Salmonella contaminations than healthy individuals. Practically, all Salmonella strains are pathogenic, as they can attack human host cells, repeat and endure, prompting a conceivable sickness (Smith et al. 2016; Dougnon et al. 2017; Garedew et al. 2018). Illness usually lasts for 4–7 days. In typhoidal fever, the bacteria can spread to the bloodstream, invading the lymphoid tissues of the intestinal tract (Barrow and Methner 2013; Cox and Pavic 2014). If the bloodstream is involved in the infection, then it can affect any organ, including the bones, gallbladder, liver and meninges, but its chances of occurrence is less than 5%. Almost, 12-72 hours is the incubation period for the Salmonella infections (Khan et al. 2019). The high-risk groups for developing infection are children less than five years of age, elderly and persons who are immunocompromised.

The complex pathogenesis of systemic *S. enterica* infections correlates with the presence of a large number of defensive, as well as offensive, virulence factors (Groisman and Ochman 1997), with more than 300 genes have been identified as regulatory genes in Salmonella (Yoon et al. 2011). Salmonella has pathogenicity island SPIs, responsible for effects like adhesion, toxicity and invasion. There are 14 types of virulence regulatory genes, including SpvR, FruR, IHF, PhoP/PhoQ, SsrA/SsrB, SlyA, Hnr, RpoE, SmpB, CsrA, RpoS, CRP, OmpR/EnvZ, and Hfq (Yoon et al. 2009). These are often associated with transfer RNA (tRNA) and mobile genetic elements.

Control and Prevention

Livestock and poultry are the main reservoir hosts to cause Salmonellosis in humans, so humans exposure can be considerably reduced by decreasing infectious pathogens of Salmonella present in these animals (Arya et al. 2017). To prevent transmission of the disease, all feeds of animals are cured before their dissemination to kill the bacteria. Animals detected positive for Salmonella, should be reported and animals related to food safety should also be regularly examined for Salmonella (Milho et al. 2018). To prevent the *Salmonella enteritidis* infection in flocks, these strategies are adopted by the egg producers in the US as a control measure. Routine disinfection in and around the poultry farms should be done. Pets and rodents should also be controlled properly (Ribas et al. 2016), and feed should be obtained from the benign foundations. There should be routine investigation for the *Salmonella enteritidis* in chickens. Pasteurization of the eggs laid by the diseased egg-laying chickens should be done, and the infected birds should be removed.

Intricate food safety insurances are necessary for the control of Salmonellosis. This can be implemented by the prevention of ready-to-eat foods during their processing and refrigerated foods should also be sufficiently protected by controlling the survival and growth of Salmonella. All the persons dealing with the raw and cooked food and utensils should be cleaned with water and soap, and different food items such as meat, milk and vegetables are advised to be cooked at designated temperature to kill all the microbes. We should introduce the culture of germ-free slaughtering of animals, consumption of pasteurized milk, and completely cooked items to decrease the risk of being infected (Bao et al. 2015). Many serovars of Salmonella are extra resilient to antibiotics (das Neves et al. 2016). When antibiotics are inevitably required, then some of these can be used in humans for treatment and control of infections. For the prevention of this causative agent, judicial use of antibiotics among the humans and animals is imperious (Shrestha et al. 2016). Surveillance programs and subtyping of isolates are important for the control of Salmonella. By investigating outcomes of Salmonella subtyping from animals used for food, ecological illustrations, and humans, public health administrators can lure deductions about foundations of human disease and emphasis on control strategies consequently.

Immunization against Salmonella Species of Public Health Significance

To lessen the Salmonella infections, vaccination of chickens is necessary for Salmonella and other interference infections. Two licensed vaccines are largely available against Salmonella (Gayet et al. 2017), but till now no one has been executed at the country level. These vaccines are live attenuated,Ty21a (Fraser et al. 2007) and Vi capsular polysaccharide (Gayet et al. 2017). They cause lower immunogenicity in younger children and, therefore, the use of these vaccines is reduced in the high-risk individuals and no one is authorized to use in children of less than two years of age. The drawback of these vaccines is that they are directed against *S. typhimurium*, with no cross immunity for other three invasive types of serovars of *S. enterica*, *paratyphi* A, *typhimurium* and *enteritidis*.

In Pakistan, typhoid conjugate vaccine (Typbar TCV) is largely practiced and a research team is working on mass vaccination against the outbreak to target a local town, and quite healthy response is achieved (Qamar et al. 2020a). Pakistan became the pioneer state and the first country to introduce the WHO recommended typhoid conjugate vaccine into routine vaccination programs and this vaccine has high efficacy, with added benefit of long-lasting immunity. The vaccine is now being administered to the individuals aged 6 months or older since 2019, with fewer adverse effects (Qamar et al 2020b).

Conclusion

Salmonellosis is an important zoonotic disease of animal origin; poultry meat, eggs and their products are the

common sources of transmission to humans. Salmonellosis has posed a serious threat to the public health (Zdragas et al. 2012), as it is thought to be the most frequent food borne pathogen worldwide. During the period of 1983-87 in US, one third outbreaks of human Salmonellosis were linked with poultry chicken and eggs (Tauxe 1991). In Pakistan, poultry chicken is the main type of meat being consumed, as in many other countries. Apart from commercial poultry farming, backyard poultry is also reared in urban and rural areas to meet the nutritional requirements and they are also vulnerable to Salmonella infection (Bhuvaneswari et al. 2015). Salmonella has the ability to attach with any part of gastrointestinal tract in the absence of other microflora, where it can colonize and multiply. It can also be disseminated to the environment, humans and other animals via feces (AL-Kubaisi et al. 2020; Santana et al. 2020). Therefore, fecal swabs or samples are used as true representative to confirm the presence of Salmonella in the intestinal wall of poultry chicks (El-Fakar and Rabie 2009).

Irrational use of antibiotics in poultry and livestock farming has resulted in the rise of antibiotic resistance among food producing animals, which envisage a major threat to production industry. The rise of MDR Salmonella causes an escalating risk for health of human population. Out of the 10 serotypes that CDC has commonly reported from human infections in the US, 8 comprise at least a few isolates that showed resistance to five or more antimicrobic drugs (Varma et al. 2005). MDR *S. typhimurium* isolates commonly exist in two resistance forms including, (i) resistance to ampicillin, streptomycin, kanamycin, tetracycline and sulfamethoxazole or, (ii) resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, the resistance type is typically associated with *S. typhimurium* DT104 (Soler et al. 2006). Regarding antibiotic resistance, production of extended spectrum Beta Lactamase invokes a major threat to livestock and poultry production system. This threat not only affects animals, but also humans, which are at risk of being infected in direct food chain (Witte 1998). A current study appraises the presence of extended spectrum Beta lactamase in gut *S. typhimurium* in poultry food chain. Double Disc screening test was employed to differentiate ESBL producing organism from non ESBL producing *S. typhimurium* (Nadimpalli et al. 2019).

Hence, proper legislation holds the key to lessen the risk of antimicrobial resistance among animals and human populations. The principal factor for inhibiting the spread of antimicrobial resistance throughout the food chain is identical to the non-resistant foodborne pathogens that may include the appropriate food management, and food formulation methods. Additionally, there is substantial proof that the use of antimicrobials for the treatment of food animals, their growth promotion and prophylaxis, increase the prevalence of resistance in human pathogens (Singer et al. 2007; Saharan et al. 2020).

Immunization against different Salmonella serovars in human population is the ultimate solution for public health challenge. Pakistan, being the first country in the region following typhoid conjugate vaccine, has permitted the use of vaccine for the individuals, even above 6 months of age.

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MOLECULAR EPIDEMIOLOGY AND FUNCTIONAL GENETICS OF *MYCOBACTERIUM TUBERCULOSIS* IN HUMANS AND ANIMALS

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INTRODUCTION

Tuberculosis (TB) is listed in the top 10 worldwide killing diseases of 21st century (WHO 2019). The global TB report 2019 had declared 10 million morbidities, 1.5 million mortalities and 0.484 million drug resistant TB cases in 2018 (WHO 2019). *Mycobacterium tuberculosis* (*Mtb*) is the causative agent of TB, which can form lesion(s) in any organ, such as lungs (Pulmonary TB) bones, lymph nodes, brain, kidneys and joints (extra-pulmonary TB) (Golden and Vikram 2005; Kumar et al. 2007; Rockwood 2007; Behera 2010). *Mtb* is an obligate intracellular bacterium of the family Mycobacteriaceae. Mycobacteria are non-spore forming, aerobic, straight rods or slightly curved and non-motile. Colony morphology varies among species. The rod shaped (bacilli) appearance of *Mtb* by scanning electron microscopy (Fig. 1.1A) and the acid-fast staining of sputum sample (red rods) by light microscopy (Fig. 1.1B) can be observed. The characteristic features of the *Mtb* include: dormancy, slow growth complex cell envelope due to thick waxy coating and genetic homogeneity. The cell envelope of *Mtb* contains an additional layer beyond the peptidoglycan, which is highly rich in lipids, glycolipids and polysaccharides. Generation time of *Mtb*, in growth medium or infected animals, is usually around 24 hours. Thus, the growth of mycobacterial species is slower compared to other bacteria. This slow growth of *Mtb* makes the TB disease chronic in nature.

Tuberculosis is an aerosol transmitted disease that spreads from infected population to healthy one (active TB) and may be sub-clinical *i.e.,* does not show symptoms (latent/dormant TB). In the latent state, *Mtb* remains inactive within the infected tissue. As immunity decreases through ageing, poor diet, diabetes and HIV, the inactive bacteria become reactivated, causing an outburst of the disease. Active TB (ATB) has many communal symptoms which are low grade pyrexia, continuous coughing, blood spattered phlegm, weight loss, tiredness, chest pain and night sweats (Loddenkemper et al. 2015).

Tuberculosis can be over-come to some extent by *Mycobacterium bovis* BCG vaccination, which was developed and isolated by Calmette and Guérin in Lille, France (Calmette et al. 1927). The variable efficacy and response of BCG vaccine led to ineffective disease control (Andersen and Doherty 2005). The first anti-tuberculous drug (streptomycin) was developed during World War II by Selman Waksman (Waksman 1964). In the beginning, therapy with streptomycin seemed highly effective, but problems arose when resistance developed rapidly against this drug (Kaufmann and Parida 2007). In most cases, TB can be diagnosed and treated successfully with combination therapy of different antibiotics. However, total drug resistant (TDR), multidrug-resistant (MDR) and extensively drug resistant (XDR) tuberculosis remain a public health issue and a health security threat. World Health Organization estimated 600,000 new rifampicin resistant cases of TB (WHO 2018).

The genome of *Mtb* is rich with G+C nucleotide pair (Bohlin et al. 2019). It is thought that progenitor of MTBC comprises of *Mtb*, *M. bovis*, *M. bovis* BCG, *M. microti* and *M. africanum*. This MTBC originated from soil bacterium, lacking inter-strain genetic diversity and nucleotide changes. The human *Mtb* is mainly originated from the bovine tubercle bacilli after domestication of cattle (Sreevatsan et al. 1997). Several studies have shown that *Mtb* can even infect bovines, though the severity of disease is less (Ameni et al. 2013). However, in biomedical research, H37Rv strain of *Mtb* has been used that has retained almost all the virulent features of clinical isolate. However, unlike clinical isolates, H37Rv is prone to genetic manipulation and anti-TB drugs. Shining rod shaped bacilli of *Mtb* have been shown in Fig. 1.

History

The origin of genus Mycobacterium has dated back to approximately 150 million years. Recent molecular techniques provided data of its origin in East Africa about 3 million years ago (Gutierrez et al. 2005) and current *Mtb* strains are believed to be instigated from these ancestors (Sreevatsan et al. 1997). Researchers have forecasted that main genetic variation among these strains was from 250 to 1000 years ago (Hirsh et al. 2004). The bacteriologist, Robert Koch, had claimed first time *Mtb* as the etiological agent of TB by delivering a lecture on Uber Tuberculosis on 24th March, 1882. In the past era, several names were designated to TB, depending upon the clinical symptoms *e.g.,* phthisis (health decline), scrofula (enlarged degenerated lymph nodes), consumption (severe weight loss), white plague (pallor skin in infected patients) and

pott's disease (extra pulmonary tuberculosis affecting spine) (Daniel 2006). The earliest evidence of *Mycobacterium tuberculosis* infection dated back to 9000 years in a woman and infant buried in Eastern Mediterranean (Philipp et al. 1996).

Transmission and Pathogenesis

A thorough understanding of *Mtb* transmission is necessary for the development of effective TB control policy. As TB is an aerosol transmissible disease, inhalation is the main portal of transmission. Multiple factors are also involved in its transmission *e.g.* immune status of the person exposed to infection, stage of infectiousness, as severely infected patients shed more tubercle bacilli, and period of exposure. Aerosol droplets (1-5 µ in diameter) transmit mycobacteria through sneezing, spitting, coughing and speaking (Cole et al. 1998; Long and Schwartzman 2014). Around 40,000 droplets have been discharged in a single sneeze. Each droplet is considered as a disease transmitting moiety on its inhalation (Klettner et al. 2012). Most of the times, infection is subclinical and the disease persists in dormant form (Cheigh et al. 2010). Yates et al. (2016) have demonstrated the transmission cycle of *Mtb* and a schematic figure has been drawn by taking concept from their paper and is shown in Fig. 1.2a.

The main feature behind effective pathogenesis of *Mtb* is its biological adaptation in a highly mutable environment and its slow growth under *in vitro* and *in vivo* conditions. Both impart its survival within macrophages necessary for the infection development (Bhardwaj 2014). The infection commences when *Mtb* enters alveolar macrophages and replicates within its phagosome (Kumar et al. 2007). Macrophages crack to eradicate *Mtb* via phagocytosis by considering it as a foreign entity. This happens by the fusion of phagosome with lysosome to form phagolysosome, which is an acid-killing trap for *Mtb*. On the other hand, a thick waxy capsule around *Mtb* shields it from noxious elements. The bacterium multiplies within macrophage till bursting of the immune cell and dissemination of infection throughout the body. A recent research has described that a mutant *Mtb* strain, by knocking out of Rv3167c (a transcription factor), over-expresses phthiocerol dimycocerosates (PDIM), which is an *Mtb* virulent factor and endorses necrosis, resulting in phagosome mediated escape of *Mtb* (Quigley 2017). Delogu et al. (2013) have described the pathogenesis of TB and a schematic diagram has been plotted by taking concept from their research study, which is shown in Fig. 1.2b.

Epidemiology and Topographical Spread

Tuberculosis is a poverty associated disease and most commonly affects young individuals (Glaziou et al. 2015). Recent TB statistics demonstrates that around 10 million people got TB, of which 3.2 million (32%) women, 1.2 million (12%) children and 5.6 million (56%) men are diagnosed with this vicious infection (WHO 2019). Unemployment, food insecurity, psychological circumstances, poor housing conditions, illiteracy and congested social setups are the main aspects in high load TB countries (WHO 2014a). A total of 95% deaths, reported in 3rd world countries, are caused by TB (WHO 2014a). Persons with human immunodeficiency syndrome (HIV) or cases of other immunocompromised anomalies, such as silicosis, renal insufficiency and diabetes, are more susceptible to tuberculosis infection (Glaziou et al. 2013). However, a universal drop in TB cases was detected by the induction of direct observation treatment strategy (DOTS) and increasing the therapeutic consequences for recurred and newly emerged TB cases. In the recent global TB report of WHO, the data have been collected from different regions of continents and presented in Table 1.3 of this chapter, courtesy of Table 8.5 of "The global TB database 2020" (https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf).

Tuberculosis has caused more deaths than human immunodeficiency syndrome virus (HIV) in 2015. Around 60% of high TB burdened areas are China, Pakistan, India, Indonesia, South Africa and Nigeria. Especially, the highly populated and distressing part of world for TB infection is the South East Asian region with maximum number of reported MDR-TB cases (with 54% treatment coverage). African regions have 39% HIV positive TB patients and 48% overall treatment coverage. American, Western and European regions are provided with maximum treatment coverages, which are 81, 84 and 78%, respectively. Global epidemiology of tuberculosis and its progress towards achieving global targets has been presented by the Center of Disease Control and prevention (CDC). The map is taken from the morbidity and mortality weekly report (MMWR) of the Center for Disease Control and Prevention (CDC) and shown in Fig. 1.3 (MacNeil et al. 2019).

The *Mtb* can also cause TB in animals, including reptiles, rodents, birds and elephants. In animals, almost all the ruminants and non-ruminants' species can be infected with bovine TB. An interesting case report demonstrated the TB cross-transmission from animal to human and back to animal. The pathogenic bovine TB strain was isolated from the cattle, which got infection from humans. Actually, this person got bovine TB bacterial infection through exposure in his childhood (Fritsche et al. 2004). Tubercle Bacilli are shed almost in every secretion and excretion, such as respiratory tract secretions, milk, feces, tears, urine and other body fluids of infected animals.

Bovine TB is caused by *M. bovis,* with similar presentation of lesions is observed in pulmonary and extra-pulmonary level, just like humans. In cattle, *Mtb* takes entry through the inhalation route, however, the activity level of infective organism depends on the health of immune status. In case of suppressed immunity, the infection disseminates to the extra-pulmonary organs like spleen, kidneys, liver and lymph nodes and this is known as military TB. Debilitation, emaciation or general body weakness, loss of weight, low grade fever and anorexia are general bovine TB symptoms (Ayele et al. 2004). Even researches have declared that eating dairy products of milk from infected animal, or drinking raw milk of such animal can also transmit bovine TB to humans (Davies 2006).

Table 1.3: Status of core elements of multi-sectoral accountability in 2020 for 30 high TB burden countries, WHO regions and globally (Global TB database 2020)

a) National strategic plan (NSP) for TB and annual TB report

High TB burden countries and WHO regions	Number of countries and territories	NSP Exits		Representatives of civil societies and affected communities were actively involved in NSP development		NSP was developed or updated since the UN high level meeting on TB in September 2018		Annual TB report available publicly	
High TB burden countries	30	30	100%	29	97%	25	83%	27	90%
Africa	47	42	89%	40	85%	32	68%	39	83%
The Americas	45	32	71%	21	47%	16	36%	21	47%
Eastern Mediterranean	22	17	77%	11	50%	12	55%	16	73%
Europe	54	25	46%	21	39%	14	26%	30	56%
South-East Asia	11	11	100%	9	82%	8	73%	9	82%
Western Pacific	36	21	58%	14	39%	15	42%	19	53%
Total	215	148	69%	116	54%	97	45%	134	62%

b) High-level review mechanism(s)

High TB burden countries and WHO regions	Number of countries and territories	NSP Exits		Representatives of civil societies and affected communities were actively involved in NSP development		NSP was developed or updated since the UN high level meeting on TB in September 2018		Annual TB report available publicly	
High TB burden countries	30	16	53%	12	40%	15	50%	7	23%
Africa	47	26	55%	24	51%	22	47%	11	23%
The Americas	45	13	29%	6	13%	8	18%	2	4%
Eastern Mediterranean	22	6	27%	3	14%	5	23%	1	5%
Europe	54	19	35%	14	26%	16	30%	7	13%
South-East Asia	11	7	64%	4	36%	6	55%	3	27%
Western Pacific	36	15	42%	11	31%	12	33%	6	17%
Total	215	86	40%	62	29%	69	32%	30	14%

Reverse Zoonosis: *Mtb* Infection in Bovines

Humans also serve as the potential source for TB transmission to animals *via* reverse zoonosis (Hackendahl et al. 2004; Messenger et al. 2014). Transmission of *Mtb* to grazing cattle in Central Ethopia was through a traditional practice of spitting crushed tobacco from farmer's mouth directly into grazing cattle, which is a common practice and was considered the main route of *Mtb* transmission in cattle. 16srRNA locus sequence of isolates was used for differentiating the non-tuberculous mycobacteria (NTM) from the tuberculous mycobacteria (TM) (Ameni et al. 2011). TM were then used for region of difference (RD) typing, a standard method of differentiation TM at species level (Parsons et al. 2002). The RDs (RD4, 9 and 10) were selected by designing their primers for PCR analysis and declared 27% isolates were *Mtb* (Brosch et al. 2002; Berg et al. 2009). It was also concluded that *Mtb* infection in cattle mostly occurred in high human TB burden areas (Ameni et al. 2011).

Transmission of *Mtb* from human to cattle was through inhalation of cough droplets of active pulmonary TB patients or the ingestion of their sputum/urine contaminated pasture. The interesting point reported was less virulent form of disease in cattle (Ameni et al. 2013). From Ameni et al. (2013) studies, spoligotyping pattern of MTBC species and its schema of image has been drawn and shown in Fig. 1.4a.

Three isolates of *M. bovis* were isolated from two oxen of a farmer with active pulmonary tuberculosis. These three isolates had the same spoligotype pattern and were SB1176. The other two isolates were *Mtb* and were from cattle owned by farmers with active tuberculosis. These isolates had different spoligotype pattern and were SIT149 and SIT53.

The study of Villarreal-Ramos et al. (2018) has experimentally supported the claim of Ameni et al. (2013) reports regarding attenuation of *Mtb* in cattle (Villarreal-Ramos et al. 2018). Villarreal-Ramos et al. (2018) have suggested bovine model as one health approach for new TB biomarkers/vaccine candidates' development. RNA-seq/transcriptome profiling was done from whole blood of *M. bovis* AF2122/7 and *Mtb* H37Rv infected cattle (n=4) at day 14 and 42. Top 10 differentially expressed genes in animals at 14 and 42 days by infecting with *M. bovis* and *Mtb* have been shown and intrigued below in Fig. 1.4b by considering the findings from Villarreal-Ramos et al. (2018) studies.

M. bovis (AF2122/97) and Mtb H37Rv strains were used for infection. For transcriptional analysis, first 10 differentially expressed genes (DEGs) were studied at 14 and 42 days of infection (FDR < 0.05). Log_2 fold change (Log_2FC) values are shown on y-axis of the graph for the selected genes which means the relative expression of each gene in *M. bovis* infected animals to *Mtb* infected animals and vice versa. The x-axis represents the days at which this study was conducted.

Vaccines for TB

The reasonable, resourceful and effective way of controlling any disease in a population is through vaccination (Khatoon et al. 2017). The whole organism-based vaccines (attenuated and killed) have a drawback of

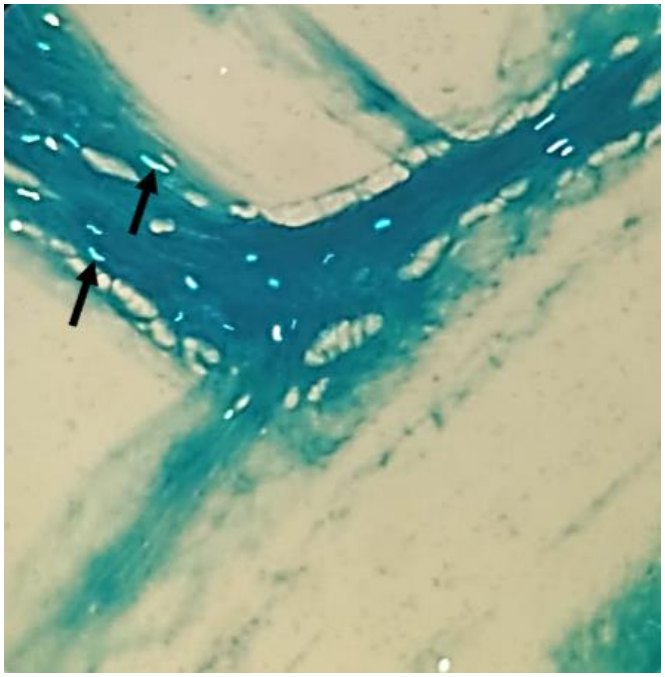

Fig. 1: Shining rod shape bacilli of *Mtb* (courtesy by Dr. Tahir Rasool, DHQ Hospital, Faisalabad): Arrow head depicts truant auramine-rhodamine (AR) stained acid-fast *Mtb* bacilli by using fluorescent microscope. The slide was prepared from the sputum sample of a TB positive patient.

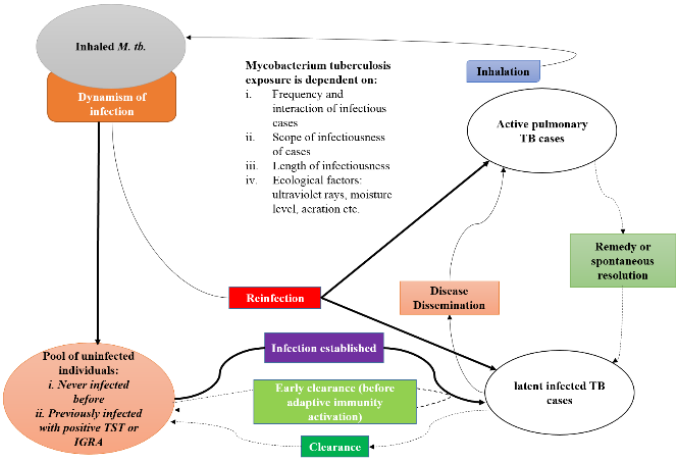

Fig. 1.2a: Schematic illustration of *Mtb* transmission cycle

containing non-antigenic potential fractions. Being the most widely used vaccine, BCG has been given over 4 billion times. However, BCG can induce a disseminating disease in individuals with mutation in immune modulating genes and infants with clinically active HIV (Hesseling et al. 2007). Moreover, BCG can be reverted back to virulent form, so the vaccine is not effective for worldwide TB control. BCG is a weakened strain of *Mycobacterium bovis,* which was developed between 1906 and 1919. It is still in use and is the only available vaccine against TB. This vaccine can prevent the neonates and children from the spread of TB and mortality. However, the use of BCG vaccine has some serious limitations and its efficacy is still questionable. The controversies about BCG include: i) fails to provide protection against TB in adults *i.e.* pulmonary TB, ii) fails to provide protection

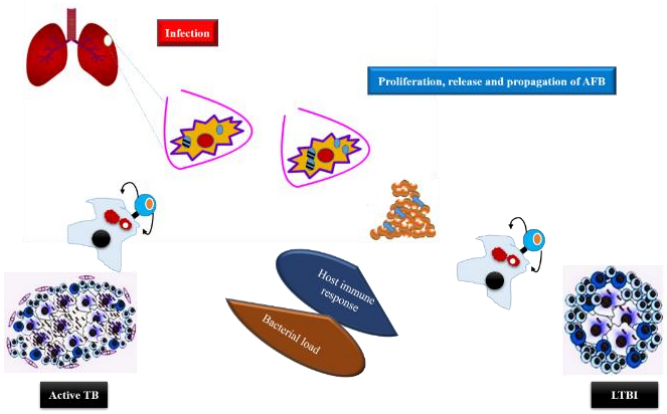

Fig. 1.2b: Pathogenesis of tuberculosis: Figure represents the aerosol inhalation of *Mtb* which then move in lungs, where they encounter with the host innate immune system (alveolar macrophages). If the *Mtb* overcome macrophages, they start multiplying and disseminate to other tissues (military TB) via blood and lymphatic streams. Only the T cell immunity (cell-mediated) can undermine the infection and in >90% cases overt signs of TB become covert (Latent TB). However, if cell mediated immunity is subverted by somehow, then active bacterial replication starts and heavy tissue damage occurs (active TB) (Delogu et al. 2013).

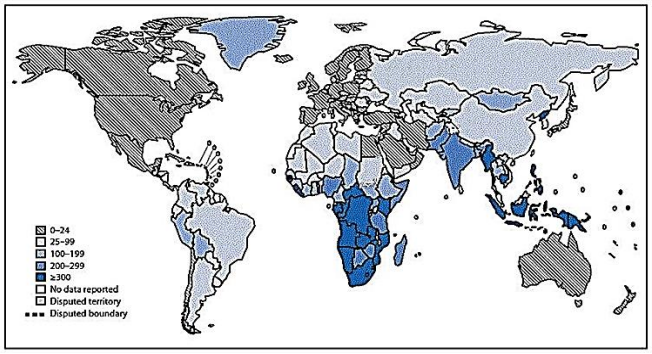

Fig. 1.3: Annual TB incidences (per 100,000 population), by region- worldwide, 2017 (MacNeil et al. 2019)

against chronic TB, iii) has no booster for repeating vaccine and, iv) difficulty in diagnosis of actually diseased and vaccinated individuals by common diagnostic test *e.g.* tuberculin skin test (Crampin et al. 2009; Glick et al. 2014). Consequently, *Mtb* has undergone to dormant state in most cases after BCG vaccination and latent chronic infection has been established. The latent *Mtb* becomes active when the strong immune status of person becomes weakened *e.g.,* individuals with genetic immune defects, aged people and immunosuppressant drugs taking people. Thus, there is a strong need to develop vaccines that can either act as BCG's booster or substitute the BCG (Hesseling et al. 2007).

Moreover, to overcome these issues of BCG, an alternative or ancillary vaccine is essential which is safer, more immunogenic and induces longer lasting protection. An answer could be peptide vaccine(s) that identify the peptide epitopes on immunogens to generate the immunogenic response. For this purpose, synthetic versions of peptide epitopes have been used by engineering the vaccine. Contrary to customary vaccine,

peptide vaccines are non-infective, totally synthetic, zero risk of pathogen activation and free from unwanted effects, which are common while using whole organism based attenuated vaccine(s) (Glick et al. 2014).

Currently, 12 vaccines for TB are in different phases of clinical trial (Zhu et al. 2018). These could be weekend pathogenic strain, viral vaccine, recombinant BCG vaccines, DNA vaccines and subunit/peptide vaccines (Kaufmann 2014; Zhu et al. 2018). The concept of peptide vaccine has been aroused during the past one or two decade(s). The reason is to generate non-infective and broad-spectrum vaccine by using only immunogenic part of organism instead of using full organism. These subunit vaccines contain only the immune system stimulatory part of pathogen. Currently, these vaccines are becoming more precise due to the use of only a high binding affinity epitope, instead of using full peptide, that can stimulate the production of antibodies or pro-inflammatory and inflammatory cytokines (Glick et al. 2014; Bellini and Horváti 2020; Gong et al. 2021). Since subunit vaccines contain only the essential part of antigens, the risks of adverse reaction to the vaccine are lower. Firstly, it has to be effective, and an epitope (antigenic determinant) must constitute a short stretch of adjoining amino acids. Secondly, the peptide vaccine must have the same 3D conformation as its isolated epitope in the intact viral particle. Conventional vaccines have undesirable antigens that have no or little contribution in providing protective immunity and also these antigens sometimes complicate the situation by inducing atopic responses. Peptide vaccines are an attractive alternative strategy that relies on the use of short peptide fragments to engineer the induction of highly targeted immune responses. While talking about peptide vaccines, both CD4+ and CD8+ T cell epitopes are presented on the surface of an antigen presenting cell (APCs) for binding with MHC molecules. For designing peptide vaccines, databases are available for the prediction of T and B cell epitopes. The accurate selection of these epitopes is necessary, so that the shortlisted epitopes could have a strong binding affinity with MHC molecule for eliciting immune response (Glick et al. 2014; Bellini and Horváti 2020; Gong et al. 2021).

Peptide/subunit Vaccines for TB

Subunit vaccines were designed on immunoinformatic logics and evaluated by *in silico* analysis that provides confidence for their *in vitro* and *in vivo* analyses. In the study of Dhivya et al. (2018), T cells epitopes were obtained from polyketide and non-ribosomal peptide synthesis proteins of *Mtb*. These epitopes were used as potential vaccine candidates for designing vaccine by immunoinformatics approach. Polyketide and non-ribosomal proteins belong to the class of small molecule metabolites of *Mtb,* used in bacterial envelope organization, virulence, and pathogenesis. A total of 41 proteins from both classes of proteins were analyzed computationally for obtaining possible overlapping peptides. Similarly, in another research work (Hossain et al. 2017), a computationally identified and characterized *Mtb* Ag85B T-cell epitopes-based subunit vaccine was designed. Computational tools for generating T-cell epitopes predicted an epitope, 181- QQFIYAGSLSALLDP-195, that could bind to at least 13 MHC molecules, demonstrating the widened nature of epitope. Moreover, these workers also analyzed the "Allele Frequency Database," for the spread of HLA alleles throughout worldwide population and found that both HLA-I & II alleles are frequently present in TB- endemic regions (Hossain et al. 2017).

Choi et al. (2018) used the concept of subunit vaccine against *Mtb* in which ESAT-6 protein was combined with PE/PPE protein CD4+ epitope. Then this combination was used against HN878- highly virulent strain of *Mtb*. In response to that, CD4+ cells of mice released copious amount of IFNɤ and IL-2 *i.e.,* the cytokines considered protective against *Mtb* infection. Moreover, CFU count of *Mtb* in spleen was also found reduced in this study. This means that this subunit vaccine works well against virulent *Mtb* strain (Choi et al. 2018).

Another subunit vaccine H56/CAF01 was tested against *Mtb*. The study revealed that CD4+ T cells memory generated by H56/CAF01 vaccination was lost in natural infection of *Mtb*. The possible reason was the changes in ESAT-6 and Ag85B proteins of *Mtb* in lungs. This leads to high expression of KLRG1 expression on CD4+ T cells surface and decreases the production of TNFα and IFNɤ. This subunit vaccine, however, provides excellent protection against *Mtb* re-infection with excessive amount of IL-1 and IL-17A production. Both these cytokines provide protection to counter TB infection (Lindenstrøm et al. 2017).

One more subunit vaccine was designed and tested according to the infection states of *Mtb,* such as active, latent and reactivation. In all these states, metabolic states of bacteria were variable. In this work, single-, two-, poly- and multi-stage vaccines were developed against TB infection and tested in mice. The results showed that multi-stage vaccine provided more immunity than all others and this was due to the detection of IFNɤ and IL-2 producing CD4+ T cells in mice (Ma et al. 2017).

Genomic Organization Features

The genome of *Mtb* was studied generally using the H37Rv strain. A total of 4,411,529 base pairs (bp) are present in *Mtb* genome rich in G+C contents of approximately 65.6%. High G+C content in *Mtb* genome is apparent with highest percentage of GTG initiation codons of 35% compared to 9% in *Bacillus subtilis* and 14% in *E. coli* (Cole et al. 1998). An integrated genomic map of 4.4 mega base (Mb) in circular form has been shown in Fig. 1.7, the resource is copied from Tuberculist open access web server and ordered libraries of cosmids and bacterial artificial chromosomes (BACs) (Philipp et al. 1996; Brosch et al. 1998). *Mtb* genome is also rich in repetitive DNA (insertion/transposon) sequence. The insertion sequences in *Mtb* H37Rv genome are mostly slot in to the intergenic regions. Around 3,924 open reading frames (ORF) are present in genome, constituting ~91%

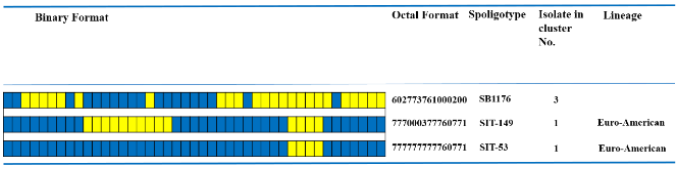

Fig. 1.4a: Spoligotype patterns of MTBC species (*M. bovis* isolates from *Bos Taurus*).

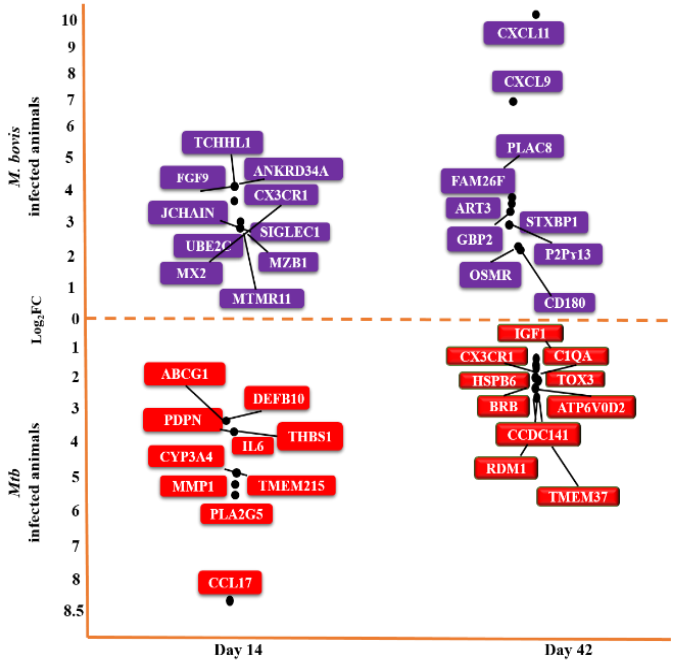

Fig. 1.4b: *M. bovis* or *Mtb* infection mediated transcriptome analysis (RNA sequencing), using whole blood non-activated sample.

of coding capacity. On the other hand, virulence transfer by innate plasmids of *Mtb* is quite easy than chromosomal gene transfer. Moreover, *Mtb* genome encodes around 190 transcriptional regulators, which include: 11 two-component system, 13 sigma factors and more than 140 other transcription regulators.

Functional Genetics of *Mtb*

Nuclear Localized Proteins

Nuclear localized proteins have nuclear localization signal (NLS) to move inside host nucleus and make chromosomal conformational changes. Because of these changes, these proteins are sometimes called nuclear modulators. Rv2966c is a nuclear localized protein of *Mtb* that interacts with host chromatin through non-CpG methylation and has histone (H3 & H4) binding. Rv2966c localizes to the nucleus and then binds to DNA sequences of host chromatin, methylates cytosines in the non-CpG region. They have also described the significance of their study in a sense that it was the first one to explore such a protein of *Mtb* that can methylate host DNA in non-canonical manner (Sharma et al. 2015). Similarly, Rv1988 protein was discovered to influence the host genome by methylating the non-tail arginine of histone H3. Rv1988 is

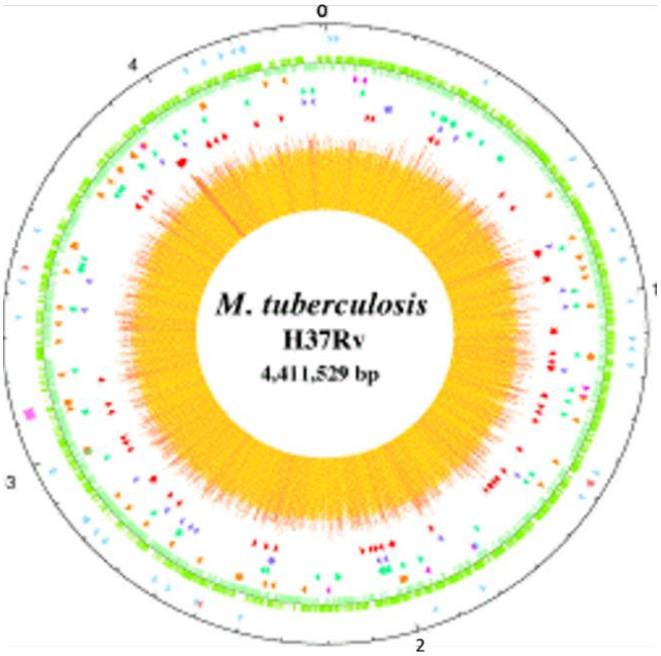

Fig. 1.7: Circular representation of the *Mtb* chromosome illustrating the location of each gene/protein-coding in H37Rv strains: The figure is taken from Tuberculist open access web (http://genolist.pasteur.fr/TubercuList/) server. The image depicts the full genomic map of *Mtb* chromosome, including; protein coding, non-coding, synonymous or non-synonymous substitutions and IS6110 elements insertion.

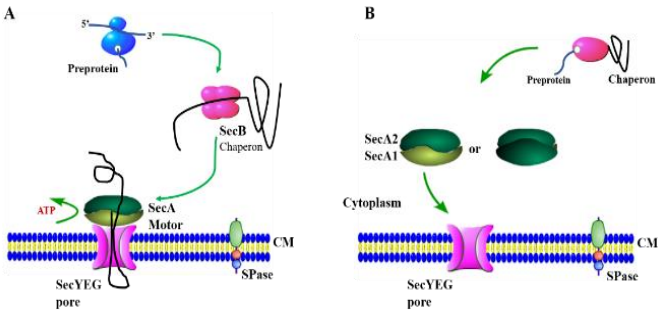

Fig. 1.8.2a: Model of protein secretion: A) It shows the canonical secretion pathway of Sec system. *Mtb* ribosome (blue) synthesized a pre-protein and delivered to chaperon which is SecB (a complex type of protein- pink). This SecB maintains the protein in folded form and passed on to SecA. SecA is complex (light/dark green) that acts as motor by consuming ATP. SecA binds with SecYEG pore and then hydrolyse ATP and use its energy to deliver the protein to SecYEG. SecYEG acts as cell membrane pore through which formed protein can go out for functioning. B) In this suggested model, instead of SecB, just chaperon is a protein to maintain the folded protein and deliver it to SecA. Here SecA is in complex form as a heterodimer (SecA1/SecA2). Both after complexing uses ATP and bind on SecYEG pore to deliver out the synthesized protein from *Mtb* cell. CM- cell membrane, SPase-signal peptidase (Prabudiansyah et al. 2015)

an outgoing (secreted) mycobacterial protein that is functionally characterized as methyltransferase and localized to the nucleus of host cell to interact with host chromatin material. Rv1988 methylated histone H3 at arginine 42 position (H3R42) and suppressed the expression of genes involved in the defense against *Mtb*.

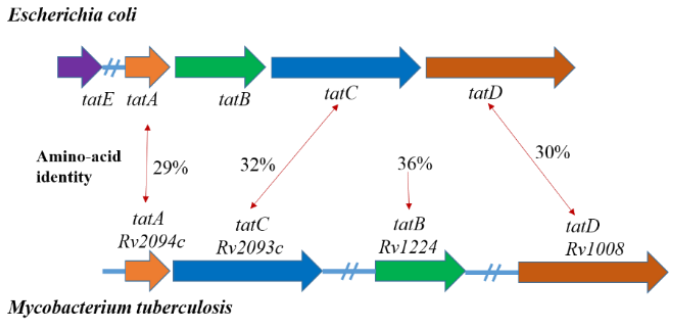

Fig. 1.8.2b: Organization of *Mycobacterium tuberculosis TAT* genes: Comparative analysis of genetic arrangement of *TAT* genes between *Mtb* vs *E. coli*. Percent identity of the respective homologous for TAT proteins has been shown. A 16bp intergenic region has separated the *TATA* and TAT*C* genes in *Mtb* (Saint-Joanis et al. 2006).

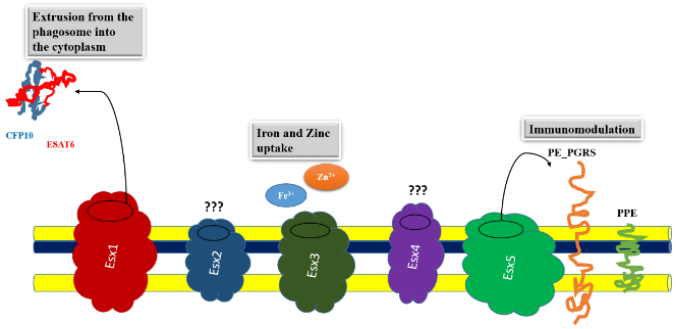

Fig. 1.8.2c: Esx (T7SS) Secretion system(s): Five components have been shown schematically. Esx1 is involved in phagosomal lysis within macrophages that liberate *Mtb* within cellular cytoplasm. Esx3 is involved in iron and zinc uptake or homeostasis. Esx5 gives growth to bacteria. Esx2 and 4 function is still in exploration (Delogu et al. 2013).

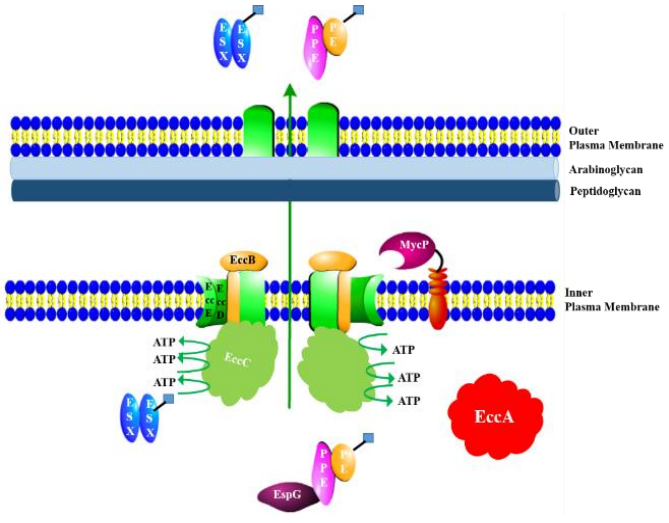

Fig. 1.8.2d: Proposed model of *Mtb* special type 7 secretory system (T7SS). Ecc- ESX conserved components.

A non-tail histone methylation at arginine 42 position (H3R42me2) happened at the entry and exit point of DNA in the nucleosome. However, within regulatory sites in the N-terminal tail, this methylation is not present. Knock out of Rv1988 in *Mtb* decreases bacterial survival in the host, and experimental expression through plasmid cloning of Rv1988 in non-pathogenic *Mycobacterium smegmatis* adversely affects the infected mice health. Thus, it can be inferred that Rv1988 is an important growth regulatory virulence factor and uses non-canonical pathway to regulate host cell transcription (Yaseen et al. 2015). Rv3423 is another nuclear localized protein, acting as histone acetyltransferase. Bioinformatics predictions showed that Rv3423 acetylates histone H3 at lysine K9 and K14 positions. The protein is necessary for intracellular growth of bacteria. The histone acetyltransferase activity of Rv3423 influences the expression of host anti-inflammatory genes to inhibit bacterial clearance and to promote intracellular survival of bacteria (Jose et al. 2016). Mycobacterial phosphatase PtpA, which acts in the host cytoplasm, can also localize to host nucleus and control the expression of host cell proliferation controlling genes. PtpA is an effector protein of *Mtb,* secreted outside the bacterial cell and regulates the immune regulatory proteins, such as p-p38, p-VPS33B and p-JNK, by dephosphorylating them in the host cytoplasm. Hence, it suppresses the host innate immunity. The most significant host gene controlled by PtA is GADD45A *i.e.* PtpA that can bind directly to the GADD45A promoter (Wang et al. 2017).

Secretory systems

Currently, much of the data are available on *Mtb* genomics and proteomics. Pathogenesis of TB is largely dependent on secretory (exported) proteins of *Mtb*. These proteins are exported into host cell(s) and modulate its functions, especially those related to immunity (Majlessi et al. 2015). Three main secretory systems are present in *Mtb,* which are involved in exporting their secretory proteins to the external environment (DiGiuseppe and Cox 2007). These include: general secretory (Sec) system, twin arginine translocation (TAT) system and Type VII secretory system (T7SS) or Esx secretory system. Sec system has Sec A, D, E, F, G and Y proteins, which are unfolded in nature with N-terminal signal sequence across the bacterial cell membrane (DiGiuseppe and Cox 2007). SecA2 (member of SecA) has great importance in imparting virulence to *Mtb* (Lenz et al. 2003). Sec system, the diagrammatic illustration of which is shown in Fig. 1.8.2a, has been drawn by taking indication from Prabudiansyah et al. (2015), the study describing SecA1 and SecA2 interaction in *Mtb.*

TAT is another system, independent of Sec, which translocates folded proteins. Its member proteins have similar N-terminal sequence, with specialty of double arginine motif (Frain et al. 2019). It has been hypothesized that they are involved in pathogenesis of TB by directly acting on host cell membrane and disturbing its signaling or release some lipid moieties that are consumed by *Mtb* (Muñoz-Elías et al. 2006). Rv2525c is the member of TAT system, and is highly virulent for mice (Saint-Joanis et al. 2006). This protein has structural homology with transglycosidase enzyme involved in bacterial wall synthesis (Kelley et al. 2000). Organization of TAT genes in *Mtb* and its comparison with *E. coli* has

been shown in Fig. 1.8.2b. This figure has been strategized by taking notion from Saint-Joanis et al. (2006) research.
The third category of secreting proteins *i.e.* T7SS/Esx is a very special type; its proteins are smaller in size and lacking secretory signal (Sorensen et al. 1995). Most of the proteins in this system are functionally hypothetical. The two common members are ESAT-6 and CFP-10, which are considered immunodominant antigens (Wards et al. 2000). The whole Esx family has two novel features; 1) all member proteins have almost 100 amino acids sequence with Trp-X-Gly (WXG) motif in the center of protein that gives helix turn helix structure (Renshaw et al. 2005), 2) transmembrane proteins with FtsK–SpoIIIE-like ATPase family (Pallen 2002). Fig. 1.8.2c demonstrates the organization of Esx system, designed as per concept taken from the study of Delogu et al. (2013).
Specialized protein transport mechanism has been used by *Mtb* to deliver protein in its inner and outer membrane. Type VII secretion system (T7SS) is the one among transport systems that was identified in 2003 in *Mtb*. A total of 5 T7SS systems have been recognized in *Mtb* and commonly designated as Esx-1, Esx-2, Esx-3, Esx-4 and Esx-5 (Gröschel et al. 2016). Almost all Esx have common features, including small secreted protein pair (each of ~100 amino acids) with conserved Trp-X-Gly (WXG) motif (Vaziri and Brosch 2019). This helps the secretory pair to make helix-turn-helix conformation. This system also has some conserved transmembrane proteins that help in the secretion of secretory pair, hence called Esx conserved components (Ecc) (Famelis et al. 2019). The idea of plotting Fig. 1.8.2d has been taken from the Houben et al. (2014) manuscript; designed and entitled "Take five-Type VII secretion systems of Mycobacteria".

Conclusion

Tuberculosis (TB) is a highly contagious disease of human beings and bovines, caused by *Mycobacterium tuberculosis* (*Mtb*) and *Mycobacterium bovis* (*M. bovis*), respectively. Both species share almost 99% genomic identity and can infect each other's host with less severity of disease than infecting their own host. This observation leads to the development of Bacillus Calmette–Guérin (BCG) vaccine (a modified form of *M. bovis*) for human TB. Currently, BCG is the only available vaccine for TB; it offers no booster and efficacy in adults. The promising adjunct or BCG complement remains major challenge to overcome TB menace. Potent vaccine against *Mtb* that can be used safely in adults and also as a booster is still in question, even after 100 years discovery of BCG. Therefore, exploring the new vaccine candidates for TB vaccine development is the need of time and this can be done by researching more on epidemiology and function genetics of *Mtb*.

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PATHOGEN, HOST CELL RESPONSE, DIAGNOSIS AND THERAPY OF BRUCELLOSIS

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INTRODUCTION

Brucellosis is one of the most ubiquitous zoonosis with global distribution (Gul et al. 2015; Massis et al. 2019). Etiology of this disease is Brucella that is a facultative intracellular pathogen. In Brucella genus, 11 species are recognized (Gul et al. 2013; Mesureur et al. 2018). The disease may affect bovine, caprine, ovine, swine, and humans. Due to lack of hygienic processes, public health measures, even national animal health managing policies, the disease is more common in developing countries (Thakur et al. 2002; Farouk et al. 2017; Hasan et al. 2021). Brucellosis causes abortion, reduced fertility, decreased milk production and cost of replacement (Khan and Zahoor 2018). Serious socioeconomic issues can be posed by the disease to livestock owners (Khan et al. 2020). Due to rapidly increasing intercontinental tourism and animal trade, there are high chances that the disease could spread in developed countries (Imtiaz et al. 2018). Brucellosis in humans frequently results in a typical undulant fever, with osteoarthritis as usual impediments (López-Santiago et al. 2019). The intracellular biology of the Brucella is the consequence of complicated interfaces with host that is mandatory to determine a role of pathogen existence and multiplication. In spite of the risk to the public health, there are no effective vaccines to counteract many of them. In this chapter, advances in the field of the pathogen, host cell response, diagnosis and therapy of brucellosis are described.

History, Spread and Pathogen

Huge economic losses are rendered by the Brucellosis (Shahzad et al. 2018), especially in food animal production sector. The economic losses in animals due to Brucellosis are primarily due to abortions, occurring during the last trimester, decreased milk yield, transient infertility and perinatal mortalities (Gul et al. 2015; Zeng et al. 2019; Khan et al. 2020). The disease is endemic in the buffalo and cattle, causing approximate economic loss of US $ 344 billion to the animal industry (Pal et al. 2020). In some countries of the world, animal Brucellosis has been eradicated, but in many other countries it remained uncontrolled (Gomez et al. 2013). Due to its zoonotic aspect, high incidence in humans has been reported from different countries (Wang and Jiang 2020), including Yemen (89.96%), Kenya (203.07 cases per 100 000), Syria (47.26%), Greece (42.96%) and Eritrea (21.82%). However, this disease continues to exist, particularly in Africa, India, South and Central America, Middle East and the Mediterranean region (Baldane et al. 2012; Wang and Jiang 2020; Akya et al. 2020). In the past, in humans, 0.5 million new cases have been reported due to Brucellosis annually (Franco et al. 2007; Pal et al. 2020). On the basis of published reports about Brucellosis, Iran stands second in the world with an annual prevalence of 98-130 people/100,000 populations (Marvi et al. 2018).

It is documented that David Bruce from Royal British Medical Staff examined it as “Malta fever” or “Mediterranean fever”, while the British troops were enduring high fever for long time. David Bruce was able to culture the bacterium liable for the disease in 1887. Afterwards, Themistocles Zammit’s discovered that people rearing goats, and drinking their milk also exhibited similar signs, as those of Mediterranean fever patients. Then, Zammit was able to reproduce the disease in healthy goats, who also isolated Brucella from milk and blood. He concluded that the personals of British Army could have caught the disease by drinking milk from contaminated goats (López-Santiago et al. 2019). Based on these results, though, a decision to ban goat’s milk consumption in the British army was made in 1906, however, Malta fever was not eradicated but doubts evolved regarding the use of cheese, and ice-cream made from contaminated milk. Findings of Zammit demonstrated that Brucellosis is commonly transmitted via oral route. Later, other routes were documented (parenteral, respiratory, or by contact) and the disease was believed to be the occupational hazard (Mantur et al. 2007; Gomez et al. 2013; Gul et al. 2015; Shahzad et al. 2017). It has been reported that following possible risk factors can be responsible for human brucellosis: i) eating contaminated animal products, ii) occupational exposure, and iii) contact with diseased animals or their products and/or discharges (Pal et al. 2020).

Classification of Brucella spp. is established on host inclination and virulence (Cloeckaert et al. 2002). The genus Brucella consists of seven species based on primary host and antigenic variation: *Brucella abortus* (cattle), *B. melitensis* (sheep and goats), *B. ovis* (sheep), *B. canis* (dogs), *B. neotomae* (wood rats), and *B. suis* (hogs). *Brucella abortus* causes abortion spontaneously in bovines, thus leading to major monetary losses to livestock farmers. Currently, *B. melitensis* REV.1 or *B. abortus* RB51 strains are being utilized to vaccinate caprine and ovine or bovine, respectively (Atluri et al. 2011; Shahzad et al. 2018; Dadar et al. 2019; Celli 2019). Causative agent of Brucellosis can survive for two to four months under natural environment but would die in 10-20 minutes at 60°C, by disinfectants of peroxides, iodine

or chorine. Bacteria of Brucellosis form intracellular phagocytotic vesicles to escape from the effects of antibiotics (Ugalde et al. 2000). The membranes are made of cellulose, peripheral cytoplasm membrane and the outer membrane, with outside enveloped cytoderm and peptidoglycan; it has well known antigen involved phosphatide, lipopolysaccharide and proteins distributed at out membrane. For example, different peptides have been reported, such as 10 ku/kd, 16.5 ku/kd, 19 ku/kd, 25-34 ku/kd, 31-38 ku/kd and 89 ku/kd, especially genes of omp25 and omp31 ecode 25-34 ku/kd proteins (Fig. 1).

Lipopolysaccharide of Brucella strains are in both smooth and rough forms (Corbel 1990). The rough strains, comprising no or low O polysaccharide (OPS), usually are less potent than smooth strains and are also less challenging to complement strike (Ko and Splitter 2003). However, sometimes spontaneously virulent strains, like *B. canis* and *B. ovis,* are rough stains. Virulence factor can be identified by two aspects of Brucella LPS. First, less immunogenic LPS amount in the Brucella than enterobacterial LPS. Whereas, non-pyrogenic Brucella LPS are unable to stimulate the alternate perfect route to a substantial level and is a very mild mitogen B cells (Sangari and Aguero 1996). Furthermore, 10 times more Brucella LPS is needed for interferon (IFN) production and lethality compared to bacterial endotoxins (Keleti et al. 1974; Ko and Splitter 2003). Thus, Brucella LPS low biological activity is necessary for the survival of Brucella in phagocytic cells (McQuiston et al. 1999). Second, OPS-deficient Brucella mutations are vulnerable to complement-mediated lysis and polymyxin B, as *in vivo* and *in vitro B. abortus* phosphomannomutase (pmm) transposon mutants were attenuated (Allen et al. 1998; Ko and Splitter 2003) and were susceptible to complement-mediated killing.

Brucella infection begins via ingestion or inhalation of the causative organisms through the oral, nasal, and pharyngeal cavities (Morgan and Corbel 1990). Following their entry into the mucosal epithelium, the bacteria are carried out to the regional lymph nodes, either in free form or within phagocytic cells. The propagation and proliferation of Brucella in liver, spleen, lymph nodes, mammary glands, bone marrow, and sex organs takes place through macrophages (Godfroid et al. 1998). In general, humans get *B. abortus*, *B. melitensis*, and *B. suis* infections and usual pathological manifestations include endocarditis, arthritis, spondylitis, meningitis etc. (Ko and Splitter 2003).

Pathophysiology

Brucella enters into the animal body via oral cavity; it comes-across several hurdles, like saliva that is rich source of antibodies, neutrophils, plasma cells, complement molecules, etc. After passing through the mucosal barriers of digestive system, the pathogen is defensed by the intestinal mucosa, containing proteins as well as immune cells (Mowat and Agace 2014) involved T lymphocyte and B lymphocyte in gut associated lymphoid tissue (GALT), such as mesenteric lymph nodes (MLN) and Peyer's patches (Forchielli and Walker 2005). Mucosal cells along with phagocytic cells in these tissues recognize Brucella pathogens. Dendritic cells (phagocytic cells) and macrophages (antigen-presenting cells, APCs) are capable to engulf Brucella and take them to the nearby local lymph node (López-Santiago et al. 2019). As soon as these cells engulf Brucella, APCs move to the lymph node to introduce the bacteria to the lymphocytes and then deliver it to the proper activation signal.

Host cell response to antigen

Innate immune responses

Brucella spp. infect phagocytizing cells and disrupt intracellular trafficking pathways. It allows antigen to invade defensive processes to induce an intracellular environment which is favourable for existence and multiplication of the antigen and to provide a means for propagation. After breaking the mucosal obstacles, Brucella affects intraepithelial phagocytic or submucosal cells and sabotage intracellular operating pathways (Pappas et al. 2005; Gomez et al. 2013). This pathway permits Brucella spp. to invade defensive mechanisms of host phagocytosis to create an intracellular environment that could play a role for the survival and duplication of pathogen and to support distribution of host cells (Adams 2002). The most important virulence factor of Brucella spp. is its capability of existence and multiplication within the phagocytic cells, in addition to the processes which lead to death of cells at intracellular level. Brucella spp. can affect various cells, such as epithelial cells, monocytes, macrophages, B lymphocytes, DC, etc. The antigen is depicted by macrophages, where it is recognized as intracellular processes, i.e., phagosome-lysosome fusion (Pizarro-Cerda et al. 1998) and respiratory burst via components, such as LPS and those of the type-IV secretion system (Franco et al. 2007). The Brucella spp. are intercellular bacteria, which favors their survival and tenacity by dogging the host immune system (Skendros et al. 2011; Gomez et al. 2013).

Adaptive immune responses

Adaptive immune reactions are essential for aiding the memory purposes in vaccination. In Brucellosis, purposes of the adaptive immune response can be classified into three mechanisms: i) γδ T, CD4+ and CD8+ produce IFN-γ by triggering macrophages against the bactericidal activities to obstruct the survival of the Brucella intracellularly; ii) cytotoxicity of γδ T and CD8+ cells kill the macrophages infected with the Brucella (Bessoles et al. 2011; Zheng et al. 2018); iii) Th1-type antibody isotypes, such as IgG2a and IgG3, opsonize the pathogen to enable phagocytosis (Ghaderinia and Shapouri 2017). The key role of T cells in Brucella immunity is the excretion of IFN-γ for the stimulation of cytotoxic T-lymphocyte activity and bactericidal activity in the macrophages. The importance of CD4+T and/or CD8+T cells in Brucella immunity has been presented as histocompatibility complex (MHC) class I and II (Fig. 2).

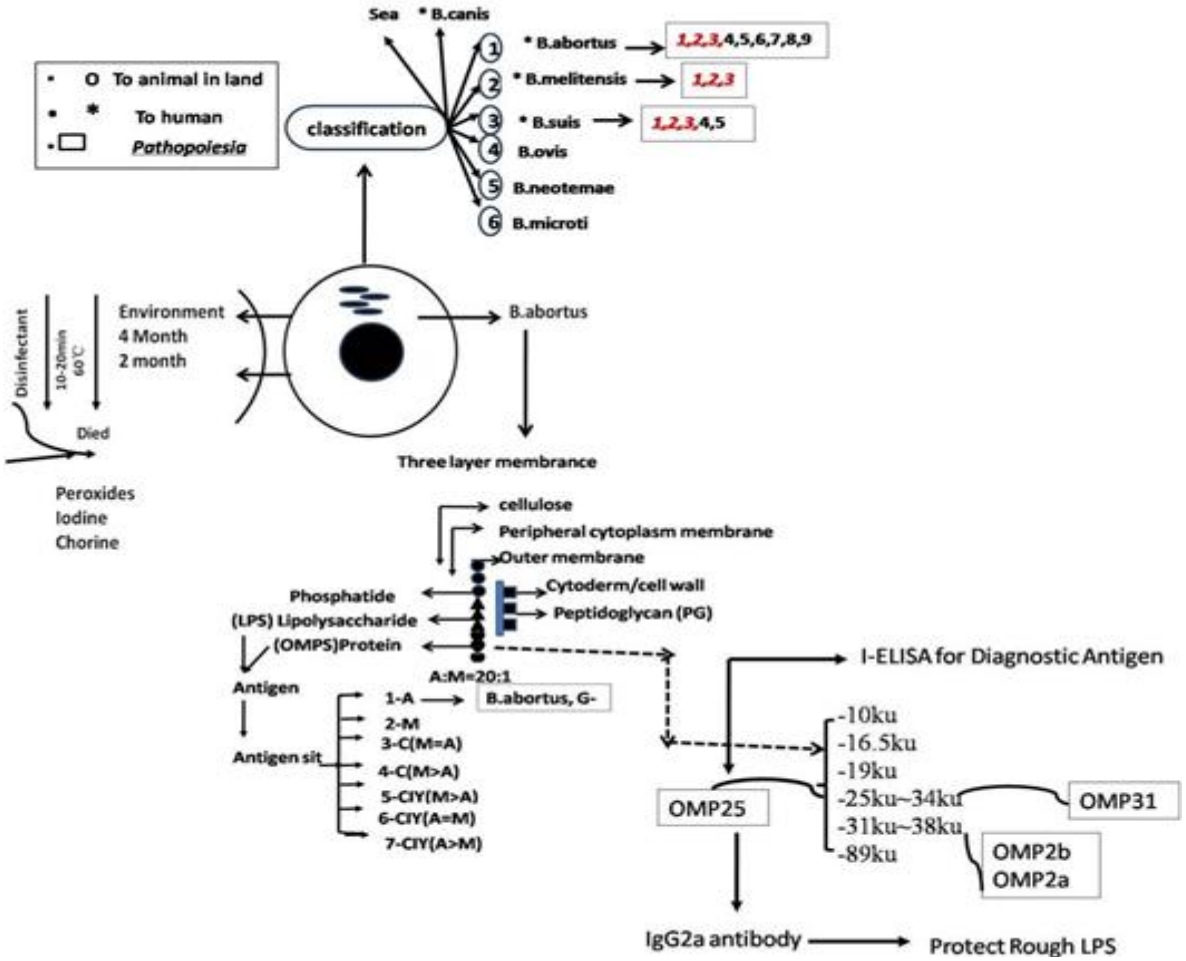

Figure 1: Pathogenesis of Brucellosis in the body.

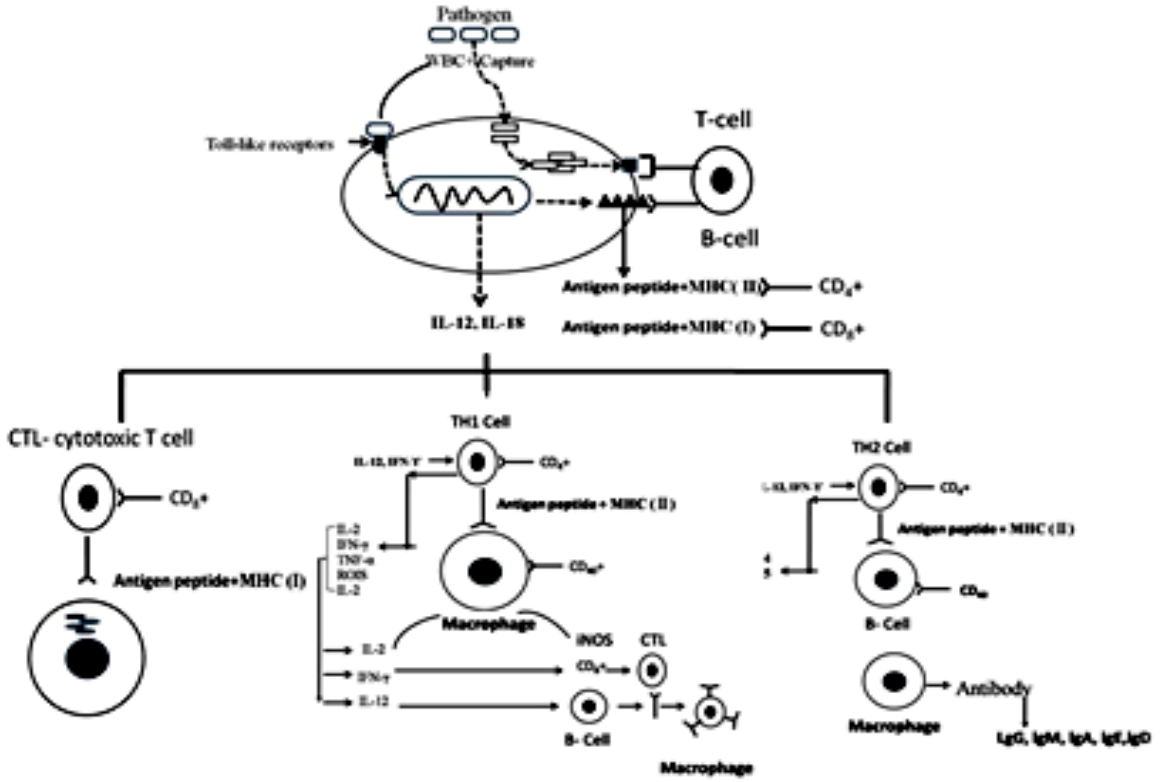

Figure 2: Mechanism of presented histocompatibility complexes.

Macrophages and T-cells play a vital role in the defense. The helper T-cell-arbitrated defense is mainly linked with a Th1 T-cell reaction and perseverance with a Th2 response (Yingst and Hoover 2003; Perkins et al. 2010; Skendros et al. 2011; Gomez et al. 2013). Precisely, results have indicated defensive aids for TNF-α, IFN-γ, and IL-12 against Brucellosis (Murphy et al. 2001; Brandao et al. 2012). Cytotoxicity of T cells and T-cell derived cytokine-mediated orchestration of the immune response in defense against the Brucellosis is important (Araya et al. 1989; Huy et al. 2021). Role of dendritic cells in adaptive and innate immunity and their survival at the level of mucosal surfaces renders them important in the study of the Brucellosis (Iwasaki 2007). The dendritic cells have been shown to be permeable to brucellae multiplication and infection (Bosio and Dow 2005). Brucella has been proved to control the reaction of these cells, i.e., dendritic cells (Iwasaki 2007; Imtiaz et al. 2018). Lastly, natural killer cells are cells with cytotoxic abilities and have ability to produce IFN, but a title role for these cells in the control of acute Brucellosis is not clear (Vivier et al. 2011; Gomez et al. 2013).

Humoral immunity

Accurate defensive processes of humoral immunity against intracellular pathogens, like Brucella, lacking in β-cell activity specify that this cell type is not essential for the defense at the level of primary infection, yet antibodies from the vaccinated and immunized or exposed animals provide necessary defense to the animals not exposed to the disease (Goenka et al. 2011). Moreover, results of a previous study indicate that antibodies possess a protecting role compared to re-infection with Brucella spp. (Gomez et al. 2013). The results further indicate that the innate immunity mechanisms, that herald expansion of humoral immunity, are adequate to overcome the primary Brucella infection and the synergetic and/or repressive impacts of antibodies need to be studied (Titball 2008).

Diagnosis

Clinically, the disease in animals often characterized by clinical signs, such as abortion, retained placenta, arthritis, orchitis, and epididymitis with excretion of the Brucella spp. in discharges and milk (Shahzad et al. 2017). There are different methods for diagnosis of Brucellosis, but the gold standard test remains the culture isolation (Ko and Splitter 2003; Gul et al. 2015). Serum agglutination tests and milk ring test are being used for the screening of the patient. Important isolation sources are milk and vaginal discharge from infected animals. Moreover, when there is abortion, then organs of aborted fetus, including stomach content, lymph nodes, etc. are the best sources for isolation of the bacteria (Singh et al. 2014). Phage typing, a very handy tool for species and biovars characterization along with biochemical tests, has been in use (Singh et al. 2014). Additionally, different serological tests involving lgM isotype, lgG1 and lg A have also been reported (Weynants et al. 1996).

With advancements in the field of diagnosis, many laboratory tests, such as 16s rRNA, ELISA and PCR across the world are in use; these tests help in the development of molecular markers which are specific and sensitive assays for the detection of Brucella spp. (Shahzad et al. 2017; Imtiaz et al. 2018). PCR–based methods that point out the molecular markers are more helpful and practical in nature than other assays and may take sometime to be fully functional and applicable in the field. PCR–based methods are quick, simple, possess high sensitivity and less hazardous (Singh et al. 2013) for Brucella detection, especially those using the 16S rRNA as targets (Shahzad et al. 2018) and the bcsp31 genes (Singh et al. 2014; Imtiaz et al. 2018) are highly sensitive for genus Brucella.

Therapy

Brucellosis is usually treated with antibiotics, like rifampin, streptomycin, gentamicin and doxycycline. However, the effect of treatment is usually limited. So, vaccine development is the best way for treatment, prevention and control of Brucellosis.

In human Brucellosis, most commonly implicated agents are *B. abortus*, *B. melitensis*, and *B. suis* (Franco et al., 2007; Wattam et al. 2009; Gomez et al. 2013). The virulence of these organisms is variable, with *B. melitensis* being at the top. Vaccination is the most effective and low-cost solution for the prevention of the disease (Oyewumi et al. 2010; Imtiaz et al. 2018). There are two main procedures to produce immune-protection against Brucellosis, vaccination of the animals/humans with live-attenuated organisms or subunit antigens (Gomez et al. 2013). However, the success of this type of immunization approach is influenced by multiple factors, including pathogen biology, efficacy, safety, and adequate levels of immunization.

The first vaccine used in cattle to control Brucellosis was the S19 vaccine (Imtiaz et al. 2018). This vaccine is a live attenuated when administered via action of cytotoxic-T-lymphocytes it produced protective immunity (Levitz and Golenbock 2012), however, it is very difficult to differentiate between infected and vaccinated animals, as both types of animals show a similar serological response (Al-Dahouk et al. 2005). Another vaccine, RB51, was unstable (Moriyon et al. 2004). Presently, live attenuated *Brucella* vaccines are being used to control the disease in animals, however, major difficulty of their wide application is about human's safety against them (Ficht et al. 2009; Goodwin Pascual 2016; Zhang et al. 2017; Lalsiamthara and Lee 2017).

Brucella melitensis Rev.1 is also a live attenuated vaccine, commonly used in animals for the control of *Brucellosis* (Levitz and Golenbock 2012; Avila-Calderon et al. 2013). The presence of smooth LPS in the vaccinal strain Rev-1 might make it difficult to differentiate between infected and vaccinated individuals, and may also interfere in the test-and-slaughter policy (Khan et al. 2017). Disadvantages of live attenuated vaccines are that, being

pathogen for humans and animals, they may i) lead to the development of resistant to streptomycin, ii) could cause abortion in pregnant animals, and iii) produce specific antibodies against LPS that may impede diagnosis (Gwida et al. 2010; Khan et al. 2017; Imtiaz et al. 2018).

It has been documented that subunit vaccines are safe and efficient against *B. abortus* in both humans and animals (Dorneles et al. 2015). Various subunit (Ghasemi et al. 2015), DNA (Leclercq et al. 2003; Al-Mariri et al. 2010) or live vector vaccines have been produced (Cabrera et al. 2009). Humoral, as well as cellular, immunity both play a significant role in protective immunity against *Brucella* infection, though cell-mediated immunity is likely to perform an important role in the safety, as *Brucella* is a pathogen that is present intracellularly (Gul et al. 2015). The IFN-γ is secrete by the CD4+ and CD8+ T lymphocytes, and is reported to play an important role in the control of Brucellosis (He et al. 2002). When DNA vaccine is used to immunize animals, both humoral, as well as cellular, immunity is produced against many pathogens (Villinger et al. 2004; Donnelly et al. 2005), thus, the effectiveness of DNA vaccine against *B. abortus* is augmented by encoding various genes, like SOD, L7/L12, and BCSP31 (Da-Hai et al. 2007; Imtiaz et al. 2018). Similarly, recombinant flagellar proteins (FlgJ and FliN) and DNA vaccine encoding BAB1_0270, BAB1_0278, BAB1_0278a (Sislema-Egas et al. 2012; Li et al. 2016) were used to produce a good immune retort and safety against *B. abortus* infectivity (Escalona et al. 2017).

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FOODBORNE MICROORGANISMS

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INTRODUCTION

Food microbiology deals with beneficial and harmful microorganisms affecting the safety and quality of raw and processed meat, milk, poultry, and egg products. Food contamination results in 600 million cases of foodborne diseases every year, with around 0.75% of the global annual death are due to such diseases (Milani 2013). In general terms, food microbiology is related to studying microorganisms that contaminate, modify, process, and spoil food products. These include fermentative, probiotics, as well as pathogenic bacteria, like *Salmonella* species, *Campylobacter jejuni*, *Shigella* spp., *Vibrio* spp., *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum* and viruses, such as Norwalk and Hepatitis A. Fungi, like *Aspergillus spp*., *Penicillium spp*., and *Fusarium spp.*, are associated with mycotoxins production in foods. Bacteria account for about 66% of foodborne diseases, while chemicals cause 26% of the illnesses. Each of the viruses and parasites is responsible for about 4% of foodborne diseases (Desta and Addis 2015). Many microorganisms produce different diseases inside or outside the human body (Bintsis 2017), with most pathogens cause zoonotic diseases (Ejo et al. 2016). Food products are susceptible to contamination at different stages of processing, including production, handling, distribution, preparation, transportation, and human consumption (Hemalata and Virupakshaiah 2016). However, food contamination depends mainly on the food handler's health status, hygiene, knowledge, and food hygiene practice (Aklilu et al. 2015). In food microbiology, specific terms describe different conditions, mostly related to the pathogen that contaminates food products. Examples are foodborne illness, food poisoning, and food intoxication; each is related to pathogen growth inside the food products (Abebe et al. 2020).

Factors affecting growth of microorganisms inside foods

Microorganisms mostly require a suitable environment for growth inside or outside food products, whether bacteria, virus, fungi, or parasites. Many factors, which may affect microbial growth in meat, eggs, poultry and dairy products, are classified as intrinsic or extrinsic factors (Rolfe and Daryaei 2020).

Intrinsic factors

pH

Most microorganisms grow at an incubation pH range of 6.7 and 9.0 and hence, food products are stored under alkaline (pH~9) or low acidic (pH~6) conditions (Kim et al. 2019). Food pH levels can significantly affect growth of microorganisms and many bacteria grow at environmental pH from 4.5 to 9.0. Most yeasts and molds can grow in acidic and alkaline environments, a pH range from 2.0 to 10.0, but the minimal optimum environment for growth of most microorganisms needs to have a neutral environment, and a small number of them can grow at a pH lower than 4.0. Bacteria are more fastidious about nutrition or culture requirements than fungi. That is why bacteria, especially pathogenic, are more susceptible to pH alterations than yeasts or molds (Rolfe and Daryaei 2020). The pH range of meat after slaughtering of the animal is nearly acidic (4.0–5.6) due to elevated lactic acid levels from glycogen, indicating that meat products are more susceptible to bacterial spoilage contamination than mold and yeast (Ray and Bhunia 2007).

Water activity or moister content

The water content of any food product has a tremendous role in the deterioration of its quality, shelf life and preservation, which is a critical point in industrial food processing. Drying (dehydration) or desiccation is one of the oldest food preservation methods, removing water or preventing moisture binding to prevent pathogen growth. The water requirement for the growth of a microorganism is known as water activity (aW). Without water, most microorganisms cannot grow inside food products. Other methods that are now being used are heating, freeze-drying, and the addition of salt and sugar (Rolfe and Daryaei 2020). The aW of pure water is 1.0, but most foodborne pathogenic bacteria require aW between 0.75 and 1.0. Molds grow slowly at 0.62 aW, but bacteria require aW of about 0.86, except for toxin-producing species such as *Clostridium* and *Staphylococcus aureus* (Iulietto et al. 2015).

Redox potential

The redox potency of a substrate evaluates its power to receive or give electrons. A compound that donates an

electron is oxidized. Microorganisms exhibit various levels of sensitivity to the redox potential of their growth environment. Aerobic microorganisms need a more oxidative environment (more oxygen), but aerobic microorganisms require a more reduction environment (lack of oxygen) (Lb et al. 2007). Many other intrinsic factors may alter microbial contamination of food products like nutrient contents, antimicrobial constituents and biological structures of food particles.

Extrinsic factors

Temperature

Storage temperature is an important factor that can affect or control bacterial growth. Microorganisms, either individually or as a group, can grow at different temperature ranges. So, it is essential to know the growth temperature range of microorganisms that aids in choosing the right temperature for product storage. This control is essential to improve the shelf-life and nutritional value of food products (Preetha and Narayanan 2020).

Presence/concentration of gases

Gas concentration inside the food products, especially oxygen (O_2), carbon dioxide (CO_2), and ozone (O_3) levels, mostly control the growth of microorganisms in food (Preetha and Narayanan 2020). Levels of these gases regulate the growth of aerobic and anaerobic bacteria; aerobic microorganisms require higher oxygen levels, while anaerobic bacteria prefer a low quantity of oxygen for their growth (Pellissery et al. 2020).

Relative humidity

The relative humidity of the storage environment is essential from the viewpoint of aW within foods and growth of microorganisms. Also, the microbial contents of food play a vital role in its shelf-life (Esbelin et al. 2018).

Foodborne Pathogens

Most foodborne diseases result from the administration of food or water polluted with microorganisms or their metabolic products. Bacteria are considered important causes of foodborne disease outbreaks all over the world. Viruses causing foodborne diseases are Norovirus and Hepatovirus. Fungal species, including *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., are associated with foodborne illnesses through production of mycotoxins. Also, protozoa, such as *Toxoplasma gondii*, *Cryptosporidium spp*, *Cyclospora cayetanensis*, *Cystoisospora belli*, *Sarcocystis* spp., *Giardia* spp., and *Entamoeba histolytica,* are associated with foodborne diseases (Bintsis 2017; Gourama 2020).

Foodborne Bacteria

Gram-positive bacterial pathogens responsible for foodborne diseases include *Staphylococcus aureus*, *Clostridium botulinum, C. perfringens, Bacillus cereus,* and *Listeria monocytogenes*. These bacteria, except *L. monocytogenes* and *C. perfringens*, are capable of living on food and liberate toxins, causing food poisoning once consumed. *Clostridium botulinum*, *C. perfringens*, and *B. cereus* produce spores, enabling them to resist heat and other untoward conditions. Foodborne disease-causing Gram-negative bacteria include *Salmonella* spp., *Campylobacter* spp., pathogenic *Escherichia coli*, *Shigella* spp., *Yersinia enterocolitica,* and *Vibrio* spp. (Table 1). Every bacterial species has specific nutritional, temperature, and humidity requirements (Ag et al. 2020).

Gram-positive Foodborne Pathogens

Staphylococcus aureus

Staphylococcus aureus is a facultatively anaerobic Gram-positive species of the family Micrococcaceae, which comprises several subspecies (Abraha et al. 2018; Abebe et al. 2020). *S. aureus* is the most frequent causative agent of foodborne diseases worldwide with a high incidence, second to salmonellosis (Abebe et al. 2020). It is present on the skin surface, nose, and mucous membranes of healthy animals and humans (Abebe et al. 2020). During food preparation and processing, *S. aureus* can contaminate food products and grow at a wide pH range (4.2–9.3; optimum 7.0–7.5), temperatures (7.0–48.5°C; optimum 30.0–37.0°C), and up to 15% NaCl (Kadariya et al. 2014). Also, it can cause many infections and foodborne illnesses (Wang et al. 2017). These bacteria are critical in the food industry causing food intoxication, food poisoning, and food spoilage, mainly due to staphylococcal enterotoxin (2020). *S. aureus* survives for a long time in foods, particularly those that need handling during processing and fermented foods such as cheese (Addis 2015).

Transmission

The primary source of transmission of *S. aureus* is through contaminated food during handling, processing, preparation, wrapping, mincing, and storage of animal-based products (Wang et al. 2017). *S. aureus* also contaminates poultry, pork, seafood, and bakery products, mainly infected with enterotoxigenic strains (Karimi et al. 2019), raw meat, milk, pork, and moist food comprising starch and proteins (Wu et al. 2016).

Pathogenesis

Staphylococcus aureus possesses different virulence factors that enhance its attachment to the extracellular matrix components, damage host cells, and fight the immune system (Fluit 2012). It also produces extracellular active substances, such as coagulase, protease, hemolysins,

Table 1: Common pathogenic bacteria associated with foodborne illnesses

Pathogens	Pathogen characteristics and its toxin	Commonly associated foods
Bacillus cereus	Gram-positive bacilli, spore-forming, aerobic Cereulide toxin (emetic syndrome, emetic toxin) Production of toxin in the small bowel (diarrheic syndrome)	Rice and grain-based foods Meats, milk, pasta, desserts, cakes, sauces
Campylobacter spp.	Gram-negative, microaerophilic, and catalase-oxidase positive Invasion of intestinal cells	Raw meat and poultry, under-pasteurized milk, contaminated water
Clostridium botulinum	Gram-positive bacilli, spore-forming, anaerobic *Botulinum* neurotoxin	Home-tinned vegetables and meats, honey, milk products, fish, fermented seafood
C. perfringens	Gram-positive bacilli, spore-forming, anaerobic *Clostridium* enterotoxin	Meat products
E. coli (pathogenic)	Specific *E. coli* serotypes, such as O157:H7, produce Shiga toxins which may cause serious infections. Toxin production (EPEC, EIEC, EAggEC, ETEC, EHEC, and DAEC)	Meat products, raw or underpasteurized milk and juices, leafy vegetables, fish, contaminated water
Listeria monocytogenes	Intestinal epithelial cell invasion and dissemination to other tissues and organs	Raw vegetables and salads, cheeses, raw or under-pasteurized milk, deli beef, chicken, smoked fish
Salmonella spp.	Gram-negative bacilli, Intestinal cell invasion causing gastroenteritis, Typhoid fever (typhi and paratyphi serovars)	Eggs, meats, dairy products, fruits, spices, melons, raw/untreated tree nuts
Shigella spp.	Intestinal cells invasion and toxin production	Shellfish, crustaceans, fruits, vegetables
Staphylococcus aureus	Gram-positive cocci Staphylococcal enterotoxins	Milk and dairy products, egg and meat products, salads such as tuna, chicken, potato, and macaroni, cream-filled pastries, confectionaries
Vibrio species	Gram-negative, non-spore-forming, curved, rod-shaped *Vibrio cholerae:* Cholera toxin *Vibrio parahaemolyticus*: production of adhesins and cytotoxins *Vibrio vulnificus*: production of adhesins and cytotoxins	Contaminated water, vegetables, and seafood Shellfish, raw fish, shrimp, and oyster Shrimp, fish, oysters, and mussels
Yersinia enterocolitica	Intestinal cell invasion	Beef, fish, milk products, oyster, pork, poultry

nuclease, lipase, acid phosphatase, fibrinolysis, enterotoxins, and toxins, that cause toxic shock syndrome (Tsepo et al. 2016).

When food products are stored at room temperature for a while, the bacteria start growing and producing enterotoxins. These enterotoxins are heat-stable proteolytic enzymes that might be present in food with bacteria's absence (Argaw and Addis 2015; Adugna et al. 2018). There are 23 staphylococcal enterotoxins (SEs) (Wu et al. 2016; Wang et al. 2017), which stimulate the vomiting center of CNS, inhibit intestinal water and sodium assimilation (Desta and Addis 2015) and cause acute gastroenteritis (Abebe et al. 2020).

Symptoms

The incubation period of *S. aureus* is short, about 2 to 4 h only. After eating infected food, several diseases could be produced by this type of bacteria, ranging from skin disease to life-threatening severe infection such as septicemia, endocarditis, necrotizing pneumonia, and toxic shock syndrome (Wang et al. 2017; Che Hamzah et al. 2019). Most of these diseases are characterized by nausea, vomiting, chills, hypothermia, cephalalgia, and abdominal cramps with or without diarrhea (Dhama et al. 2013; Kadariya et al. 2014; Wang et al. 2017). Death occasionally occurs in children, old aged, and immunocompromised patients (Wang et al. 2017).

Clostridium botulinum

Clostridium botulinum is motile organism, using peritrichous flagella, and produces botulinum neurotoxins, of which seven types are recognized, A through G, according to the toxin's antigenic specificity of each strain (Lund and Peck 2013). Types A, B, E, and F cause botulism in human beings, while types C and D cause botulism in birds and mammals. Type G has not yet been implicated in any botulism case (Lund and Peck 2013). Thermal processing is the most widespread method used to inactivate *C. botulinum* spores and produce shelf-stable, low-acid, moist foods (Tumpanuvatr et al. 2015).

C. botulinum is present in soils, fresh water, marine sediments, and the intestinal tracts of animals. Food sources commonly sampled include honey, which should not be fed to infants less than one year of age, fish, meat, vegetables, and infant foods. Botulinum toxin has been reported in various foods, including canned food, chicken and chicken giblets, ham, lobster, luncheon meat, sausages, and smoked and salted fish (Bintsis 2017).

Pathogenesis

Botulinum toxin, produced by *C. botulinum*, is the causative agent of botulism, and it is the most toxic substance known to be lethal to animals and humans. Botulinum toxin is a single polypeptide chain that is the most potent toxin at present, with as little as 30–100 ng being potentially lethal (Francisco et al. 2018). Botulinum toxin is a zinc-dependent protein of 150 kDa, composed of two subunits; a 100 kDa heavy chain and a 50 kDa light chain. Eight strains of *C. botulinum* have been described, depending on the toxins they produce. These strains are A, B, C1, C2, D, E, F, and G. Serotype C2 produces an enterotoxin, types C and D are bacteriophage coded, and all except C2 produce neurotoxins (Zaragoza et al. 2019).

Botulinum toxin is produced intracellularly and appears outside only after the lysis of bacterial cells. It is synthesized at first as a nontoxic protoxin and needs trypsin or other proteolytic enzymes to be activated (Tamang et al. 2017).

Symptoms

The symptoms of botulinum neurotoxin ingestion appear 12–36 h after consuming contaminated food and initially include nausea and vomiting. The more characteristic neurological signs follow later, including visual disorder and acute flaccid paralysis that begins with the facial muscles, head, and pharynx, descending to involve the thorax and limb muscles. Paralysis might lead to death from respiratory failure caused by upper airway or diaphragm palsy (Cai et al. 2007; Harris et al. 2020). The minimum toxic dose of *C. botulinum* neurotoxin has not been determined, but from a human health and food safety standpoint, there should be no tolerance for the neurotoxin itself or conditions allowing growth of the organism in food (Harris et al. 2020)

Clostridium perfringens

Clostridium perfringens belongs to the family Bacillaceae and is a major cause of foodborne disease. Clostridia are non-motile, encapsulated, rod-shaped cells that produce protein toxins and form spores resistant to various environmental stresses, such as radiation, desiccation, and heat (Grass et al. 2013; Gul et al. 2016). Vegetative cells grow at temperatures from 6 to 50°C, but 43 to 47°C is the optimal range. Growth requires a minimum aW of 0.93, a NaCl concentration less than 5–8% depending on the strain, and a pH of 5.0–9.0, optimum is 6.0–7.2 (Freedman et al. 2016). *C. perfringens* is the most prevalent clostridial species found in human clinical specimens, excluding feces. It has been implicated in simple wound infections to myonecrosis, bacteremia, brain abscesses, clostridial cellulitis, gangrenous cholecystitis, intra-abdominal sepsis, intravascular hemolysis, pneumonia, postabortion infection, and thoracic and subdural empyema (McClane et al. 2012). Spores of the organism and cells are frequently associated with dust contamination on many surfaces, including foods such as meat and shellfish, resulting from its ubiquity throughout the environment (Uzal et al. 2010).
C. perfringens is a soil habitant and occurs naturally in the intestinal tract of many homeothermic animals and humans. There are many *C. perfringens* isotypes, but type A contains the enterotoxin gene (*cpe* gene) responsible for causing food poisoning. Types B–E occasionally possess the *cpe* gene (Freedman et al. 2016; Rood et al. 2018).

Pathogenesis

The disease is due to enterotoxin production (Skjelkvåle and Uemura 1977; Sarker et al. 2000). Enterotoxin is produced in the small intestine after the intake of about 10 million *C. perfringens* cells. About 6–24 h (mostly 8–12 h) after consuming contaminated food, the symptoms begin with acute abdominal pain, nausea, and diarrhea. Heat-treating contaminated food kills intestinal flora, while spores of *C. perfringens*, a common species of the intestinal flora, persist (Andersson et al. 1995). Botulism is primarily self-limiting and lasts for about one day, but death primarily due to dehydration occurs in very young and elderly patients (Uzal et al. 2015).

Symptoms

Symptoms typically include abdominal spasms, diarrhea, emesis, and pyrexia (Leung et al. 2017). The less severe form of the disease usually lasts for 12 to 24 h. The symptoms may last for 7 to 14 days in infants and aged people. The gastroenteritis form is characterized by watery diarrhea and mild abdominal pains, and the Pigbel form (enteritis necroticans) causes abdominal spasm, distension, occasional bloody diarrhea, emesis, and patchy necrosis in the small intestine (Freedman et al. 2016).

Bacillus cereus

Bacillus *cereus* is a rod-shaped, Gram-positive, motile, spore-forming, facultatively anaerobic, and beta-hemolytic bacterium. It is commonly found in food, soil, and fresh and marine water (Bacon and Sofos 2003). *B. cereus* spores have appendages, pili, or both and are hydrophobic. That is why they can attach to different surfaces and are difficult to remove by cleaning or sanitation (Bintsis 2017). *B. cereus* can produce two different types of toxins, enterotoxigenic or emetic.

Transmission

Transmission of *B. cereus* most commonly occurs through ingestion of contaminated food, soil, and water, either with the bacterium or bacterial toxin, fried rice, rice dishes, vegetable, milk, meat, and poultry products (Bintsis 2017).

Pathogenesis

The emetic form of the toxin is heat stable and secrets a highly toxic cereulide peptide (a cyclic dodecadepsipeptide) during the stationary phase. It is highly resistant to acidic conditions and heat (at 12°C) and responsible for producing the emetic strain. Diarrheal strains of *B. cereus* are heat liable and synthesize three enterotoxins: hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K (CytK). HBL, a tripartite dermonecrotic permeability factor, may induce detachment of the retina and blindness (Bintsis 2017).

Symptoms

By excreting two different bacterial toxins, *Bacillus cereus* causes two types of food poisoning. The first type is the

emetic form, characterized by acute abdominal pain, profuse diarrhea, nausea, emesis, and pyrexia, and this form has an incubation period of about 0.5–6 hours (Martinelli et al. 2013). The second type is the enterotoxigenic diarrheal form, characterized by acute nausea, vomiting, and diarrhea, but incubation period of this type is about 1 to 5 hours.

Listeria monocytogenes

Listeria are Gram-positive, non-spore-forming, rod-shaped, motile, facultatively anaerobic bacteria (Şanlıbaba et al. 2018). *Listeria* has ten different species, but human and animal Listeriosis is caused by *L. monocytogenes* (Abebe et al. 2020). This type of bacteria can survive at different pH ranges (4.4–9.4), high salt, and temperatures from 0°C to 45°C (Rodrigues et al. 2017). *Listeria* is a major cause of foodborne illness that usually is present in nature and is one of the most virulent pathogens (Olaimat et al. 2018).

Transmission

The most common transmission route is the intake of raw food, vegetables contaminated from soil or water containing the bacteria, and animal-based products contaminated with *L. monocytogenes* (Şanlıbaba et al. 2018). However, the risk of infection in young, old, and immunocompromised patients is higher than the other people (Dhama et al. 2013).

Pathogenesis

The most significant virulence factor in Listeriosis is evading the immune system and immune cells, causing infection (Dhama et al. 2015). *L. monocytogenes* has D-galactose residues on its surface that can adhere to receptors on the host cell, generally M cells and Peyer's patches of the intestinal mucosa. When these bacteria bind to the host cells, they can pass through the intestinal membrane, move into the bloodstream and produce septicemia. Then these bacteria enter the monocytes, macrophages, or polymorphonuclear leukocytes (Sisay and Addis 2015). The most critical point associated with this bacterium is that intracellular organism can cross through barriers such as the intestines, blood-brain barrier, and placenta and produce infection from each of these barriers (Ranjbar and Halaji 2018).

Symptoms

Symptoms usually last for 7 to 10 days (Sisay and Addis 2015), and include influenza-like symptoms such as pyrexia, tiredness, and gastrointestinal problems like nausea, emesis and diarrhea. In severe cases, it causes a life-threatening infection like septicemia, meningitis, meningoencephalitis, abortion, stillbirth or fetal infection (Jami et al. 2014; Reda et al. 2016).

Gram-negative foodborne pathogens

Salmonella Species

Salmonella are Gram-negative, anaerobic, and non-spore-forming bacteria, belonging to the family Enterobacteriaceae (Chlebicz and Śliżewska 2018). The genus is composed of more than 2500 serotypes (Musa et al. 2017). More than 150 serotypes can produce foodborne Salmonellosis (Dhama et al. 2013) and are cosmopolitan in nature. *Salmonella* are described as an important cause of human and animal infection. These bacteria most likely grow in the intestinal tract of humans and animals. The latter can act as a reservoir of foodborne Salmonellosis (Sisay and Addis 2015).

Transmission

Contaminated food and water are the primary causes of Salmonella infection (Dhama et al. 2013). Also, animal-based products such as meat, milk, pork, and poultry can cause foodborne Salmonella infection (Musa et al. 2017). Carcass contamination after slaughtering (Girma 2015) and eggshell or egg content contamination with this bacteria may occur either from the hen's reproductive system and from fecal or environmental contamination.

Pathogenesis

Several factors influence the host susceptibility to infection, such as infectious dose, route of infection, immune state of the host, and virulence of the bacteria either through plasmid, toxin, fimbriae, or flagella that help the bacteria to initiate the infection (Kemal et al. 2015). The pathogenicity of *Salmonella* infection starts from the bacterial invasion of the host cell. This complex invasion process is facilitated by producing several chromosomal genes that help invade the host cell, depending on the presence of virulence plasmid.
The target cell of *Salmonella* infection is the micro fold (M) cell, but the infection mechanisms include bacterial endocytosis, neutrophil recruitment and migration, epithelial cell cytokine synthesis, and fluid and electrolyte loss, finally producing the systemic infection (Sisay and Addis 2015). After this, *Salmonella* can reach the intestine and interact with non-phagocytic cells, such as normal intestinal mucosal cells. This binding is accomplished by adhesive structures of the fimbriae that accelerate this binding, promote invasion of the epithelial cell and beginning of gastroenteritis (Kemal et al. 2015) and produce local damage without septicemia. However, infection occurs in the micro-fold cells in the Peyer's patches by fimbriae adhesion, resulting in the sloughing and damage of the target cell membrane and bacterial internalization through membrane-bound vacuole and producing vesicles inside the cell (Sisay and Addis 2015).

Symptoms

The incubation period of *Salmonella* infection is about 12 to 72 hours (Dhama et al. 2013). However, signs vary from

gastroenteritis to septicemia, and the severity of the disease is dependent on the immune state and susceptibility of the host and the virulence factor of the bacteria (Kemal et al. 2015). Other signs include nausea, vomiting, abdominal cramp, watery, greenish fetid, or bloody diarrhea with mucus, headache, fatigue, sickle cell anemia, reactive arthritis, and osteomyelitis (Desta and Addis 2015). However, Salmonellosis can cause a severe problem in young, old, and immunocompromised patients (Dhama et al. 2013).

Campylobacter spp.

Campylobacter is a Gram-negative, microaerophilic, and catalase-oxidase positive organism. The genus *Campylobacter* comprises more than 25 species and eight subspecies (Hagos et al. 2019) but *C. jejuni* and *C. coli* are the most important foodborne pathogens that can produce foodborne illness (Dhama et al. 2013).
This bacterium is the most important causative bacterial agent of foodborne diarrheal disease in humans globally (Wieczorek et al. 2018), which results from contamination of food of animal origin (Dadi and Asrat 2008). *Campylobacter* can grow and colonize in most homeothermic animals, especially in poultry (Mughal 2018). It is responsible for 15% of foodborne disease-associated hospitalizations and 6% of foodborne disease-associated deaths (Desta and Addis 2015).

Transmission

Campylobacter infection is principally transmitted via contact with infected animals, or fomites, water, or during carcass handling in the slaughterhouse (Chanyalew et al. 2013), while the main route of *Campylobacter* transmission is from contaminated food handling, preparation, and consumption, especially from poultry (Abebe et al. 2020), through indirect fecal contamination (Khoshbakht et al. 2016), consumption of contaminated meat or raw unpasteurized dairy products, for example, milk and cheese (Dhama et al. 2013).
Cross-contamination occurs during ready-made food preparation by food handlers (Dadi and Asrat 2008), but about 30% of all infection cases have been reported due to poultry product consumption (Chlebicz and Śliżewska 2018).

Pathogenesis

The pathogenesis of *Campylobacter* infection is not entirely understood because several mechanisms of pathogenesis are speculated. *Campylobacter jejuni* virulence is associated with bacterial motility, mucus colonization, invasion of epithelial cells, toxin production, adhesion, internalization, and translocation (Desta and Addis 2015; Asuming-Bediako et al. 2019). Flagellae, encoded by a flagellin gene (fla A), enable the bacterium to reach attachment sites in the intestine. Several virulence factors in *Campylobacter* have been identified, which improve its motility, intestinal adhesion, colonization, toxin production and invasion. After this step, bacteria can penetrate the intestinal mucosa and begin toxin production, especially cytotoxins, and cause disease.
When the bacteria start multiplication in the mucosal surface of intestinal epithelium and cells of the lamina propria, it results in self-limiting diarrhea and abdominal pain. However, the heat-labile toxin is the common cause of diarrhea produced by this bacterium (Hadush and Pal 2013).

Symptoms

The incubation period **of** *Campylobacter* infection is 3–5 days (Dhama et al. 2013). *Campylobacter* causes traveler's diarrhea (Hagos et al. 2019), characterized by watery and bloody diarrhea, abdominal cramps, pyrexia, malaise, and emesis. These signs are more severe in young-aged children than in adults due to excessive loss of proteins, nutrients, and electrolytes (Asuming-Bediako et al. 2019). The bacteria may also produce megacolon, dehydration, and septicemia in young and immunocompromised patients (Omara et al. 2015).

Pathogenic *E. coli* groups

E. coli is a Gram-negative, non-spore-forming, facultative anaerobic bacillus of the family Enterobacteriaceae. It is one of the normal flora in the lower intestine of warm-blooded animals and humans (Eggesbø et al. 2011). However, it can also cause intestinal disturbances and illness in humans that range from self-limited intestinal trouble to renal failure and septic shock. *E. coli* is also a causative agent of many diarrheal diseases in both humans and animals, such as traveler's diarrhea. It can also produce bacteremia, pneumonia, cystitis, and peritonitis. *E. coli* is divided into 150–200 serotypes based on the somatic (O), flagella (H), fimbriae (F), and capsular (K) antigens. That is why antimicrobial resistance is the most common problem associated with *E. coli* treatment.
E. coli related to diarrheal diseases are divided into six groups: enteropathogenic *E. coli*, enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), and diffusely adherent *E. coli* (DAEC). However, the most common cause of human intestinal infection is EHEC, which produce Shiga-like toxin (Devleesschauwer et al. 2019). *E. coli* O157:H7 is one of the familiar serotypes that contain pathotypes, causing foodborne infection in humans (Ashebr and Alemu 2019).

Transmission

The most frequent transmission mode of *E. coli* O157:H7 is through consuming contaminated food and water or person to person. Transmission can also occur by consuming contaminated raw, undercooked meat of bovine origin (Ashebr and Alemu 2019) and contaminated raw milk and unpasteurized dairy products (Dhama et al. 2013).

Pathogenesis

E. coli virulence factors are Shiga-like toxin and adherence factor; both are considered an *E. coli* mechanism of pathogenesis (Saeedi et al. 2017). A specialized gene, known as the intimate gene, is responsible for the intimate attachment of bacteria to the intestinal cell that will cause attachment and erosion of the microvilli of the brush border enterocytes (Abreham et al. 2019). *E. coli* destroys microvilli and alters the cytoskeleton structure of the enterocyte associated with the accumulation of polymerized actin and cytoskeleton proteins beneath the site of bacterial attachment (Karmali et al. 2010). This adhesion is either through fimbrial or non-fimbrial pathways, which causes bacterial binding and colonization after *E. coli* starts proliferation and pathogenesis.
E. coli O157:H7 can bind to the intestinal mucosal tissue and secrete several enzymes, proteins, and toxins (Saeedi et al. 2017). Production of Shiga toxin, either Shiga-like toxin 1 (stx1) or Shiga-like toxin 2 (stx2), will start bloody diarrhea, sloughing of intestinal epithelial cells and hemolytic uremic syndrome (Kiranmayi and Krishnaiah 2010).

Symptoms

After an incubation period of 2–10 days, clinical signs appear, including diarrhea, abdominal pain and crump, vomiting, hemorrhagic colitis, hemorrhagic uremic syndrome, and bloody diarrhea (Desta and Addis 2015).

Shigella spp.

Shigella are Gram-negative, facultatively anaerobic, non-motile, and non-spore-forming bacteria that can grow at temperatures between 6°C and 48°C and pH of 4.8 to 9.3 (Mikhail et al. 2021). *Shigella* is a genus of the family Enterobacteriaceae and is composed of four serogroups, of which A, B, and C are composed of 38 serotypes, while serogroup D has only one serotype (Bacon and Sofos 2003). In developing countries, *Shigella dysenteriae* type 1 is the most common serotype that causes infection (Alamdary and Bakhshi 2020).

Transmission

Shigellosis most frequently occurs after consuming contaminated food and water, but Shigella is commonly found in poor hygienic and sanitation environment. The primary transmission route is through direct contact (Al-Dahmoshi et al. 2020). *Shigella* could be present in different foods such as milk, meat, chicken, salad and shellfish, but the most common outbreaks occur in eateries, homes, schools, sorority houses, and commercial airlines (de W Blackburn and McClure 2009).

Pathogenesis

After ingestion, *Shigella* moves to the small intestine and goes down through the large intestine, where it invades the host cells, causes injury to the colonic mucosa, and produces enterotoxins. After that, it starts mucosal invasion by using transcytosis and uses M cells to move through the basolateral epithelium, primarily accountable for stimulation of intestinal lymphoid tissue by antigen recognition. Transcytosis produces macrophage and cellular apoptosis. It releases inflammatory cytokines, Interleukin (IL)-1 and IL-8, contributing to intestinal inflammation and continues invasion of epithelium and immune system by using intercellular actin polymerization. Through this process, absorption of nutrients is impaired, resulting in diarrhea.
Another mechanism for cell trauma is via enterotoxins 1 and 2, which play an essential role by impairing fluid and nutrient absorption, causing diarrhea. *Shigella dysenteriae* serotype 1 also causes cytotoxicity and vascular lesions in the colon and other organs, such as the kidneys, causing problems like hemolytic uremic syndrome (HUS) (Aslam and Okafor 2021).

Symptoms

The incubation period of all serogroups of *Shigella* is about 12–50 h, followed by initiation of gastrointestinal infection. Signs may include fever, watery diarrhea, headache, malaise, fatigue, and abdominal cramp, and all four serogroups could produce symptoms, but *Shigella dysenteriae* type 1 is the most common cause of illness (Mattock and Blocker 2017).

Yersinia spp.

Yersinia are Gram-negative, rods or cocci, anaerobic and non-spore-forming bacteria, with 17 species. However, only *Yersinia enterocolitica* is a zoonotic and causative agent of food poisoning called Yersiniosis (Janowska et al. 2012; Dekker and Frank 2015). It can grow between 0°C to 40°C and pH lower than 9.0 but destroyed in an acidic medium, and the aW must not be lower than 0.96 (Bari et al. 2011).

Transmission

The primary transmission source is contaminated raw and undercooked pork, raw or unpasteurized dairy products, plants, seafood, drinking water, and contact with contaminated equipment or surface (Bursová et al. 2017; Chlebicz and Śliżewska 2018). *Yersinia* also invades the upper body part, such as the head, and meat could be contaminated and exposed by the bacteria after slaughter. So, head of the carcass is the best source of transmission after slaughtering (Van Damme et al. 2017).

Pathogenesis

The virulence factors of *Yersinia enterocolitica* include pieces of the genetic code or information contained in chromosome and the pYV plasmid of *Yersinia* pYV (virulence plasmid), approximately 70 kbp long (Bari et

al. 2011). After consuming contaminated food and water, the bacterium reaches the distal part of the small intestine and proximal part of the colon and starts proliferation, differentiation, and colonization in the environment, causing infection (Bancerz-Kisiel and Szweda 2015; Chlebicz and Śliżewska 2018).

Symptoms

Clinical signs of *Yersinia* infection include severe enteritis, gastritis, fever, stomachache, often bloody diarrhea, and appendicitis, erythema nodosum, bacteremia, osteoarthritis, purulent hepatitis, splenitis, nephritis, myocarditis, and rarely endocarditis are seen in adults (Bari et al. 2011; Rahman et al. 2011; Schaake et al. 2014). In general, the risk of this disease is low, but young children, elderly, and immunocompromised patients are more at risk.

Vibrio species

Species in this genus are Gram-negative, straight or curved rods, non-spore-forming, primarily motile, and facultatively anaerobic. The genus *Vibrio* belongs to the family Vibrionaceae. The pathogenic *Vibrio* species are *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. They are human pathogens, widely distributed in marine environments and are naturally found in the water (Hofmeister et al. 2019).

Transmission

Vibrio cholerae generally enters the human body by contaminated food consumption, such as raw or undercooked mollusks or crustaceans, or an open wound exposure to contaminated water. Food sources involved in the transmission of *V. parahaemolyticus* comprise crabs, prawns, scallops, and other seafood.

Symptoms

Vibrio parahaemolyticus infection commonly occurs within one day of the exposure and continues for three days, while *V. vulnificus* symptoms usually occur 12 hours to 21 days after exposure. These pathogens can cause fever, diarrhea, nausea, abdominal pain, and vomiting in healthy individuals. While these symptoms usually last for 2–3 days, severe cases may cause dysentery, primary septicemia, or cholera-like symptoms with the likelihood of death (Hofmeister et al. 2019).

Mycotoxigenic molds

Filamentous fungi belong to the Ascomycota phylum, which can grow on foods (crops). They produce mycotoxins, which cause chronic disease in animals and humans. Mycotoxins are toxic secondary metabolites of different filamentous fungi (Wu et al. 2014). Mycotoxins of some filamentous fungi cause various adverse effects in humans, such as intestinal symptoms, allergic reactions, immunosuppression, mutagenesis, and protein synthesis inhibition (Klich et al. 2003). Mycotoxins of the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* are the principal sources of food and feed contaminations (Sweeney 1998; Marin et al. 2013).

Aspergillus species like *A. flavus* and *A. parasiticus* are the primary fungi that produce aflatoxin in wheat, rice, peanuts, corn, and other grains. The most important mycotoxins known to contaminate food products are aflatoxins B1, B2, G1, and G2. Toxic metabolites, such as aflatoxins M1 and M2, may occur in the flesh and milk of animals given grains contaminated with type B aflatoxin. The main effect of aflatoxins is acute hepatotoxicosis, hepatocarcinoma, and stunted growth in children (Marin et al. 2013; Kaushik 2015).

Other species of *Aspergillus,* like *A. carbonarius*, *A. niger*, *A. ochraceus*, and *Penicillium verrucosum,* are the primary fungi associated with ochratoxin production in low levels in different products, such as barley, raisins, coffee products, soybean, wines, and grapes. These mycotoxins are associated with renal diseases and may accumulate in human or animal tissues upon consuming contaminated foods (Wu et al. 2014).

Penicillium citrinum and some *Aspergillus* species, such as *A. terreus* and *A. niveus*, and *Monascus ruber*, *M. purpureus*, *Penicillium camemberti*, and *A. oryzae,* can produce citrinin, which is a mycotoxin found mainly in wheat, rye, oat, barley, rice, and corn. Citrinin is associated with nephrotoxic effects in humans and animals (Húngaro et al. 2014). Some species of *Penicillium*, *Aspergillus*, and *Byssochlamys* produce patulin, a mycotoxin found in fruit such as cherry, apple, and pear. The main effect of this toxin is stomach irritation, causing vomiting and nausea (Marin et al. 2013; Húngaro et al. 2014).

Fusarium spp. produce many mycotoxins, such as zearalenone, fumonisins, and trichothecenes (deoxynivalenol (DON) and T2 toxin), mainly found in corn. Fumonisins are related to esophageal cancer and neural tube defects. Trichothecenes are immunotoxic and cause gastroenteritis (Alassane-Kpembi et al. 2016).

Foodborne Viruses

Foodborne and waterborne viral contaminations are progressively known as causes of sickness in humans. This increment is incompletely explained by changes in food preparation and consumption practices that lead to the global availability of risky food. As a result, large outbreaks may occur due to food contamination by a single food handler or source. The most common types of foodborne transmission viruses are hepatitis A virus (HAV) and Norwalk-like caliciviruses (NLV), proposing that these infections are related to most viral foodborne diseases. People might become infected without displaying symptoms, and these viruses can be transmitted indirectly via water, food, or fomites contaminated with virus-containing vomit or feces. The high frequency of NLV and, to a lesser extent, hepatitis A results in enhancing a foodborne outbreak (Koopmans et al. 2002).

Noroviruses (Norwalk)

Noroviruses are non-enveloped, single-stranded, RNA viruses of the family Caliciviridae. They produce disease in humans and other animals, primarily cattle, swine, and mice (Hennechart-Collette et al. 2015). Noroviruses (NoV) are organisms that can be carried by direct person-to-person contact or indirectly via contaminated water, food, or environments (Duret et al. 2017). Infections with NLV are considered one of the most critical causes of gastroenteritis in adults.

The incubation period of Noroviruses is one to three days. Infected persons suffer from fever, diarrhea, headache, and vomiting. In general, the illness is mild, with symptoms that continue for 2–3 days. Projectile vomiting frequently occurs in adults, and deaths from Norovirus outbreaks have been reported, but the etiologic connection needs to be affirmed. The infection rate is typically ≥45%. The virus spreads via stools and vomit, starting during the incubation period and lasting ≥ten days (Ozawa et al. 2007).

Foodborne Hepatitis

Hepatitis-causing viruses are divided into enterically transmitted (HAV, HEV) and parenterally transmitted hepatitis viruses (hepatitis B, C, D, G). Enterically transmitted viruses are the leading causes of food- or waterborne illnesses. HAV is a virus belonging to the family Picornaviridae (Koopmans et al. 2002; Lemon et al. 2018). Particles can spread via contaminated food, water, environmental surfaces, and direct or indirect contact among people (Moreno et al. 2015; Bintsis 2017).

The incubation period of Hepatitis A virus infection is 30 days, and it can cause asymptomatic or symptomatic infection. The disease has non-specific symptoms, such as headache, fever, nausea, fatigue, and abdominal disturbances, and the symptoms and signs of hepatitis appear 1–2 weeks after infection (Pintó et al. 2012). The probability of having symptoms with HAV infection is dependent on the infected individual's age. Most infections are asymptomatic among children younger than six years, and children with symptoms rarely develop jaundice. Infection is ordinarily symptomatic among older children and adults, and jaundice occurs in most patients (Koopmans et al. 2002; Pintó et al. 2012).

Foodborne Parasites

Protozoa are unicellular microorganisms, larger than bacteria, that lack a rigid cell wall but with an organized nucleus. Like viruses, protozoa do not multiply in foods, only in living tissues, and they are important causative agents of human diseases. The virulent form of protozoa is a cyst (Robertson et al. 2014), which may be transmitted from humans to animals, from humans to humans, or from animals to humans. A few parasites cause foodborne and waterborne illnesses. Protozoa live and reproduce within the infected animal and human hosts and are often excreted in feces. The most common foodborne parasites are *Toxoplasma gondii, Cyclospora cayetanensis*, and *Trichinella spiralis*. Foodborne parasites pose a burden in low- and middle-income countries, where parasitic infection cycles from food sources have been emphasized (Yoshida et al. 2011).

Toxoplasma gondii

Toxoplasma gondii is a member of the family Sarcocystidae, and phylum Apicomplexa. It is an obligate intracellular pathogen that is considered one of the most common parasitic (protozoal) infections of humans and other homeothermic animals (Hill and Dubey 2002). The infectious stages of *T. gondii* are oocysts, bradyzoites, and tachyzoites, and transmission may be horizontal or vertical. Horizontal transmission of *T. gondii* involves consuming undercooked meat containing bradyzoites or ingestion of vegetables or fruits, contaminated water with oocysts, tachyzoite transmission by blood transfusion, or milk and cheese consumption (Dubey et al. 2014). Vertical transmission of *T. gondii* tachyzoite may be via the placenta or semen (Lopes et al. 2013).

The symptoms of Toxoplasmosis include pyrexia, rash, cephalalgia, myalgia, and lymph node swelling that may persist for more than a month (Hill and Dubey 2002). Ocular Toxoplasmosis causes blurred or reduced vision, redness in the eyes, pain, and sensitivity to light. In immunocompetent individuals, Toxoplasmosis is generally subclinical or asymptomatic (Wang et al. 2017). *T. gondii* contagions cause abortion or fetal developmental disorders in pregnant women and small ruminants (Dubey 2016). Immunocompromised people are at a high risk of severe health problems (Weiss and Dubey 2009; Wang et al. 2017).

Cyclosporra cayetanensis

Cyclospora is a genus of protozoa under the family Eimeriidae. The only species of the genus is *Cyclospora cayetanensis,* which infects humans through the fecal-oral route. *C. cayetanensis* is widespread and was a critical causative agent of foodborne enteric disease outbreaks in many countries. It can cause prolonged illness, lasting for six weeks or longer in immunocompetent and immunocompromised people, with symptoms including vomiting, diarrhea, nausea, anorexia, bloating, abdominal discomfort, malaise, pyrexia, and tiredness (Ortega and Sanchez 2010).

Trichinella Species

Trichinella nematode spp. cause a parasitic disease, known as Trichinellosis, a worldwide foodborne zoonosis caused by eating raw or undercooked meat containing the infective larvae (Bai et al. 2017). The primary sources of infection are the domestic pig (pork) and its products (Hassan et al. 2019), but meat of equines and wild boars played a substantial role in outbreaks in Europe. *Trichinella* can infect more than 150 species of animals, including humans (Dupouy-Camet 2000).

Conclusion

Foodborne diseases are of universal concern, and a joint program by all nations and international agencies is a requirement to recognize and control all foodborne diseases that jeopardize human health and international transactions. Most foodborne diseases are avoidable, notwithstanding being complicated in their biology, analysis, and epidemiology. Public health and regulatory agencies, the food industry, and consumers must make continuous efforts to preclude food contamination on the farm, during processing, and in eateries and households. With appropriate education programs on food safety for all involved people, foodborne diseases could be controlled or their occurrence reduced.

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EMERGENCE OF ANTIMICROBIAL RESISTANCE AND INTERACTION BETWEEN HUMANS, ANIMALS AND ENVIRONMENT

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INTRODUCTION

Antimicrobial resistance (AMR) is defined as the newly found resistance of a group of micro-organisms to an antimicrobial agent against which they were originally sensitive. Unnecessary usage of antimicrobials in the treatment of infectious diseases and as growth promoters in animals may result in the development of resistant microbial strains (Holmes et al. 2016). Micro-organisms acquire resistance to antimicrobials via genes' mutations and through acquisition of new antimicrobial resistant genes from other bacteria. Transmission of this resistance can also occur from animals to humans and from humans to humans (Jindal et al. 2015). Resistant bacteria may be transmitted from food animals, either through the food chain, the environment or through direct contact with these animals. Such transmission may lead to the emergence of potentially challenging infections (Lekshmi et al. 2017).

AMR is a natural evolutionary process normally observed in bacteria, however, antimicrobial abuse, including extensive use, misuse or overuse can lead to an acceleration of this natural phenomenon, and an exacerbation of unwanted effects (Hwang and Gums 2016). Poor management practices in the treatment of infectious diseases also lead to the emergence of AMR in both animals and humans (Ayukekbong et al. 2017). To tackle the issue of AMR in micro-organisms and to combat its threat to public health, a complete understanding of the mechanisms underlying the emergence of AMR is essential. Such an understanding will aid in the development of better diagnostic and therapeutic approaches to bacterial infections (Lammie and Hughes 2016).

This chapter presents a review of number of factors attributing to the emergence and dissemination of AMR among micro-organisms, as well as how natural interactions between these micro-organisms, animals, humans and the environment lead to the spread of such resistance. To limit the emergence of AMR, drivers of AMR in the animals, humans, and environment should be recognized and controlled. For this purpose, it is essential to develop appropriate interventional policies through action plans, both at national and international levels, aided by the principles of the *one health* approach and the involvement of multiple disciplines (Smith et al. 2019).

Mechanism of Acquisition of AMR

Pathogens acquire antimicrobial resistance in two ways: (a) vertical acquisition of AMR through mutations in pre-existing or previously acquired genes, and (b) horizontal acquisition of AMR through acquiring new resistant genes from other bacteria, also called the horizontal gene transfer (HGT) (Lawrence 2005). Depending on the antimicrobials, resistance can develop through either of the two mechanisms.

Vertical Acquisition of AMR

Mutations are one of several mechanisms by which bacteria become resistant to antibiotics. Division of a bacterium results in the creation of two identical copies of DNA. With each bacterial division there is a possibility of the emergence of mutation(s) in the DNA strands. These mutations can give rise to antibiotic resistance genes (Bos et al. 2015). An antibiotic resistant bacterium is then able to survive and multiply, even in the presence of the specific antibiotic to which it had become resistant (Darwinian natural selection), giving rise to a population of mainly resistant bacteria (Lenski 2017) (Fig. 1).

Natural selection of resistant bacteria can occur when an antibiotic is given to a sick animal or human as a part of the treatment of the infection. The antibiotic is unable to kill both the resistant pathogenic bacteria, as well as any commensal bacteria with resistance genes (Boerlin and Reid-Smith 2008). The use of narrow spectrum antibiotics can decrease the possibility of the selection antibiotic resistance commensal bacteria (McAdams et al. 2019).

Acquisition of AMR through Horizontal gene transfer (HGT)

Horizontal gene transfer (HGT) is the most common method by which bacteria acquire AMR. This may possibly occur through the acquisition of resistance genes and/or the exchange of genetic material of one bacterium with that of other bacteria. Plasmid-mediated conjugation is recognized as the most common mechanism by which HGT occurs. Genome sequence analysis of bacteria can confirm HGT (Husnik and McCutcheon 2018).

So, how does HGT work and what does it mean for bacterial populations? HGT is a strong and helpful tool by which bacteria are able to evade the toxic effects of antimicrobials (Gupta et al. 2019). HGT occurs through three mechanisms: conjugation, transformation and transduction (Fig. 2).

Conjugation is a mechanism by which a bacterial cell comes in contact with another bacterial cell and donates its plasmid DNA through a cell-membrane structure called sex pili. Transformation is a mechanism by which a bacterium uptakes DNA directly from its immediate environment. Transduction is a mechanism by which bacteriophages (viruses) inject their viral DNA into a bacterium.

Thus, specific AMR genes can be exchanged among bacteria in close proximity to each other through any of the aforementioned mechanisms. Those bacteria which acquire resistance genes can then transmit resistance through vertical inheritance to the upcoming generations. Unlike vertical gene transfer, mutation is not an essential part of HGT. The lack of a mutation component in HGT is advantageous for bacteria because mutations can sometimes be harmful. Transfer of plasmid through the process of conjugation is the major contributor to HGT among bacteria (Boerlin and Reid-Smith 2008). HGT occurs between the same species of bacteria, strains of the same species and between bacteria belonging to different species as well. It has also been reported that HGT can also take place among different bacterial families and orders. Factors that contribute to the success of HGT include the degree of taxonomical similarity between involved bacteria and the level of compatibility between the (donor and recipient) organisms (Rodriguez-Beltran et al. 2021).

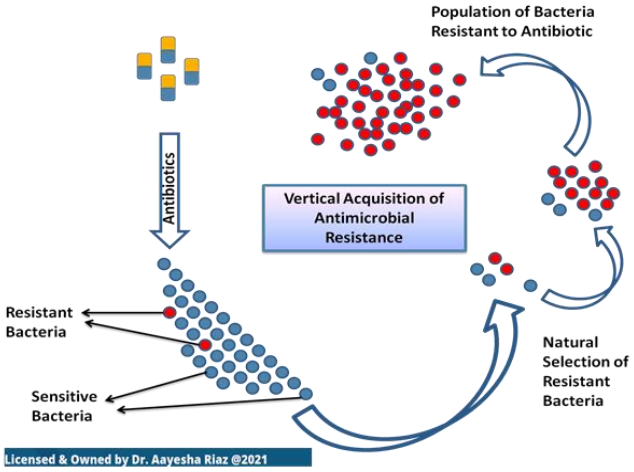

Figure 1: The process of emergence of AMR through natural selection (vertical acquisition) of resistant bacteria. Antibiotic kills most of the antibiotic sensitive bacteria, however, antibiotic resistant bacterium survives. Due to the presence of antibiotic, the sensitive bacteria no longer remain alive in the medium, and their number reduces gradually, whereas resistant bacteria increase in number over time.

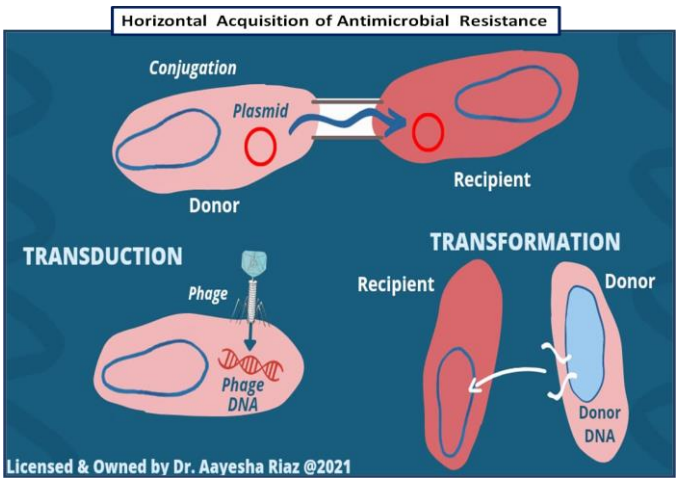

Figure 2: Horizontal acquisition of AMR by acquiring genes from outside through conjugation (from other bacteria), transduction (from a bacteriophage DNA), or transformation (directly from the bacterial surroundings).

Emergence and Transmission of AMR

Emergence and Transmission of AMR within a Micro-organism

Through the mechanisms described above, AMR eventually emerges among bacterial species. These robust mechanisms help them to evade the toxic bactericidal or bacteriostatic effects of antimicrobials. Resistance to antimicrobials was identified soon after the discovery of antimicrobials themselves (Fig. 3). Today, the menace of AMR complicates the treatment of almost all major infectious diseases afflict both animals and humans, including zoonotic diseases (Holmes et al. 2016).

Bacteria elude the effect of antimicrobials by preventing the drug from entering the bacterial cell (Reygaert 2018). They also produce enzymes (the products of AMR responsible genes), which either destroy the antimicrobial or modify the antimicrobial binding or target sites. Studies that aimed at characterizing population structures, AMR and virulence genes of bacterial spp. that afflict both animals and humans, have indicated a wide diversity of genetic determinants of resistance (Hirt et al. 2018; Panzetta et al. 2018). One study reported the emergence of AMR in a group of companion animals with urinary tract infection (UTI). They were shown to be having been infected with high-risk *Klebsiella pneumoniae* clonal lineages with resistance and virulence genes (Marques et al. 2019). In another study, variants of the tet(X) gene were identified. Bacteria with tet(X) gene can modify the structure of tigecycline, which is considered a last-resort antibiotic in the treatment of severe infections caused by extensively resistant bacteria. Two unique variants of this gene, tet(X3) and tet(X4), were also reportedly found in numerous isolates of Enterobacteriaceae and Acinetobacter collected from animals (including food animals), as well as from humans. Tet(X3) and tet(X4) were found to give bacteria the ability to inactivate all tetracyclines, including tigecycline and the newly FDA-approved eravacycline and omadacycline (He et al. 2019).

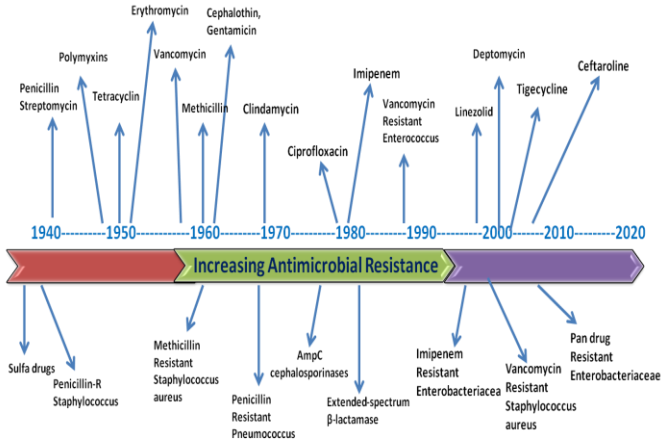

Figure 3: Timeline of antimicrobial discovery and antimicrobial resistance. Each antibiotic class is indicated above the timeline and the time when resistance was first observed for each class is shown below the timeline.

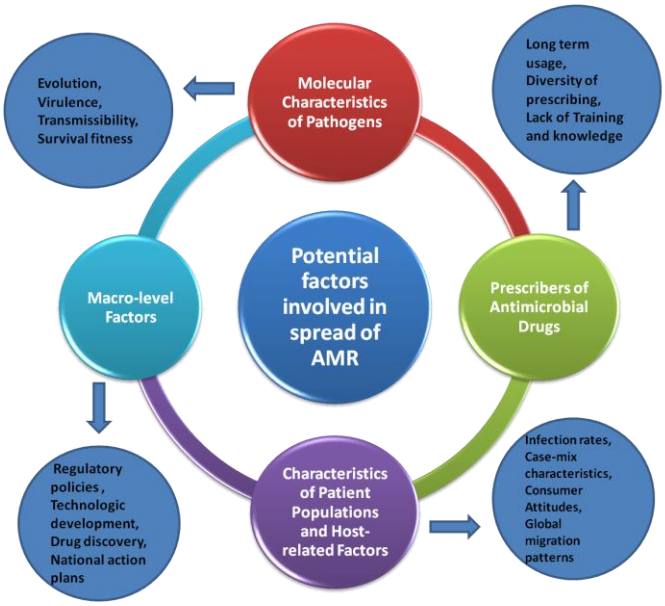

Figure 4: Potential factors involved in dissemination of antimicrobial resistance. The potential factors are indicated in inner circles and their examples are indicated in outer circles.

Other examples include the development of resistance against β-lactam antibiotics by β-lactamases producing micro-organisms that inactivate β-lactam (Sparbier et al. 2012; Worthington and Melander 2013). As widely known, most antimicrobials are produced by saprophytic organisms. The antimicrobial molecules production by saprophytic micro-organisms can affect the growth of other bacteria in the surrounding environment, even in sub-lethal concentrations. These antimicrobial molecules may influence expression of the bacterial and host genes (Andersson and Hughes 2014; Holmes et al. 2016). Emergence of resistance to synthetic antimicrobials also occurs. One example in the emergence of such resistance is resistance to fluoroquinolones. Such resistance emerges through several mechanisms. The structure of target sites may be altered, or "protection" may be "given" to the sites with DNA binding proteins. Bacterial cells may become capable of exporting the drug out at increased rates, leading to a reduction in the amount of damage caused by these fluoroquinolones. Cells may also acquire the capability to produce enzymes that inactivate fluoroquinolones (Redgrave et al. 2014; Osei Sekyere and Amoako 2017). Emergence of AMR is a continuous process in the life of micro-organisms and, thus continuous and diligent global attention is needed for both; the surveillance of AMR gene variants in both clinical and animal settings, as well as those resulting from the use of antimicrobials in food production.

Emergence and transmission of AMR within Human beings

The misuse or overuse of antimicrobials in clinical practice is a major determinant of the emergence and development of AMR in human populations. Reservoirs of AMR genes lie among the commensal microflora present in the gastrointestinal tract (GIT) and other body systems of humans and animals (Taft et al. 2018). *In vitro* and *in vivo* transfer of AMR genes between commensal microflora and pathogenic micro-organisms in the GIT has already been reported in different studies (Walsh and Fanning 2008; Scott et al. 2009). Due to a vast reservoir of commensals in humans, transfer of resistance genes from commensals to pathogens tends to be much more frequent in comparison with the *de novo* development of resistance to a pathogen, leaving the latter mechanism less impactful (Bag et al. 2019). Emergence of AMR as a result of this transfer of AMR genes to pathogens is another global health threat.

The environment, drinking water and food are also major reservoirs of microbes and determinants of AMR emergence. The irrational use of antimicrobials, or the prescription of the needless antimicrobials, is one of the main attributes of AMR in micro-organisms that afflict humans. It can cause damage to beneficial bacteria and can lead to the development of AMR in them, which may then share their resistance gene with other bacteria. In addition, an opportunity may be created for potentially harmful bacteria to replace the harmless ones (Hernando-Amado et al. 2019; Fouz et al. 2020). Some species of normal flora have "natural" resistant genes to some antimicrobials. Selective pressure allows micro-organisms with resistance genes to survive and proliferate. It has been reported that Enterobacteriaceae rapidly colonize neonatal guts soon after birth. Nearly 14% of these Enterobacteriaceae have resistance enzymes, including the extended-spectrum β-lactamase (ESβL), which inactivates β-lactam containing antibiotics. By 60 days of age, approximately 42% of babies' guts are colonized with such bacteria (Kothari et al. 2013).

Resistance genes can also be transmitted from human to human through hospitals (nosocomial infections), in the community through travel, or extended care facilities. NDM-1 (New Delhi metallo-beta-lactamase 1) is a notable example (Johnson and Woodford 2013). NDM-1 is an enzyme that renders bacteria resistant to a broad range of beta-lactam antibiotics (the carbapenem antibiotic family). Carbapenem antibiotics are considered effective

for the treatment of antibiotic resistant bacterial infections (Doi 2019). NDM-1 gene beta-lactamase enzymes are also called carbapenemases. NDM-1 was first detected in 2008 in a *Klebsiella pneumoniae* isolate from a Swedish patient in India (Yong et al. 2009; Kumarasamy et al. 2010). NDM-1 is most common in gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, and can transfer from one bacterium to another through HGT. A large-scale multi-national study in 2010 reported the emergence and spread of NDM-1 gene carrying bacteria in United Kingdom, Pakistan and India (Kumarasamy et al. 2010). In the community, the transmission of AMR can also occur through the oral-fecal route. Failure to conduct appropriate biological waste management and adequate WASH (Water, Sanitization and Hygiene) plays an important role in the transmission of bacteria, particularly resistant gram-negative organisms like pathogenic *E. Coli* (WHO 2018). Spread of infection due to ESβL-positive Entero-bacteriaceae and methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with travel, contamination associated with hospitals and healthcare personnel, as well as poor WASH conditions (Holmes et al. 2016). During the last ten years, the collective human microflora has acquired AMR in an unprecedented scale (Bassetti et al. 2017). It is essential to interrupt chain of transmission of resistance from human to human by interventions like mass drug administration and vaccinations (Wegener 2012).

Emergence and transmission of AMR among Animals and Human

Antimicrobials are widely used in domestic animals and livestock for the treatment of infectious diseases. In some countries, these are used in sub-therapeutic doses as growth promoters. In many others, use of antimicrobials as growth promoters is banned (Hosain et al. 2021). Humans can acquire antibiotic resistance genes from several sources of animal origin, including livestock, poultry, wildlife and pet animals. AMR can be transmitted from animal to animal or animal to human by direct infection with resistant organism from an animal source. Other sources of bacterial resistance include HGT of resistance genes from agriculture/livestock to human pathogens. In this regard, food-borne pathogens have a higher impact on health at the population level (Walsh and Fanning 2008).

Direct contact with animals can also be a cause of spread of antibiotic resistant micro-organisms. Persons who are involved in direct handling or management of animals (veterinarians, animal handlers, shepherds, milk men, cleaners, manure handlers, laborers and animal receivers) are typically at high risk (Rao 1998; Tang et al. 2017). Studies have revealed that manure may also contain resistant micro-organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Casey et al. 2013). Also called livestock-associated MRSA (LA-MRSA), these resistant bacteria are carried or harbored by livestock (cattle, buffaloes, sheep, goat and poultry) and companion animals (dogs, cats, pet animals) and are easily transmittable to humans (Graveland et al. 2011). Studies have shown that these new lineages are commonly found in the guts of workers and veterinarians dealing with these animals. These workers and veterinarians can then potentially transmit these resistant bacteria to their un-exposed family members, friends and other community/society members. This indicates that exposure to livestock greatly increases the risk of developing (MRSA) infection (van Cleef et al. 2011).

There is a positive but complex association between the consumption of antimicrobials and the emergence, development and transmission of resistance between humans, animals, and the environment. There are direct and indirect routes of AMR transmission, which are associated with the use of antimicrobials in animals and resistance in humans (Graham et al. 2019). Studies examining LA-MRSA, such as CC 398 and mecC MRSA, have reported a shared lineage of AMR genes among humans and farm animals, indicating that at some time point resistance genes were transmitted from humans to farm animals (Lepuschitz 2015). Similarly, sequencing analyses of the ST5 MRSA lineage revealed that they may have originated as a result of human to poultry transmission (Bernier-Lachance et al. 2020). There is also evidence of MRSA lineages that were transmitted from animals to humans (Takano et al. 2013).

Third-generation cephalosporin resistant *Escherichia coli* and *Salmonella typhimurium* DT104 strains have been shown to be spread from livestock to humans via food consumption and their genes shared through conjugation (Dechet et al. 2006; Lin et al. 2019). Animals transfer AMR bacteria to humans indirectly through food. Ingestion and occasional colonization of resistant micro-organisms in the gut can lead to the development of AMR in humans (Marston et al. 2016). Salmonella, *E. coli*, Campylobacter, and Listeria species are the most common food-borne bacterial pathogens.

Milk and other dairy products, as well as meat (beef, mutton, and poultry meat), can carry pathogens that may have developed resistance to antimicrobials. Shiga toxin-producing *E. coli* (STEC) is a food borne pathogen of zoonotic importance that causes outbreaks of diarrhea, hemorrhagic colitis, and hemolytic uraemic syndrome (HUS) in humans. Irshad et al. (2020) reported the presence of STEC in 43.5% of raw meat samples (of cattle and goats) collected from meat shops. They found that positive isolates had one or more of the STEC virulence genes; stx1, stx2, eae and/or ehxA. Antibiotic susceptibility profiles indicated AMR levels ranging from 33.3 to 100% against lincomycine, cephradine, neomycin, streptomycin and doxycycline (Irshad et al. 2020). These antibiotics are widely used for treatment, prevention, and as growth promoters in food producing animals in Pakistan (Rahman and Mohsin 2019). Other studies involving the surveillance of retail meats such as beef, chicken and turkey have detected the presence of Enterobacteriaceae. Handlers, buyers and sellers of these products are thus at serious risk of becoming infected with typical AMR resistant bacteria (Miranda et al. 2008; Gelbíčová et al. 2019; Díaz-Jiménez et al. 2020).

Emergence and transmission of AMR via Environment

The emergence of AMR depends on many environmental factors. Most importantly, these include population densities, the level of health care provided, immigrations, travel, tourism, sanitation, the occurrence of cultural and religious gatherings involving animals (such as Eid-Al-Ad'ha in Islam and worship of cattle in Hinduism) and the presence of animals in close proximity to humans (Berndtson 2020). Some examples of environmental transmission of AMR have been discussed in previous sections. In this section, mechanisms of environment-related spread and transmission of AMR have been discussed. Some antimicrobial drugs (either taken for the treatment of infections or consumed as food source) are not fully digested, absorbed and processed in the animal or human gut. Therefore, 40-90% of these ingested drugs are excreted to environment in urine and/or in faeces (Berndtson 2020; Wang et al. 2020). This means that human sewage and animal manure can both be sources of antibiotic resistant micro-organisms. Animal manure is also used as fertilizer, potentially spreading micro-organisms to crops and into runoff water (Kumar et al. 2013). Different studies have detected the presence of antibiotics in small amounts in crops grown in fields fertilized with animal manure and into runoff water (Hu et al. 2010; Kang et al. 2013). This clearly indicates the importance of waste processing to control environment–human transmission. Many potentially pathogenic resistance micro-organisms and antimicrobial metabolites have been isolated from sewage systems, even after treatment, which indicates the need of preventive measures to be taken (Talebi et al. 2007; Luczkiewicz et al. 2010a; Luczkiewicz et al. 2010b; Heck et al. 2015).

Potential factors involved in spread of AMR and methods of control

Potential factors involved in dissemination of AMR are numerous and diverse. It is very important to control these factors in order to control the AMR transmission and spread. These can be divided into 4 groups, as shown in Fig. 4.

Molecular characteristics of pathogens

Molecular characteristics of pathogenic micro-organisms, including their evolution, pathogenicity, virulence, transmissibility, and survival potential, are key factors in the spread of AMR. These factors can be detected by laboratory diagnostic techniques (Baker et al. 2018). However, uncertainty in diagnosis may lead to misuse or overuse of antimicrobial and higher rates of emergence of resistant microbes (Harbarth and Samore 2005). Evolutionary engineering, inhibition of microbial AMR gene expression, probiotics, and rapid and improved diagnostic tests can help to control the potential determinants of AMR (Andersson et al. 2020; Pollock et al. 2020).

Prescribers of antimicrobial drugs (Physicians)

To control the emergence of AMR, physicians need to change their prescription patterns by avoiding the use of antibiotics in the treatment of non-bacterial infections, as well as avoid any unnecessary long-term use of antimicrobials. These are promising means of reducing antimicrobial selection pressure. Studies have shown that shortening the duration of treatment with antimicrobial agents reduces the risk of development of AMR (Marston et al. 2016). With the increased awareness of prescribers of the emergence of AMR, an overall reduction in prescriptions of antimicrobials has been reported. This has resulted in some reduction in antimicrobial resistance. This also indicates that to overcome antimicrobial misuse or overuse, educating the prescribers and other health care providers is crucial (Abera et al. 2014; Llor and Bjerrum 2014).

Characteristics of patient populations and host-related factors

These factors include rate of infection and case-mix characteristics, consumer behavior and attitude towards usage of anti-microbials and increased immigrations or global migrations (Harbarth and Samore 2005). Host related factors which can increase antimicrobial use and AMR include, increased numbers of surviving immune-compromised patients, longer life expectancies, along with the increased susceptibility to infection of older populations (Yoshikawa 2002). These factors can be controlled through screening, increased surveillance of antimicrobial usage and AMR dissemination, better control of chronic infectious diseases, public information campaigns, vaccination and the implementation of WASH guidelines (WHO 2018).

Macro level factors

These are factors related to the quality of each country's health-care system, as well as its policy making practices. Key measures include, the formulation of regulatory policies designed to control the use of antimicrobial drugs and enforced implementation of infection control practices (Weese et al. 2015). Other factors include technological development and drug discovery. An effective strategy to combat AMR requires both global and national action plans (WHO 2016). Challenges linked to the control of the dissemination of AMR include, poor healthcare regulations, politicization, lack of predictability in decision-making in case of any outbreak, the ability to identify key stakeholders and the ability to implement regulations at smaller and individual levels. If implemented correctly, healthcare regulations would have a powerful influence and help to control antimicrobial drug use in the future (World-Bank 2017). It is also of extreme importance to restrict the purchase of over-the-counter antimicrobial drugs without a medical prescription (Parsonage et al. 2017).

Novel Approaches to control or slow down the Emergence of AMR

There is a dire need of the development of novel approaches to more efficiently curb the emergence and spread of AMR. In recent years, metal nanoparticles (NPs) have gained attention of scientists and researchers due to their high and long-lasting antimicrobial activity (Bogdanović et al. 2014; Kruk et al. 2015). Zinc doped CuO NPs, used against multi-drug resistant (MDR) bacteria, have shown profound antimicrobial effect against both antimicrobial susceptibility and MDR-strains of *E. coli* and *S. aureus* (Malka et al. 2013; Ali et al. 2017).

Commercially available bacteriophages, such as commercial Listeria phage products, (ListShieldTM and ListexTM P100), can be used to reduce the growth of Listeria. It was reported that treatment with ListexTM P100 considerably reduced the growth of *Listeria monocytogenes* (Pietracha and Misiewicz 2016). Also, a mixture of two bacteriophages (P433/1 and P433/2) used to treat infections induced by the *E. coli* strain P433 in pigs was shown to be very promising. Both bacteriophages showed a high capacity to lyse bacteria *in vitro* (Zhang et al. 2015).

Bacteriocins can also be used as potential alternatives to antibiotics. Bacteriocin is a group of ribosomal proteins with antimicrobial properties (Yang et al. 2014). Some of the bacteriocins exhibit narrow spectrum of activity, whereas others exhibit broader spectrums (Cotter et al. 2013). Lantibiotics have been the most extensively studied bacteriocins. They include nisin, lacticin 3147, mersacidin, lacticin 481 and staphylococcin C55, amongst others (Willey and van der Donk 2007). Some lentibiotics have been shown to target vancomycin-resistant Enterococci and MRSA (Alves et al. 2020; Reinseth et al. 2020). Due to their distinct mode of action and wide spectrum of action, bacteriocins have attracted considerable attention in the area of antimicrobial research.

Conclusions

Antimicrobial resistance has become an enormous global public and animal health concern. It is vital that measures be implemented in order to curtail the emergence of AMR in micro-organisms, animals, humans and the environment. Needless use of antimicrobials is the single biggest cause of emergence of AMR, which then is disseminated via animals, humans, agricultural practices and/or environmental contamination. Our understanding of antimicrobial resistance is far from complete. A thorough understanding of factors involved in AMR, such as pathogen-host and pathogen-drug interactions, mutation rates of pathogens and AMR transmission rates is crucial for our ability to prevent the emergence of AMR. AMR cannot be controlled by a single measure. There should be several overlapping and synergistic stern strategies with the involvement of multiple stakeholders at the national and global levels. The prudent and safe use of antimicrobials in humans and animals can only be achieved through the prescription of antibiotics limited to appropriate and fully justifiable cases.

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Udder Pathophysiology and Public Health

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INTRODUCTION

Mastitis is an inflammatory disease of milk secretary tissues of animals, with relatively higher incidence among the disorders in milch animals. Mammary glands infection results in production loss to dairy industry (DeVliegher et al. 2005; Bachay et al. 2011; Ali et al., 2016).

Bacterial pathogens are most prominent infectious agents throughout the world (Bradley 2002; Unnerstad et al. 2009). Mastitis is caused by a wide range of bacteria, mainly *Staphylococcus aureus* and Corynebacterium species. It severely affects milk quality and production of infected animals and has tendency to spread rapidly within the herd and to other animals. Compositional changes in milk of infected animal with mastitis depend upon inflammatory response of mammary glands, extent and pathogenicity of infectious agent, along with amount of infected tissue, leakage of blood, various enzymes, proteins, salts, decreased concentration of lactose and fat in milk (Kitchen 1981; Osteras 2000; Eckersall et al. 2001).

Mastitis is linked with enzymes, as lactate dehydrogenase and alkaline phosphatase enzymes are increased in milk from animals infected with subclinical mastitis, which is mainly due to tissue damage provoked by subclinical mastitis (Batavani et al. 2007). Mastitis is also linked with minerals, as the milk pH, sodium content, electrical conductivity and malondialdehyde concentration is higher, while the concentrations of other minerals like calcium, zinc, iron, magnesium, potassium and phosphorus are decreased in mastitic animals (Mehrzad et al. 2002).

Shape of udder, asymmetric udder with larger rear quarters than front quarters, udder injuries, udder oedema, housing, feeding, breed and management have been known as mastitis risk factors (Schukken et al. 1994; Slettbakk et al. 1995; Waage et al. 2001; Svensson et al. 2006; Compton et al. 2007). Mastitis has great economic importance, as it may lead to the culling of affected animals. In dairy production, improved management and different control measures have greatly decreased the incidence and prevalence of contagious mastitic organisms. Inflammation of mammary tissue (mastitis) is a multifarious disease that affects dairy animals and causes huge economic losses in the form of decreased milk yield (3-5%) and impairs milk quality which indicates serious risk for human health (Costa 1998; Marjan et al. 2009).

Mastitis

Mastitis is an inflammatory disease of milk secretary tissues of animals, with relatively higher incidence among the disorders in milch animals. Mammary glands infection results in production loss to dairy industry (DeVliegher et al. 2005; Bachay et al. 2011). It affects the milk quality and is also hazardous from the public health prospective. It also leads to an increased culling rate of affected animals from the herd (Parker et al. 2007). It is a multifactor disorder. The disease occurs when pathogens penetrate the teat orifice, enter into the mammary gland and thrive in udder, causing its inflammation (Pacha et al. 2020).

Clinical mastitis exhibits systemic reaction of mammary parenchyma, discoloration of udder, hardened quarters, abnormal secretions, flakes and clots in milk and loss of appetite of the affected animal (Akers and Nickerson 2011). On the other hand, sub-clinical infection without showing clinical picture remains vague to dairy animals. Increase in milk somatic cell count is typical finding in subclinical mastitis (Schwarz et al. 2020). Subclinical mastitis can be diagnosed by culturing of bacteria from milk, but routinely it is not a good test (Hamann 2002 and Akersted et al. 2007; Pyorala 2009).

Mammary gland

Mammary gland is a complex organ by both in configuration and function. Milk is sterile and free from pathogens when it is secreted by the mammary gland and is infected with different microorganisms when flows out of the udder. Milk production is a complex process and is under the influence of hormones and some other factors which affect milk yield (Silanikove et al. 2006). Generally, various pathogenic and non-pathogenic microorganisms cause changes in colour, taste and aromas of milk and certain pathogens result in food borne diseases. Pathogenic organisms which are responsible for deterioration of milk

and its products quality mainly enter milk through improper milking, handling, storage and finally unhygienic condition of employee.

Bovine mammary gland is highly susceptible to new intra-mammary infections around calving, and many infections which occur at this stage result in clinical mastitis. Histological examination of infected mammary glands demonstrates a severe inflammatory response. It has been suggested that marked leukocyte infiltration is harmful to the mammary tissues (Nickerson 2009).

Etiology and Microbiology

Bacterial pathogens are most prominent infectious agents throughout the world (Bradley 2002; Unnerstad et al. 2009). Mastitis is caused by a wide range of bacteria, mainly *Staphylococcus aureus* and Corynebacterium species. *Streptococcus dysgalactiae* acts as an environmental pathogen (Ali et al. 2008; Unnerstad et al. 2009; Waller et al. 2009; Fakhar-uz-Zaman et al. 2009; Ibrahim et al. 2011). Mastitis causing bacteria are divided into two groups, as Gram positive and Gram-negative bacteria based on Gram staining, with Gram-positive bacteria withhold crystal violet stain and Gram-negative bacteria lack peptidoglycan layers and teichoic acid is incorporated within the peptidoglycan. The major Gram-positive bacteria include *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Streptococcus agalactiae* (Piepers et al. 2009). Among Gram negative mastitis pathogens, *E. coli* acts as the primary pathogen which causes mastitis in dairy herds (Piepers et al. 2009; Djabri et al. 2002; Unnerstad et al. 2009).

Among a wide range of pathogens causing mastitis in buffaloes and cows, *Staphylococcus aureus* and *Streptococcus agalactiae* are the most frequent etiological agents and induce extensive and wide variety of pathologies in lactating animals (Djabri et al. 2002; Wilson et al. 2004; Marjan et al. 2009; Cheng et al. 2010).

Staphylococcus aureus is the major pathogen identified worldwide (Guler et al. 2005; Pyorala and Taponen 2009). Mastitis caused by *Staphylococcus aureus* is a highly contagious disease (Bachay et al. 2011). In dairy animals, *Staphylococcus aureus* causes hardness of udder (Nagase et al. 2002; Soliman 2018). *Staphylococcus aureus* infection also forms abscesses in mammary glands. These occluded areas become refractory to antibiotics (Pacha et al. 2020).

Staphylococcus chromogenes, *Staphylococcus hyicus* and *Staphylococcus aureus* are the common pathogens isolated from secretion and teat canal keratin. Isolation of *Staphylococcus aureus* is linked in tie stalls and *Streptococcus dysgalactiae* are associated with teat injuries (Unnerstad et al. 2009). Intra-mammary infections with *Staphylococcus aureus* may result in clinical or sub-clinical mastitis and is usually associated with increase in somatic cell count. *Staphylococcus aureus* is a problem under different field conditions. This pathogen is contagious. So, control of *Staphylococcus aureus* mastitis remains important and necessary (Waller et al. 2009).

Phenotyping and genotyping techniques have been developed for *S. aureus* isolates, such as gene typing (Wang et al. 2009), and amplification of specific gene regions (Javid et al. 2018; da Silva Soares et al. 2021). Histological and molecular studies are, therefore, valuable tools in assessing the damage caused by mastitis pathogens to secretory tissue of the udder in bovine mastitis.

Immune cells and Somatic Cell Count

Udder Lesions

The risk of increased milk somatic cell is higher in animals with increased udder lesions, along with increasing amounts of concentrates in feed, moving animals to confined housing before the calving day and use of restraint procedures at milking (Svensson et al. 2006). Other risk factors for clinical and subclinical infection are dirty udder after calving, udder edema and breed immunity/predisposition (Compton et al. 2007).

The movement of polymorphonuclear leukocytes during inflammatory process, triggered by clinical and subclinical mastitis pathogens, is crucial against infections (Sordillo 2018; Fakhar-uz-Zaman et al. 2009; Abera et al. 2010). The measuring of milk somatic cell count is the standard way for diagnosis of subclinical mastitis, along with various other inflammatory tools like electrical conductivity, enzymes and proteins (Hamann 2002 and Akerstedt et al. 2007; Pyorala 2009).

Intra-Mammary Infectious Agents and Somatic Cell Count

Coagulase-negative *Staphylococci* are the cause of higher percentages of milk leukocytes and damage the mammary parenchyma (Pyorala and Taponen 2009). Intra-mammary infections with *Staphylococcus aureus* are associated usually with increased milk somatic cell count. *S. aureus* remains a problem in a variety of locations and under different management conditions. This pathogen is contagious and spreads easily within dairy herds (Barkema et al. 1999; Barkema et al. 2006; Marjan et al. 2009). Milk somatic cells consist of macrophages, neutrophils, lymphocytes and epithelial cells (Lindmark-Mansson et al. 2006; Fakhar-uz-Zaman et al. 2009).

Staphylococcus aureus cause both clinical and subclinical mastitis, which increases milk somatic cell count (Taponen and Pyorala 2009). When somatic cell count increases in milk, a decrease in proteins together with breakdown and less absorption of calcium occur in milk from animals infected with clinical or subclinical mastitis. Milk somatic cell counts are usually used as diagnostic tool. The mean somatic cell count in healthy buffaloes is 3.64×10^5, while in cattle it is 3.40×10^5, while in mastic animals these values are 62.69 and 41.34×10^5, respectively (Shah et al. 2017; Kirkeby et al. 2021; Leitner et al. 2000a; Pillai et al. 2001; Moroni et al. 2005; Dhakal 2006; Lindmark-Mansson et al. 2006; Suriyasathaporna et al. 2006 Piepers et al. 2009; Fakhar-uz-Zaman et al. 2009).

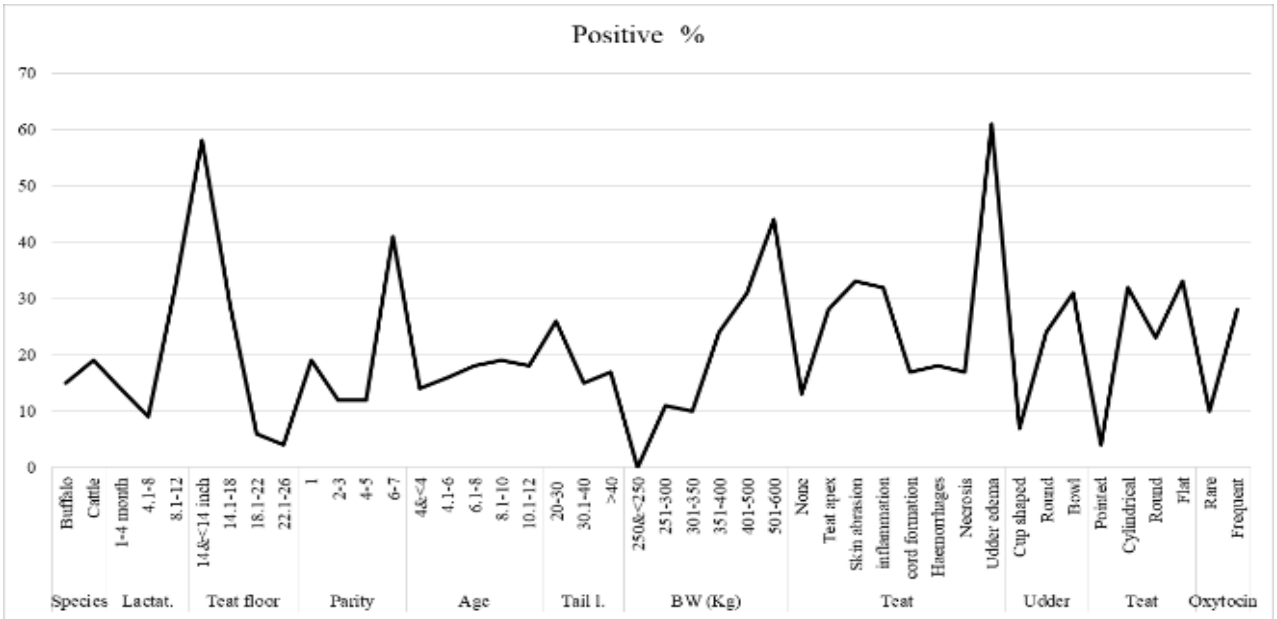

Table 1: Overall bivariate frequency analysis of different parameters in infected and healthy cattle and buffaloes to CMT test (Hussain 2011).

Staphylococcus aureus cause both clinical and subclinical mastitis, which increases milk somatic cell count (Taponen and Pyorala 2009). When somatic cell count increases in milk, a decrease in proteins together with breakdown and less absorption of calcium occur in milk from animals infected with clinical or subclinical mastitis. Milk somatic cell counts are usually used as diagnostic tool. The mean somatic cell count in healthy buffaloes is 3.64 x 10^5, while in cattle it is 3.40 x 10^5, while in mastic animals these values are 62.69 and 41.34 x 10^5, respectively (Shah et al. 2017; Kirkeby et al. 2021; Leitner et al. 2000a; Pillai et al. 2001; Moroni et al. 2005; Dhakal 2006; Lindmark-Mansson et al. 2006; Suriyasathaporna et al. 2006 Piepers et al. 2009; Fakhar-uz-Zaman et al. 2009).

Some pathogens release metabolic by-products, or toxins when they enter and multiply in the mammary tissue. These factors are released either directly or indirectly by different pathogens that serve as chemotactants for the leukocytes (Ávila et al. 2020). If somatic cells move rapidly from the blood vascular system and can reduce the inflammatory stimuli (pathogens), then further movement of leukocytes stops, and somatic cells number returns to physiologically normal level. However, when the pathogens can survive after the immediate host response, then the inflammatory process continues, resulting in increased somatic cell migration between the mammary secretary cells towards the alveolar lumen (Capuco et al. 2003).

Mastitis severely affects milk quality and production of infected animals and has tendency to spread rapidly within the herd and to other animals. Compositional changes in milk of infected animals with mastitis depend upon inflammatory response of mammary glands, extent and pathogenicity of infectious agent, along with amount of infected tissue, leakage of blood, various enzymes, proteins, salts and decreased concentration of lactose and fat in milk (Adkins and Middleton 2018; Osteras 2000; Eckersall et al. 2001).

Mammary infections reduce income from dairy animals. Various environmental and individual risk factors including milking technique, hygienic husbandry practices and use of antibiotics to treat mastitis during dry period and lactating stage relate to mastitis (Bradley and Green 2001). Different strategies including selection of those animals which have resistance against the disease, removal of animals which are susceptible to disease and environmental hygiene can be used to lower the prevalence of mastitis.

More than 90% of the total somatic cells which come from blood into mastitic milk are the neutrophils (Depreester et al. 2017). Somatic cells act as natural defence mechanism and first line of defence against invading pathogens in the mammary gland and include eosinophils, monocytes, lymphocytes, macrophages, neutrophils and a few epithelial cells (Riollet et al. 2000; Pillai et al. 2001; Hussain et al., 2012). Milk somatic cells reflect inflammatory process in the udder of infected animals during infection and initiate the immune system. Milk somatic cell count is a golden standard and quick tool to measure inflammation by cytological investigation and is directly related to immune system of mammary gland triggered by different pathogens (Riollet et al. 2000; Leitner et al. 2007). Milk somatic cell count also increases with increase in stage of lactation. Occurrence of increased leukocytes in milk samples is indicative of presence of subclinical infection. Quarters with somatic cell count greater than 200,000 per ml are at the greatest risk of clinical mastitis (Green et al. 2004; Dhakal 2006; Suriyasathaporna et al. 2006; Paape et al. 2007; Gargouri et al. 2008).

Milk production and leukocytes

Milk production is decreased as the population of milk leukocytes increases in animals (Pacha et al. 2020; Piepers

et al. 2009). The milk production is also dependent on parity, breed and lactation stage of cattle (Durr et al. 2008).

The mammary parenchymal DNA content is higher before calving and localization of thymidine in mammary tissue is more in dry cows than in lactating animals (Fabris et al. 2020). Administration of bovine somatotropin (bST) increases mammary epithelial cells, showing nuclear proliferation during mid-lactation stage. The number of mammary epithelial cells within the mammary gland decreases with increase in lactation stage, indicating the loss of milk production along with increase in mammary cell apoptosis (Capuco et al. 2003).

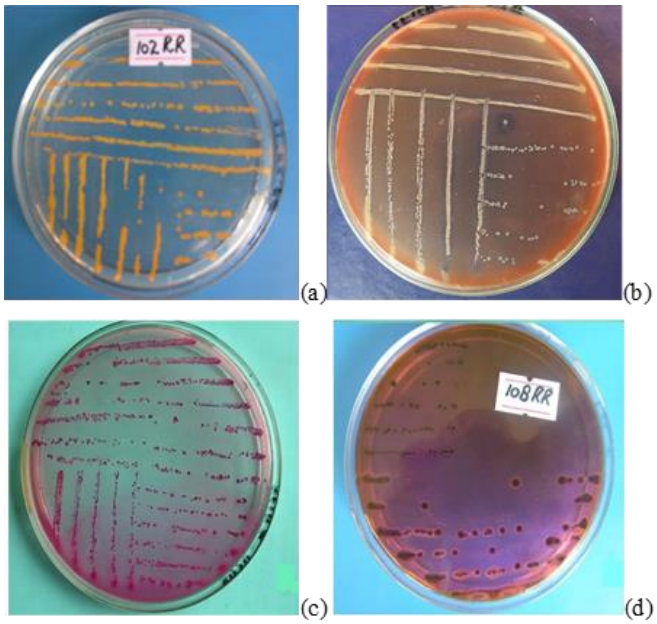

Figure 1: (a) Creamy white to yellow and golden colonies of *S. aureus* on Staph 110 agar medium, (b) Small pin point colonies of *Streptococcus agalactiae* on 5% sheep blood agar medium, (c) Rose pink colonies of *E. coli* on MacConkey's agar (A) and metallic shin on EMB agar (D) isolated from cattle and buffaloes (Hussain 2011).

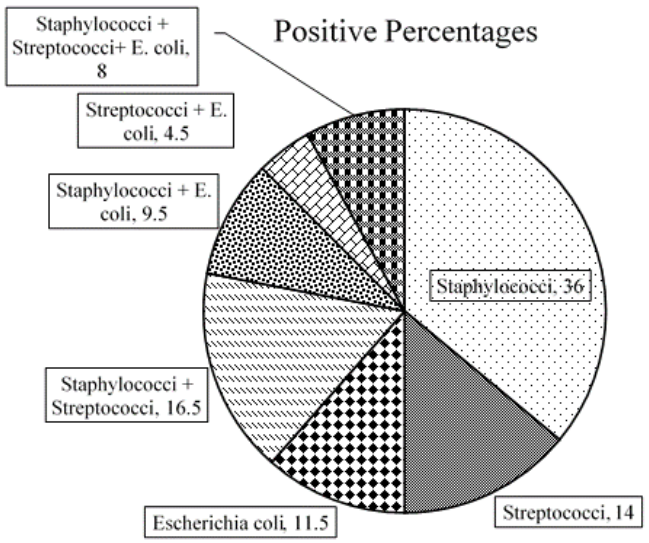

Figure 2: Overall prevalence of different pathogens recovered from mastitis animals at abattoir (Hussain 2011).

Free radicals, Enzymes and Hormones

Free radicals of mastitis cause damage to mammary glands and decrease the milk production (Moradi et al. 2020). The secretion of milk and proteins in udder by secretory cells is controlled by several systemic and multiple steroid hormones (Suriyasathaporna et al. 2006; ELsayed et al. 2009; Ibrahim et al. 2011; Cardoso et al. 2020).

Mammary glands infected with algae indicate inflammatory response, along with mononuclear infiltration of eosinophils and polymorphonuclear cells (Benites et al. 2002). *Staphylococcus chromogenes* and *Staphylococcus epidermidis* decrease milk production due to damage in udder tissues and increased connective tissue stroma (Santos et al. 2009). Cows infected with *Brucella* show hyperplasia of mammary lymph nodes and medullary plasmacytosis, with infiltration of lymph nodes with macrophages (Beytut et al. 2009). The total milk somatic cell and neutrophil counts are higher in mastitic than healthy animals. However, the percentages for macrophage and lymphocytes are lower in milk of mastitic than healthy animals.

When the mammary gland is invaded by pathogenic bacteria, these cells gather in the udder to decrease microorganisms under the effect of various cell communication products (Piepers et al. 2009; Damm et al. 2017; Malik et al. 2018). The inflammatory process of mammary tissue is directly related to influx of neutrophils. In the mammary gland alveoli, neutrophils produce and release toxic chemicals which lead to decreased milk production and are termed as somatic cells.

Mastitis and Enzymes

Streptococcus dysgalactiae invasion does not influence the mammary epithelial cells viability and is dependent on specific time. Cellular damage is indicated by localization of higher amount of lactate dehydrogenase (Oliszewski et al. 2002; Chen et al. 2021). Lactate dehydrogenase and alkaline phosphatase enzymes are increased in milk infected with subclinical mastitis due to tissue damage provoked by subclinical mastitis (Batavani et al. 2007). Overall activities of enzymes in sheep mammary tissues are higher than in cattle. The activity of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and oxidoreductases are increased in all animals. In cells of ducts and secretory cells, glucose-6-phosphate dehydrogenase shows higher concentration but lower values in cells of the interstitium. The activity of both enzymes, glycerophosphate dehydrogenase and succinate dehydrogenase, are reduced in sheep and cattle. Alkaline phosphatase and ATP-cleaving enzymes have strong activity in duct cells and lactiferous ducts of mammary gland, respectively (Manocha et al. 2017). *Staphylococcus aureus* isolates from infected animals are positive for deoxyribonuclease and mannitol fermentation (Arshad et al. 2006). The level of various milk fractions, including sodium, chlorine, milk pH, milk albumin, milk lactate dehydrogenase and immunoglobulin, are increased in infected quarters as compared to healthy quarters. Milk

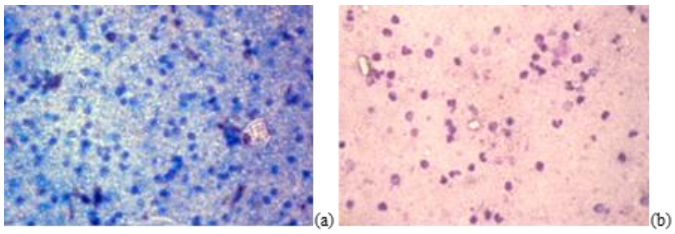

Figure 3: Somatic cells stained with; (a) Newman lampert stain, (b) Giemsa stain from milk sample of cattle and buffaloes (Hussain 2011).

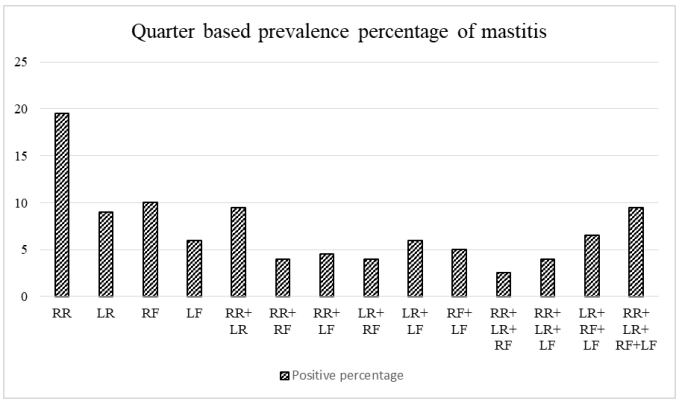

Figure 4: Overall quarter based prevalence of mastitis in animals studied at abattoir. RR, LR, RF and LF means right rear, left rear, right front and left front, respectively (Hussain 2011).

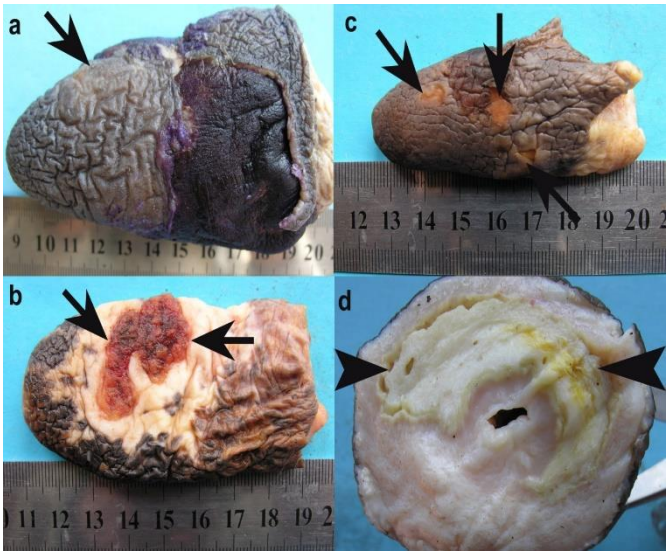

Figure 5: Various gross lesions: Inflammation (a), necrotic tissues (b, c) and fibrosis (d) in cattle and buffaloes (Hussain 2011).

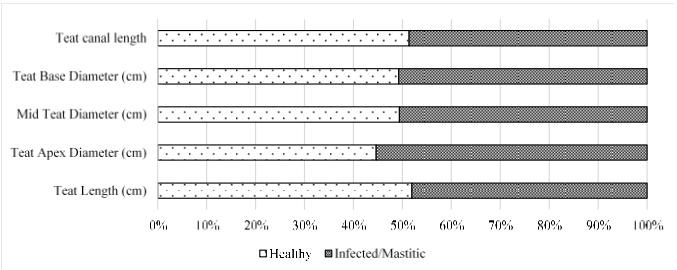

Figure 6: Measurements (Mean ± SD) of various parts of teat (Hussain 2011).

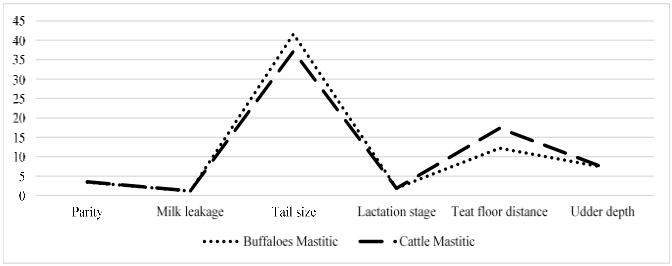

Figure 7: Analysis of various physical parameters of infected and healthy buffaloes and cattle at various livestock farms (Hussain 2011).

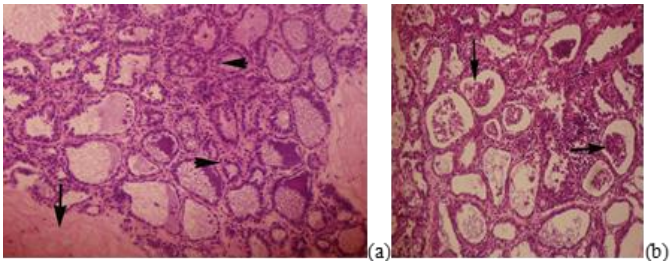

Figure 8: Section of mammary tissue showing; (a) cellular infiltration, increased stromal tissue (arrow) and atrophy of alveoli, (b) severe cellular exudate and infiltration of mononuclear cells (arrow). 200X, H & E Stain (Hussain 2011).

phosphorus, calcium and beta-lactoglobulin are decreased in infected quarters (Batavani et al. 2007). There is increased malondialdehyde in blood and milk of animals infected with sub-clinical and clinical mastitis (Kumar et al. 2007).

Histochemical Studies

The activity of an alkaline phosphatase in cattle and buffaloes, which are not infected with mastitis, is on the outer boundary of alveolar secretary cells presenting cellular activity. Tissue sections of infected animals show weak alkaline phosphatase activity (staining) on the outer membrane. In most of the infected tissues, activity of this enzyme is low, negligible or altogether absent. There is high protein staining density in tissue sections of non-infected cattle and buffaloes. However, the tissue sections of infected animals show low to absence of protein.

Mastitis and Minerals

The milk pH, sodium content, electrical conductivity and malondialdehyde concentration are higher, while the concentrations of other minerals like calcium, zinc, iron, magnesium, potassium and phosphorus are lower in mastitic animals. There is a difference in various enzymes including lactate dehydrogenase and alkaline phosphatase in milk of buffaloes and cattle. Performance of dairy industry is negatively affected the poor management, reduced nutrition and different diseases. Adequate mineral supplementation optimizes immune system function by reducing various metabolic and oxidative pressures and play crucial role in the defence mechanisms of mammary glands against mastitis. In modern dairy

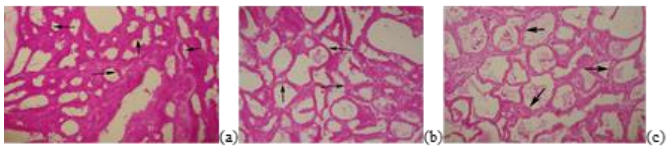

Figure 9: Section from mammary gland showing (a) high, (b) weak, and (c) no level of alkaline phosphatase activity (arrow). 200X (Hussain 2011).

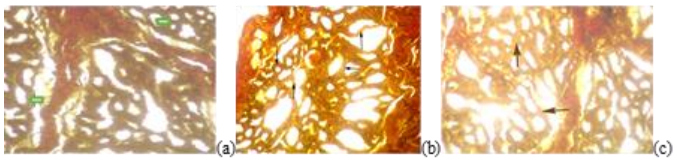

Figure 10: Section in mammary tissue showing (a) high protein staining, (b) low protein staining, and (c) negligible protein staining, all separated by trabeculae of connective tissue (arrow). 200X (Hussain 2011).

industry, animals face physiological stress when they move from the burden of foetal growth to calving, production of colostrum and milk. Suitable physiological environment is crucial for the repair process of tissue damaged during disease condition. Physiological stress at the time of transition stage favors mastitis and metritis. Various minerals play important role in reducing disease challenges and oxidative reactions (Mehrzad et al. 2002). Dietary minerals including Zn, Cu and Se play special role in ensuring efficient growth, and reduced milk somatic cells (Griffiths et al. 2007; Cortinhas et al. 2010).

Organic mineral supplementation can affect health of mammary glands and milk yield; while marginal or low levels of minerals increases the risk of mastitis and different other diseases (Ashmead and Samford, 2004; Enjalbert et al. 2006). When mastitis occurs, free radicals are released during the process of phagocytosis, which cause decrease in milk production and mammary gland lesions. Various minerals, including Zn, Cu and Se supplementations, have been associated with higher antioxidant activity, resulting in reduced somatic cell count and increased quantity of milk in dairy animals (Kellogg et al. 2004; Griffiths et al. 2007; Qayyum et al., 2016).

Mastitis and electrical conductivity

There is increased electrical conductivity in *Staphylococcus aureus* infected quarters, having high somatic cell count compared to non-infected animals (Khatun et al. 2019), due to its high salt content (Mabrook and Petty 2003). Mastitis increases the electrical conductivity and NaCl concentrations of milk (Stocco et al. 2020).

Mastitis and Risk factors

Different risk factors, including shape of udder, asymmetric udder with larger rear quarters than front, udder injuries, udder oedema, housing, feeding, breed and management, have been known as mastitis risk factors (Waage et al. 2001; Svensson et al. 2006; Compton et al. 2007; Okkema and Grandin 2021). Many risk factors of mastitis related to environment, the microflora and the animal have been investigated in spite of wide control efforts without encouraging results; however, the bovine mammary gland infection (mastitis) is a frequent and important problem among livestock herds in most of the countries world-wide. The occurrence of mastitis is influenced by managemental and different environmental factors, like housing of animals, type of milking and milking utensils, type of feeding, hygienic quality of water, health of lactating animals and execution of various preventive procedures. There is a positive correlation between clinical mastitis and udder balance, depth of udder and its attachment in different dairy animals (Sorensen et al. 2000; Zavadilová et al. 2020). Disease record, genetic and morphological investigations are crucial and efficient approaches to raise disease confrontation in animal population. Various morphological characters related to udder health play an important role and may affect susceptibility for mastitis.

Teats

All the efforts right from insemination to the lactation of animals become futile due to the infection of the mammary glands culminating in mastitis. Knowledge about pathogenic condition of milk and various structures of udder is of great value in understanding the process of mastitis in animals. The teat canal remains opened for 2 hours after milking and pathogens may reach the mammary gland through the teat canal to establish the infection. The severity of inflammatory response is dependent both upon the pathogen and host factors. The pathogens factors include the species, virulence, strain, and size of inoculums of bacteria, whereas the host factors include parity, stage of lactation, age and immune status of animals. Pathological changes soon develop in the teat and udder, reducing production potential of the animal and bringing about change in the quality and quantity of milk. Small pockets of fibrous tissue cause atrophy, replace and reduce the glandular parenchyma, making difficult for antibiotics to reach and also prevent complete removal of milk. The animal soon loses its utility as a milking animal and is finally considered suitable for beef purposes. Histopathological sections from various infected teat tissue indicate inflamed mucosa, teat polyps and fibropapiloma (Misk et al. 2018). In spite of growth inhibitory properties of keratin, bacteria are able to survive in teat canal, causing inflammation (Moroni et al. 2006; Oviedo-Boyso et al. 2007 and Ibrahim et al. 2011; Pacha et al. 2020). Animals with unbalance gland, flat and wide teat-ends, milk dropping and increased milk flow are more susceptible to intra-mammary infections. It has been reported that pathogens gain entry through teat orifice with the presence and morphology of teat injuries play a crucial role in the pathogenesis of mastitis (Chrystal et al. 2001; Okkema and Grandin 2021). There is a positive correlation between prevalence of clinical mastitis and teat length (Sorensen et al. 2000; Zavadilová et al. 2020).

Teat Shape

Shape of teats, milk leakage from teats, pointed teats, lower teat end to floor distance and increased teat canal diameter have been known as mastitis risk factors (Waage et al. 2001; Svensson et al. 2006; Compton et al. 2007; Bhutto et al. 2010; Okkema and Grandin 2021). Funnel-shaped teats had lower incidence of mastitis as compared to cylindrical-shaped (round) teats. It is difficult to explain why mastitis is higher in animals having cylindrical or round teats than those with other teat shapes. Further studies are needed to confirm it and to find the association of anatomical variations which help the pathogens to gain entry or be retained in teat canal or cistern and not cleared while milking (Sorensen et al. 2000; Waage et al. 2001; Svensson et al. 2006; Compton et al. 2007; Derakhshani et al. 2020). Teat-end shape has lower effect on milk somatic cell count than period of calving and parity of animals (Chrystal et al. 2001). Regarding teat shape, flat and round teats show higher susceptibility of mastitis than pointed teats. Animals with larger hindquarter shape have less *Streptococcus uberis*, *Staphylococcus aureus* and *E. coli* infection. *Streptococcus dysgalactiae* and *Streptococcus agalactiae* infections are higher in cattle with large pendulous udder (Bhutto et al. 2010). The prevalence of mastitis is higher in animals having round or bowl-shaped udder than those having cup shaped udder.

Mastitis, and Length and Diameter of Teats and Udder

Strong association has been found for the incidence of mastitis and teat diameter instead of milk yield (Guarín et al. 2017). Prevalence of mastitis both in buffaloes and cows is higher in quarters with small teat length, streak canal length and large teat diameter (apex, middle, and base) (Sorensen et al. 2000; Klaas et al. 2004; Bhutto et al. 2010; Guarín et al. 2017; Talukder and Ahmed 2017; Tiezzi et al. 2020; Tiezzi et al. 2020; Zavadilová et al. 2020; Derakhshani et al. 2020; Okkema and Grandin 2021). Mastitis can occur in cattle and buffaloes at advanced stages of lactation, which may be linked with continuous milking of animals for long time, putting pressure on teats, which results in breaks in epithelial lining and thus causes mastitis. Teat canal length is lower in mastitic than healthy buffaloes, while teat apex diameter, mid teat diameter and teat base diameter are higher in mastitic than healthy animals.

Oxytocin and Calves used for Milk Let Down

Prevalence of mastitis is higher in animals in which oxytocin is frequently used for milk let down. It may be due to the fact that oxytocin increases smooth muscles contraction of teat and teat cistern, ultimately ballooning of these tissues is resulted, the teat sphincter remains open for more time and in this way pathogens get chances to enter the mammary glands. Prevalence of mastitis is also increased in cattle and buffaloes in which calves are used for milk let down (Derakhshani et al. 2018; Ashraf and Imran 2020). It is also associated with teat lesions (Hultgren, 2002; Gleeson et al. 2004; Svensson et al. 2006; Compton et al. 2007; Nyman et al. 2007; Breen et al. 2009; Cerqueira et al. 2018).

Housing Circumstances, Sanitation, and Milking

Teat disinfection after milking with a low milk leukocytes count is positively associated with the incidence of clinical udder infection. The incidence of clinical mastitis due to *Escherichia coli* is associated with housing circumstances, sanitation, and machine milking. *Staphylococcus aureus* infection is related to milk somatic cell score, while the incidence of clinical mastitis caused by *Streptococcus dysgalactiae* and Streptococcus *uberis* is correlated to housing, diet, and machine milking (Tomazi et al. 2018). Other risk factors for clinical and subclinical infection are low teat height above the ground, dirty udder after calving, udder edema and breed immunity (Compton et al. 2007). Full-hand method of milking and teat dipping are not correlated with incidence of mastitis (Lakshmi et al. 2009).

Mastitis and Teat quarter involvement

Some relationship has been found in hyperkeratotic teat injures and udder shapes of infected animals. A strong association was observed between intra-mammary pathogens including *Staphylococcus aureus, S. uberis, coagulase-negative Staphylococci, S. agalactiae* and *E. coli* and the presence of hyperkeratotic teat injuries at calving (Bhutto et al. 2010). Various morphological characters related to teats play important role and may affect susceptibility of mastitis. There is higher association among various traits including location of teat (front/rear), teat length, teat morphology, teat end shape and teat canal eversion, harsh teat canal eversion, teat canal eversion and rough/cracked teat canal, teat canal eversion and teat end edema are associated with prevalence of mastitis (Wieland et al. 2020). Involvement of right rear, right front quarters, and right rear + left rear quarters has higher percentages of infection. Intra-mammary infection originating in dry period reduces milk production by about 35% compared with uninfected quarters (Bhakat 2019). In cattle and buffaloes, the right rear quarters are involved in higher percentages than involvement of other quarters alone or in combination. Mastitis can be triggered through injury or infection caused by various pathogens, including bacteria, viruses, algae, or fungi and these organisms can be categorised based on their pathogenesis, etiology or severity of infection (Qayyum et al., 2016) but majority of mastitis cases in dairy industry are due to bacterial infections (Cobirka et al. 2020).

Teat to Floor Distance and Space allowance

High prevalence of mastitis in cattle and buffaloes is observed with low teat to floor distance (Waage et al.

2001; Svensson et al. 2006; Compton et al. 2007; Bhutto et al. 2010). There is no difference between mastitic and healthy animals for teat lesions. Space allowance has no effect on milk yield, live weight, body condition score, and lying; however prevalence of mastitis is indicated in cows kept in unhygienic straw yards at low space allowance (Fregonesi and Leaver 2001).

Mastitis and Feeding system

There is no difference between mastitic and healthy animals due to feeding system. However, lesions on udder, udder shape, use of oxytocin, calf suckling and hosing space showed difference between mastitic and healthy animals (cattle and buffaloes). The cases of mastitis are higher in animals (cattle +buffaloes) having lesions like udder edema, skin abrasion and inflammation, in animals having bowl or round shaped than cup shaped udder, in animals having cylindrical udder, in animals in which oxytocin is frequently used for milk let down, where calf suckling practice is made after milking of animals, in cases of stall feeding + grazing and in animals provided less space for housing.

Miscellaneous

The milk temperature does not show any difference between mastitic and health animals. The milk pH, electrical conductivity, malondialdehyde and total dissolved solids are higher in milk from mastitic than that from healthy buffaloes. However, milk fat, milk yield, proteins, lactose and solids-not-fat (SNF) are lower for milk from mastitic than that from healthy animals. Increased risk of mammary infection is also related with increased live weight, increase in age at first calving, increase in parity, low milk flow rate and leaking of milk. The age, parity, tail size and lactation stage show no difference between mastitic and healthy buffaloes. However, live body weight, milk leakage, and udder depth show differences between mastitic and healthy buffaloes. The values for these variables are higher in mastitic than healthy buffaloes, except for teat floor distance which is lower in mastitic animals. Tail length is not associated with prevalence of mastitis.

Histopathology

Intra-mammary infusion of oyster glycogen increases milk leukocyte count six times and also increases protein and fat percentages, but reduces milk lactose concentration in lactating ewes. The infused mammary glands show increased damage or non-secretory epithelial cells along with three times increase in neutrophils, 1.3-fold plasma cells in the sub-epithelial stroma and 2.7-fold of neutrophils within the epithelial lining of alveoli. Decrease in milk yield is due to increase in somatic cells, along with loss of functional mammary cells, associated with leukocytosis. Secretory parenchyma of unbred healthy quarters is immature, showing small alveoli having limited luminal area and abundant inter-alveolar stromal area. *Staphylococcus aureus* infected tissues are less developed with small alveolar epithelial and luminal areas and with additional inter-alveolar stroma than healthy quarters. Secretory activity is also reduced in quarters infected with *Staphylococcus aureus.* Presence of abscesses is also seen in quarters infected with *Staphylococcus aureus.* Leukocytic infiltration in mammary parenchyma and in cistern lining of infected quarter is higher than that of healthy quarters (Pacha et al. 2020). Alveolar diameter (short and long), number of alveoli, maximum and minimum short and long diameters and epithelial cell population of alveoli in infected cattle and buffaloes are lower than those in healthy animals.

Histopathology for both cattle and buffaloes reveals the atrophy of alveoli in udder tissues of different degree (mild, moderate or severe), cellular exudate in the lumen of the alveoli in varying amounts, broken alveoli, cellular infiltration in udder tissue including lymphocytes, monocytes and/or neutrophils in the parenchyma, cellular infiltration in teat tissue including lymphocytes, monocytes and/or neutrophils and the proliferation of connective tissues in infected animals.

Economic importance, Characterization of Mastitis, and Public Health Concerns

Various expenses due to any disease can create such conditions where culling becomes necessary. In dairy production, improved management and different control measures have greatly decreased the incidence and prevalence of contagious mastitic organisms. Inflammation of mammary tissue (mastitis) is a multifarious disease that affects dairy animals and causes huge economic losses through decreasing 3-5% milk production and impairing its quality, which indicates serious risk to human health (Marjan et al. 2009; Freitas 2017; Jingar et al. 2017). While investigating the total expenses that are involved in mastitis prevention and its treatment, scientists have determined some strategies based on the direct impacts of the disease. In India, due to mastitis Rs. 16,072 million economic losses, along with 50% to 17.5% decrease in average milk production, have been reported. In Nili- Ravi buffaloes, mastitis decreases 438 kg milk of each animal by reducing 57 days lactation length (Yasir et al. 2020). Economic losses due to mastitis may be divided into lower milk production (70%), discarded milk due to veterinary medication (8%), and premature culling (14%) (Barkema et al. 2006; Halasa et al. 2007; Cheng and Han 2020). The degree of production losses depends on causative agent, parity of lactating animal and lactation stage at which mammary glands infection occurs. In dairy animals, production loss is harsh when clinical mastitis occurs before peak milk yield due to *S. aureus*, *Klebsiella* and *E. coli* (Grohn et al. 2004; Wilson et al. 2004). However, subclinical mastitis occurring in late stage of lactation causes highest production loss (Bennedsgaard et al. 2003; Gonçalves et al. 2018).

Mastitis affects the milk quality, and it is also hazardous from the public health prospective. It also leads to an increased culling rate of dairy animals from the herd

(Parker et al. 2007). The API Staph-kit system is excellent and accurate for the identification of *Staphylococci.* Various studies have shown its use in humans for the identification and confirmation of Staphylococcal species (Romanowski et al. 2021). The API Staph-kit system, which is urbanized for the detection of human *Staphylococci,* exactly identifies the bovine pathogensand indicates the variation in biochemical profile between animals and human pathogens (Adkins et al. 2017).

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COMMON BACTERIAL DISEASES OF FISH: PREVENTION AND CONTROL STRATEGIES

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INTRODUCTION

Bacteria are common in the aquatic environment and fish in the captive environment are exposed to a phylogenetically diverse group of bacterial pathogens. The sustainable aquaculture industry is very important because 1/3rd of the world's food comes from this industry (Ravi et al. 2007). To increase the aquaculture production, traditionally extensive fish farms have been changed to intensive ones and this intensification caused stress, which reduces the fish immunity and enhances their susceptibility to pathogenic bacteria. A disease outbreak can cause high mortality in fish that decreases their production, causing high economic loss to the fish farmers. In addition, sub-lethal diseases may affect growth rate and flesh quality and can cause undesirable visual changes. Factors that can cause infection in fish are not totally understood, but they may include: inadequate diet, poor husbandry techniques, alterations in the environment favoring the possible pathogens, reduction in resistance of the host animals to diseases, introduction of new fish species that are sensitive to local microbes or carry a microbe that is virulent to local populations. Proper understandings about the etiological agents, biochemistry, epizootiology and the relationship of stress-related environmental component are necessary for effective management and control of diseases. The improvement in diagnosing tools can provide an opportunity to identify new infective agents (Colorni 2004). Many of the bacterial species present in the aquatic habitat are vital to the natural aquatic environment, with no direct effect in inducing fish disease. In the world, there are about 125 various species of 34 different families of bacteria that have been related to different fish infections. Most of the bacterial pathogens responsible for induction of diseases in fish are Gram negative rods, but some are Gram positive rods, while a few are acid-fast rods in the aquatic ecosystem. Major bacterial pathogens of economically important fish are given in Table 1.

Bacterial populations in the environment affect not only the health status of fish stock, but also increase public health concerns because fish and their products are the potent reservoirs of human infectious bacteria. Several bacterial species have been transmitted from fish to humans by eating raw or poorly cooked food or handling the affected fish. Human infections mainly depend on the time of year, contact of patient with fish and the environment, diet and the immunity of the exposed person. Control of infectious diseases in aquaculture is more complex than that of animal diseases of terrestrial environment due to difficulty in fish observation because fish are not close enough like terrestrial animals. The aquatic environment can favor quick disease transmission, fish catching can cause stress and disease in the fish is often difficult to identify and diagnose.

Gram Negative Bacterial Diseases

Flavobacteriosis: Bacterial coldwater disease, bacterial gill disease and columnaris disease

Flavobacterial species are important fish pathogens. Species that induced significant losses in freshwater fish are *Flavobacterium psychrophilum*, *Flavobacterium branchiophilum* and *Flavobacterium columnare,* which are gram-negative, anaerobic and non-motile rods.

Etiological Agents and Diseases

F. psychrophilum causes rainbow trout fry syndrome (RTFS), bacterial coldwater disease (BCWD), peduncle disease, fry mortality syndrome, or low temperature disease and is also familiar as *Flexibacter psychrophilus* and *Cytophaga psychrophila* (Holt et al. 2012). Clinical signs are loss of appetite, listlessness, exophthalmia, eroded fin tips and skin, white patches on the fins, with some fish exhibiting fin rays separation, distension of abdomen with large volumes of ascites fluid, and pale colored gills (Cipriano and Holt 2005).

F. branchiophilum as a causative agent of bacterial gill disease was first reported by Davis (1926). The signs of disease are loss of appetite, lethargy, breathing at water top and fish will swim or present on the surface of water and orient in a "soldier-like" manner.

F. columnare causes columnaris disease (Declercq et al. 2013). This bacterium was formerly referred to as *Chondrococcus columnaris, Bacillus columnaris, Flexibacter columnaris* and *Cytophaga columnaris*. Affected fish may show lethargic behavior, loss of appetite, finrots and surface hanging. It includes both acute and long term infections and typically affects the gills, skin and fins. The disease is also known as "saddle-back disease" due to saddle-back like lesions. In tropical fish, due to these signs, the disease is also termed as "mouth fungus" or "cotton wool disease" (Bernardet and Bowman 2006).

Geographical Distribution and Host Species

Psychrophilum is known to expand constantly. The disease appears to occur in temperate regions. All salmonids and

some non-salmonid species are probably affected. This pathogen was recognized for the first time in Mexico (Castillo et al. 2017) and Argentina in 2006.
F. branchiophilum emerged from Japan, Canada, Korea, United States, Hungary, Netherlands and India, it is common in cultured fish than in wild populations (Good et al. 2010). *F. columnare* has been documented mostly in freshwater fish (cultured and wild) of Africa, Europe, North and South America, Asia and Australia.

Epizootiology

F. Psychrophilum affects mostly juvenile fish and infection is also seen in smolts and yearlings. The actual entry mode of the organism is unknown. This disease often occurs when temperature of water is 12°C or below. Once infection occurred, expired fish serve as source of transmission of pathogens horizontally to other fish. This pathogen has an incontestible ability to adjust into different environments and maintain pathogenicity (Vatsos et al. 2001).
F. branchiophilum is transmitted horizontally from fish to fish. The outbreaks of infections are common in spring season and early summer, when the temperature of water rises. *F. branchiophilum* strains (virulent and avirulent) promptly stick to gill tissues and start colonization (Ostland et al. 1994).
F. columnare can affect fish of all ages but is more prevalent in young fish. It is also transmitted horizontally from fish to fish. Handling and injuries to the skin/ mucosa may predispose fish to columnaris disease. The severity and occurrence of columnaris disease is generally higher in warm water having temperature above 20°C.

Edwardsiellosis: *Edwardsiella* septicaemia, enteric septicaemia of catfish

Edwardsiellosis is one of the infections of fish cultured in tropical side due to pathogenic *Edwardsiella* spp. (Park et al. 2012). The genus *Edwardsiella* consists of five species; *E. ictaluri* (Hawke 1979), *E. tarda* (Ewing et al. 1965), *E. piscicida* (Abayneh et al. 2013), *E. hoshinae* (Grimont et al. 1980) and *E. anguillarum* (Shao et al. 2015), all of which are Gram-negatives and belong to the Enterobacteriaceae family. Both *E. tarda* and *E. ictaluri* are most common and induce different infections; their characteristics are given in Table 2.

Etiological Agents and Diseases

Edwardsiella tarda causes *Edwaredsiella* septicaemia, which is also called as red disease of eels or emphesematous putrefactive disease of catfish. Loss of pigmentation, abnormal fluid buildup, severe haemorrhages, nodule formation, opacity of cornea, cutaneous lesions and necrosis during chronic infections are clinical signs of this disease.

Enteric septicaemia

Enteric septicaemia of catfish (ESC) and "hole-in-the-head disease" is caused by *E. ictaluri*. According to clinical signs, fish swim at the surface, and show external lesions, pale gills, exophthalmia, and ulceration. Two additional signs of the disease are acute septicaemia and chronic encephalitis.

Geographical Distribution and Host Species

E. tarda is found in both fresh and brackish water habitats. It has been reported from about 25 countries in Africa, Australia, North and Central America, Europe, Asia and the Middle East (Austin and Austin 1987). *E. ictaluri* agent has a narrow but diverse range of hosts in comparison to *E. tarda*. Experimental infections have been established in Salmonids. At least 40 fish species from more than 20 families are noted to have been infected by pathogenic *E. tarda* and approximately all fish species are prone to disease under favorable conditions.

Epizootiology

In Japan, *E. tarda* occurs in eels in the hot season at about 30°C. In the spring season of Taiwan, the disease occurs in eels when water temperatures are unsteady between 10 and 18°C. *Edwardsiella* septicemia in most fish species in the US seems to be increased by higher water temperatures (30°C and above) and the existence of high organic matter peculiarly in ponds of catfish. For *E. ictaluri*, primarily fingerlings and adult channel catfish may be affected by this disease, which results from the ingestion of pathogens from water.

Enteric redmouth disease (ERM)

It is an acute, as well as a chronic, bacterial disease caused by *Yersinia ruckeri*, a Gram-negative bacterial agent of Enterobacterium. Name of the disease "enteric redmouth" was used to differentiate it from aeromonad and pseudomon and infections that show similar pathological signs. Table 3 shows characteristics of enteric red mouth. According to clinical diagnosis, fish shows exophthalmia, skin darkening, subcutaneous hemorrhages of throat and mouth, enlarged spleen, redness and fluid in lower intestine. Degeneration of renal tubules and rise in melano-macrophages may be seen in diseased fish (Kumar et al. 2015).

Geographical Distribution and Host species

Y. ruckeri is present in the USA, Europe, South Africa, Australia and throughout the world, mainly in sites where salmonids are cultured intensively. Many invertebrates of aquatic habitat also have been found to possess *Y. ruckeri* infection.

Epizootiology

In small fish, this disease is acute, but in larger fish it occurs in chronic form. Fish that hold up disease can become asymptomatic carriers, which afterwards shed numbers of cells, hence, causing pathogen transfer within a group of fish. The severity of an outbreak increases dramatically under the unfavorable rearing situation, and also when animals are under stress.

Table 1: Major bacterial pathogens of economically important fish

Gram negative aerobic rods	Name of the disease
Flavobacterium psychrophilum	Coldwater disease
Flavobacterium branchiophilum	Bacterial gill disease
Flavobacterium columnare	Columnaris disease
Gram negative facultatively anaerobic rods	
Edwardsiella tarda	Edwardsiella septicaemia
Edwardsiella ictaluri	Enteric septicemia of catfish
Yersinia rukeri	Enteric redmouth disease
Vibrio anguillarum	Vibriosis
Vibrio salmonicida	Coldwater vibriosis
Aeromonas salmonicida	Furunculosis
Aeromonas hydrophila	Septicemia
Aeromonas caviae	Septicemia
Aeromonas sobria	Septicemia
Photobacterium damselae Piscicida	Photobacteriosis or pasteurellosis
Gram positive bacteria aerobic rods	
Renibacterium salmoninarum	Bacterial kidney disease
Gram positive facultatively anaerobic rods	
Enterococcus seriolicida / Lactococcus garvieae	Enterococcosis/ Lactococcosis
Streptococcus spp.	Streptococcusis or septicaemia
Weissella ceti	Weissellosis
Acid fast rods	
Mycobacterium spp.	Mycobacteriosis
Nocardia asteroides	Nocardiosis
Intracellular parasites	
Piscirickettsia salmonis	Piscirickettsiaceae
Pisciclamydia salmonis	Epitheliocystis
Francisella spp.	Francisellosis

Table 2: Morphological and biochemical characteristics of two *Edwardsiella* spp. *E. tarda* and *E. Ictaluri* (Hawke et al. 1981; Farmer and McWhorter 1984; Waltman and Shotts 1986; Plumb and Vinitnantharat 1989; OIE 2006).

Characteristics	*E. tarda*	*E. Ictaluri*
Gram stain	Gram negative	Gram negative
Morphology	Small straight rod	Small pleomorphic rod
Growth condition	Facultative anaerobes	Facultative anaerobes
Edwardsiella isolation media (EIM)	Black	Green
Acid from: glucose, maltose, mannose	+	+
Mannitol, sucrose, trehalose, L-Arabinose, Xylose, Rhamnose	-	-
Nitrite from nitrate	+	+
Lysine and ornithine decarboxylase, gas from glucose	+	+
Tolerance to NaCl		
1.5%	+	+
4.0%	+	-
H_2S Production		
Peptone iron sugar, Triple sugar iron	+	-
Mol % G+C of DNA	55-58	56-57

+ = Positive, - = Negative.

Vibriosis

Etiological Agents and Diseases

Vibriosis is a disease caused by bacteria of genus Vibrio. The disease is also known as boil disease (Kubota and Takakuwa 1963), salt-water furunculosis (Rucker 1963) and ulcer disease (Bagge and Bagge 1956). The two spp. of genus Vibrio are more important; *Vibrio anguillarum* causes vibriosis and *Vibrio salmonicida* causes coldwater vibriosis or hitra disease. The external signs of the disease include lethargy, weight loss and red spots on the fish and dark swollen lesions on skin that can bleed and ulcerate, causing exophthalmia. The infection spreads so rapidly in acute epizootics and fish die without exhibiting any particular disease signs (Austin and Austin 2007). The coldwater vibriosis is differentiated by extended ascites, which further causes visceral hemorrhages. The characteristics of *Vibrio* species, *V. anguillarium and Vibrio salmonicida,* investigated by Wiik and Edidius (1986), are given in Table 4.

Epizootiology

The waterborne infection is the main source of transmission of pathogens. Bacteria are continuously released from the vent and open lesions. A common entry site is by the integument with the gills. Coldwater vibriosis typically occurs when temperature of water is less than 10°C.

Table 3: Morphological and biochemical characteristics of enteric red mouth.

Characteristics	*Y. ruckeri*
Gram stain	Gram negative
Morphology	Small straight rod
Growth condition	Facultative anaerobes
Substrate Utilization	
Adonitol, Arabinose, Cellobiose, Galactose, Lactose, Inositol, Dulcitol, Erythritol, Esculin, Melibiose, Raffinose, Rhamnose, Starch, Sucrose, Xylose	-
Trehalose, Mannitol, Maltose	+
Glucose	+ (17%+gas)
Lysine and ornithine decarboxylase,	+
Enzyme reaction	
Aesculinase, Chitinase, Cytochrome oxidase, Chondroitin sulfatase, Deoxyribonuclease, Fibrinolase, Elastinase, Pectinase, Phosphatase, Ribonuclease, Urease, Tributyrinase, Hyaluronidase	-
Caseinase	+(51%)
Catalase, Beta-galactosidase, Lipase	+
Arginine dihydolase	+(59%)
H_2S Production	-

+ = Positive, - = Negative.

Table 4: Morphological and biochemical characteristics of two *Vibrio* species, *V. anguillarium* and V. *salmonicida* .

Characteristics	*V. anguillarium*	V. *salmonicida*
Gram Stain	-	-
Morphology	Motile rod shaped	Non-motile Rod shaped
Growth condition	Facultative anaerobes	Facultative anaerobes
Arginie dihydrolase	+	-
Voges-Proskauer reaction	+	-
Grow at 40°C	-	-
Utilization of L-arabinose and D-sorbitol	+	-
Reduction nitrate into nitrite	+	-

+ = Positive, - = Negative

Aeromonads Diseases: Furunculosis and Motile Aeromonads Septicaemia

Furunculosis

Etiological Agents and Diseases

The causative agent of furunculosis is *Aeromonas salmonicida*. Among warm water and marine species, this variant also produces goldfish ulcerative disease, trout ulcer disease, carp erythrodermatitis and systemic infections. At the species level, four subspecies of *A. salmonicida* were recognized in the Bergeys Manual of Determinative Bacteriology (Holt et al. 1994), based on their differential properties. These are: *masoucida, achromogenes, smithia and salmonicida*. The main characteristics of *A. salmonicida* are given in Table 5. The darkened skin, lethargy, development of furuncles or boils on the musculature and skin with loss of appetite are clinical signs of the disease. After acute infection, fish showed necrotic lesions of the epidermis with rapid septicemia.

Geographical Distribution and Host Species

This disease has been recorded in Africa, Europe, Asia, North and South America. However, it is usually related to freshwater fish, but marine fish are also susceptible (Shotts 1994).

Epizootiology

The exact mechanism of transmission is not entirely understood. However, it is known that the pathogen can be transmitted horizontally both within and among populations of fish, and is present at low level in carrier fish. Susceptible fish can acquire furunculosis (within 4 to 12 days; at water temperatures of 20°C), after viable bacteria are discharged into their water supply. Chronic infections develop at temperatures below 13°C. There is some evidence that the organism can be transmitted in seawater.

Motile Aeromonads Septicaemia (MAS)

Etiological Agents and Diseases

The causative agent of Motile *Aeromonas S*epticemia (MAS) is any of three species belonging to genus *Aeromonas*, including *A. hydrophila, A. sobria. (Ingulis)* and *A. caviae.* Characteristics of different species of motile Aeromonas are given in Table 6. These all species are usually known as motile aeromonads and the disease is also known as “tail and fin rot” due to presence of fin rots (Fig. 1), ulcer disease, haemorrhagic septicaemia and red-sore disease (Thiyagarajan et al. 2014). Ulcerations, exophthalmia, haemorrhage, superficial lesions, abscesses and lesions in internal organs (liver and kidneys) are the major signs of MAS (Garcia et al. 2007).

Table 5: Morphological and biochemical characteristics of *Aeromonas salmonicida*.

Characteristics	*Aeromonas salmonicida*
Gram-Stain	Gram negative
Growth condition	Facultative anaerobes
Morphology	Non-motile rods with rounded ends 1.3-2.0µm by 0.8-1.3µm
H_2S, Indole, Voges-Proskauer, and acid production from sucrose	-
Methy red test	+
Arginine dihydrolase, D-Glucose acid and gas, D-Mannitol acid, D-Galactose acid, L-Arabinose, Maltose acid, Trehalose acid, Lipase and brown pigment production	+

+ = Positive, - = Negative

Table 6: Morphological and biochemical characteristics of different species of motile *Aeromonas* (Woo and Bruno 2011).

Characteristics	*A.hydrophila*	*A. caviae*	*A. sobria*
Gram-Stain	-	-	-
Growth condition	Facultative anaerobes	Facultative anaerobes	Facultative anaerobes
Morphology	Rods shaped	Rod shaped	Rod shaped
Motility	+	+	+
Voges-Proskauer reaction	+	-	+
Aesculin hydrolysis	+	+	-
CAMP like factor (aerobic only)	+	-	+
Pyrazinamidase activity	+	+	-
Mannitol, sucrose fermentation and Indole production	+	+	+
H_2S Production	+	-	+
Arabinose fermentation	V	+	-
Lysine decarboxylase	+	-	+
Ampicillin susceptibility	R	R	R
Carbenicillin susceptibility	R	R	R
Cephalothin susceptibility	R	R	S
Ornithine decarboxylase	-	-	-
Lysine decarboxylase	+	-	+
Arbutin hydrolysis	+	+	-
Gas from the glucose	+	-	+

R=Resistant, S=Susceptible, V=Variable, + = Positive, - = Negative.

Fig. 1: Fin rot disease in freshwater fish caused by Motile *Aeromonads septicaemi.*

Geographical Distribution and Host Species

MAS has worldwide distribution, especially in fresh and brackish water systems. It was documented in 2009 in Alabama (Pridgeon and Klesius 2011), Arkansas and Mississippi. Most fish and many aquatic invertebrates are susceptible to MAS disease, but it can also occur in vertebrates (Dias et al. 2016).

Epizootiology

Different syndromes with which aeromonds are linked vary widely, depending on the initial stimulus. Outbreaks in salmonids usually occur by sudden increase in water temperature. The relationship of individual pathogenic strain and disease outbreak is a vital factor to evaluate the outcome.

Photobacteriosis

Etiological Agents and Diseases

Photobacteriosis or pasteurellosis affects both wild and farmed fish. Its etiological agent is *Photobacterium damselae subsp. Piscicida,* from Vibrionaceae family, which is a Gram negative strain. Infected fish show lethargy, loss of equilibrium, high ventilation rates and swim on surface and finally sink before death. Skin infections are secondary to skin abrasion.

Geographical Distribution and Host Species

Photobacteriosis has a wide range of hosts and has been documented in yellowtail (*S. quinqueradiata*) in Japan, sea bass (*D. labrax*), gilthead sea bream (*S. aurata*), and sole (*S. senegalensis* and *S. solea*) in Europe, white perch (*M. americana*), striped bass (*M. saxatilis*), and hybrid striped

bass (*M. saxatilis*) in the USA, cobia (*R. canadum*) in Taiwan and golden pompano (*T. ovatus*) in China (Wang et al. 2013).

Epizootiology

This bacterium caused mortality in smaller sized striped bass in western Long Island Sound and Chesapeake. Outbreak of the disease was observed at temperature range of 14-29°C and 3-21 salinities. *Photobacterium damselae piscicida* is an obligate infective agent and can survive outside the host.

Gram Positive Bacterial Diseases

Bacterial Kidney Disease (BKD)

Etiological Agents and Diseases

The causative agent of Bacterial Kidney Disease (BKD) is *Renibacterium salmoninarum,* and this is the only specie reported in the genus that adversely affects the sustainable production of salmonid fish. This causative agent is small in size, aerobic, non-acid fast, non-motile, Gram positive bacteria. The growth of this agent is relatively slow, with the disease chronic in nature and causes mortality in juvenile salmon and prespawning adults (Evelyn 1993). Diseased fish may show no behavioral changes or appear normal, or may exhibit loss of appetite (Pirhonen et al. 2000) and lethargy. Histologically, BKD is considered as a chronic, granulomatous inflammatory disease, characterized by macrophages proliferation in infection sites.

Geographical Distribution and Host Species

This infection is found in Europe (including Iceland), Chile, North America and Japan. All fish species, belonging to family Salmonidae, are susceptible to this pathogen (Elliott et al. 2014). It has also been documented in cultured ayu *P. altivelis* (family Plecoglossidae) (Nagai and Iida 2002), sea lampreys (*P. marinus)* (Eissa et al. 2006), Pacific herring (*Clupea pallasii)* (Evelyn 1993) and sablefish (*Anoplopoma fimbria)* (Bell et al. 1990).

Epizootiology

The transmission of *R. salmoninarum* can be horizontally and vertically, and the bacteria have been isolated from both hatchery populations and wild fish populations. BKD can occur over a broad range of temperatures. Infected fish species are main source of the disease. High mortality rate has been recorded in the infective salmonids at 4-20.5°C temperatures (Sanders et al. 1978).

Enterococcosis/Lactococcosis

Etiological Agents and Diseases

Enterococcus seriolicida/Lactococcus garvieae are Gram positive, facultative non-aerobic, non-motile and ovoid-shaped bacteria that cause Enterococcosis/ Lactococcosis. Diseased fish show loss of orientation, exophthalmia and lethargy. Histologically, pathogen proliferation and necrosis occur in the affected organs.

Geographical Distribution and Host Species

is the causative organism generally survives in seawater containing high organic matter throughout the year. This disease agent is present in both fresh and marine water and becomes a major disease agent in trout of Portugal, Spain, Turkey, Italy, France, Greece and Israel, where it causes serious production losses.

Epizootiology

Microorganisms eliminated from diseased fish and contaminated diet are sources of horizontal transmission of disease from fish to fish. Moreover, organisms that survive epizootics may also be source of infection.

Streptococcosis

Etiological Agents and Diseases

Streptococcosis is primarily caused by the Gram-positive bacteria, *Streptococcus agalactiae, Streptococcus iniae, Streptococcus dysgalactiae,* and *Streptococcus ictaluri*. The characteristics of *Streptococcus* spp. are given in Table 7. *Streptococcus iniae* emerged as a major pathogen of farmed and wild fish in the 1990-2000's and has zoonotic potential. *S. ictaluri* emerged in the catfish industry in the late 2000's in USA, is phylogenetically most similar to *S. iniae* and seems unique with low virulence to channel catfish (Pasnik et al. 2009). Some of the first behavioral changes associated with the disease are lethargy and loss of appetite. Externally, fish often exhibit a darkening of the skin in color; however, acutely infected fish may die due to septicemia with few clinical signs. Dead fish, as well as survivors of recent infections, may have jaw and caudal pustules (LaFrentz et al. 2016; Shoemaker et al. 2017).

Geographical Distribution and Host Species

It is generally assumed that Streptococcosis has a worldwide distribution, having been described in fishfrom Europe, the Americas, Middle East, throughout Asia and Australia (Shoemaker et al. 2017). According to Osman et al. (2017), Streptococcosis has been reported globally in wild and cultured fish.

Epizootiology

The epizootiology of this disease is very complex. Both external environmental conditions and fish stress enhance the chance and severity of Streptococcosis (Xu et al. 2007). The horizontal mode of transmission via water with fish (i.e., carriers) is most likely considered as a source of

Table 7: Morphological and biochemical characteristics of *Streptococcus spp.*

Characteristics	Description
Gram-Stain	Gram positive
Growth condition	Facultative anaerobes
Morphology	Non-motile, spherical and ovoid in shape, mostly occurs in long chain
Voges-Proskauer,	-
Acid production from	
sorbitol	-
sucrose	+
Starch hydrolysis	+
Catalse reaction	+

+ = Positive, - = Negative.

Table 8: Morphological and biochemical characteristics of different *Nocardia spp.* (Woo and Bruno 2011).

Characteristics	*N. asteroides*	*N. crassostreae*	*N. seriolae*	*N. salmonicida*
Gram-Stain	Gram positive	Gram positive	Gram positive	Gram positive
Growth condition	Aerobic	Aerobic	Aerobic	Aerobic
Morphology	Irregular shape, Pleomorphic cell	Irregular shape, Pleomorphic cell	Irregular shape, Pleomorphic cell	Irregular shape, Pleomorphic cell
Colony colour	Beige	Pale yellow	Pale orange	Orange
Utilization of				
sorbitol	-	ND	-	+
Citrate	+	ND	+	+
Acetate	+	ND	+	+
Rhamnose	-	ND	-	-
Adipic acid	+	ND	ND	ND
Decomposition of				
Adenine, Elastin	-	ND	-	-
Xanthine, Casein	-	-	-	-
Urease, Tyrosine	-	-	-	+
Aerial hyphae	+	-	-	+

ND=Not determined, + = Positive, - = Negative

bacterial infection. The bacteria may survive for extended periods in water and sediment (Nguyen et al. 2002). Vertical transmission of both *S. iniae* and *S. agalactiae* (Pradeep et al. 2016) has been reported in tilapia.

Weissellosis

Etiological Agents and Diseases

Weissellosis is caused by *Weissella ceti,* a Gram-positive, non-endospore forming bacterium.

Geographical Distribution and Host Species

In 2007, *Weissella* spp. was first reported in rainbow trout (*O. mykiss)* in China and has been isolated as the infective agent at trout farms in both the southeastern United States (Welch and Good 2013) and Brazil (Figueiredo et al. 2012). The Weissellosis is rapidly emerging pathogen of farmed rainbow trout. The information about susceptibility of other fish species to this organism remains unknown.

Epizootiology

For Weissellosis outbreaks, high temperature is the main predisposing factor. It has been recorded in the north side of Carolina, where water temperatures vary through all seasons. The occurrence of seasonal outbreaks of this infection in North Carolina suggested that *W. ceti* has the potency to retain in sites where it has appeared, and could cause an endemic disease. In a production system, it particularly affects the larger fish (0.25-1 kg) compared to smaller fish (Welch and Good 2013). The Weissellosis quickly propagates through some undiscovered mechanisms. The main route of disease transmission remains undiscovered but is the main subject of ongoing investigations.

Mycobacteriosis

Etiological Agents and Diseases

It is a chronic to subacute but severe disease of many fish species, caused by *Mycobacterium* spp. Including *Mycobacterium marinum, Mycobacterium fortuitum and Mycobacterium cheloni.* Bacterial agents are Gram-positives, pleomorphic, acid-fast, aerobic and non-motile rods (Hashish et al. 2018). Emaciation, exophthalmia, lordosis, severe haemorrhages, lethargic behaviour and dermal lesions or loss of scales are major signs at the advanced stage.

Geographical Distribution and Host Species

This infection continues to be documented worldwide in fish populations. Wild fish, including *M. saxatilis* (Aronson 1926), *G. morhua* (Alexander 1913), *H. hippoglossus* (Sutherland 1922), *C. striatus* and *O. bonariensis,* have been reported to show Mycobacteriosis (Hatai et al. 1993).

Epizootiology

The mode of transmission of *M. marinum* between different fish species is not completely understood. The main transmission route of this disease is the oral one through ingestion of diseased dead fish or interaction with diseased fish (El Amrani et al. 2010).

Nocardiosis

Etiological Agents and Diseases

Nocardia spp. cause Nocardiosis, which is a lethal granulomatous infection of the muscles, skin and different internal tissues. Four *Nocardia* spp. have been reported that are *Nocardia asteroides, Nocardia salmonicida, Nocardia seriolae* and *Nocardia crassostreae;* their characteristics are given in Table 8. In aquaculture, this infection has caused intense economic losses, particularly in the Asia (Maekawa et al. 2018). Nodules in body organs, with or without multiple skin ulcers, lethargy and anorexia, opacity of cornea and lenticels, intraocular and periocular hemorrhage are common signs of Nocardiosis..

Geographical Distribution and Host Species

Organisms of Nocardioform caused epizootic ulcerative syndrome in freshwater fish. *N. seriolae* has been reported to cause infection in Japanese sea bass (*L. japonicus*) and yellow croaker (*L. crocea*) in China and Taiwan, causing more than 15% mortality in each species (Wang et al. 2005).

Epizootiology

The exact route of transmission for Nocardiosis is unknown. Mostly it occurs through oral cavity, but this is not primary route. The transmission of infection through contaminated feed has also been reported.

Intracellular bacterial Diseases

Piscirickettsiosis

Etiological Agents and Diseases

Piscirickettsia salmonis is a non-motile, Gram negative bacterium, belonging to the family Piscirickettsiaceae (Boone and Castenholz 2001). It causes Piscirickettsiosis, Huito disease, coho salmon septicemia and salmonid rickettsial septicemia. As a fish pathogen, it was the first rickettsia-like pathogenic bacteria to be noted. The small lesions, swollen organs and haemorrhagic ulcers appear on the skin after infection. Affected fish appear dark and lethargic.

Geographical Distribution and Host Species

This etiological agent has been isolated from Salmonids of Ireland, Chile, Norway and both the east and west coasts of Canada (Fryer et al. 1992; Brocklebank et al. 1992; Cusack et al. 2002). Other fish species may be susceptible to this organism.

Epizootiology

Piscirickettsiosis was first reported in 1989 from Salmonids of Chile. The transmission mechanism of this disease is not completely understood but horizontal transmission in both sea and freshwater has been reported (Smith et al. 2004). Vertical transmission can take place in freshwater (Larenas et al. 2003), but this route is not frequently seen in *P. salmonis*. Different strains show totally different levels of virulence.

Epitheliocystis (EP)

Etiological Agents and Diseases

Epitheliocystis (EP) is an intracellular gills and skin bacterial infection that results in hypertrophy of the cells of host (Nowak and LaPatra 2006). The causative agents are obligate intracellular, Gram negative bacteria, mostly from phylum *Chlamydiae* and are also considered as γ- and β-proteobacteria (Kurahashi and Yokota 2007; Katharios et al. 2015; Seth-Smith et al. 2016). *Candidatus Clavichlamydia salmonicola* (Karlsen et al. 2008) and *Candidatus Piscichlamydia salmonis* (Draghi et al. 2004) were isolated from Salmonids, but not from other species of fish. All causative agents of Epitheliocystis are host-specific. Infected fish show white nodular lesions in the gills and skin and disturbed or imbalance gas exchange, ammonia excretion and salt reduction. The clinical signs are loss of appetite, increased mucous production, fish swimming at the surface of water, abnormal swimming pattern and lethargy.

Geographical Distribution and Host Species

Epitheliocystis was initially reported by Hoffman et al. (1969) in bluegill sunfish (*L. macrochirus*). This disease has been recorded in about 90 fish species from 14 countries (Nylund et al. 1998); the infected fish species include: brown trout (*S. trutta*) (Guevara et al. 2016); catadromous fish such as barramundi (*L.calcarifer*); marine fish species e.g. broad-nosed pipefish (*S. typhle*) (Fehr et al. 2013) or sharpsnout sea bream (*D. puntazzo*) (Katharios et al. 2008); and freshwater fish species like silver perch (*B. bidyanus*) (Frances et al. 1997), white sturgeon (*A. transmontanus*) (Groff et al. 1996), carps (family Cyprinidae) (Nowak and LaPatra 2006) and striped catfish (*P. hypophthalmus*) (Sood et al. 2017). The Chondrichthyes species like leopard shark (*T. semifasciata*) can also be infected by this pathogen (Polkinghorne et al. 2010).

Epizootiology

Infection carrying fish are considered to be the main source for horizontal transmission. Wild Salmonids may be the source of the disease in farmed Salmonids, with

translocation or stocking actions of fish may be contributing to transmission (Guevara et al. 2016). From eggs to fingerlings, vertical transmission has been supposed to occur in barramundi. The amoebae have been considered as a vector in the past (Corsaro and Greub 2006). Although other species of chlamydiae grow in amoebae, so far no infection agents of this disease have been able to be cultured in amoebae.

Francisellosis

Etiological Agents and Diseases

Francisellosis, caused by the *Francisella* species, has been documented recently; severe granulomatous disease has also been reported to becaused by *Francisella* spp. *F. noatuensis, F. piscicida* and *F. victoria. Francisella* spp. are non motile, Gram negative, facultative intracellular bacterial pathogen and pleomorphic coccobacilli, having 0.5-1.5 µm diameter. Phylogenetic data have classified this organism to the genus Francisella and also classified it in the γ sub-division of the proteobacteria. Infected fish can show different clinical signs, such as exophthalmia, loss of appetite, lethargy, petechia, abnormal behavior of swimming, dark colored body and haemorrhagic nodules on the skin.

Geographical Distribution and Host Species

In tilapia fish, this disease has been reported from continental US, Latin America, Taiwan and Hawaii, three lined grunt of Japan, cod in Norway, Atlantic salmon of Chile (Woo and Bruno 2011). Francisella species have global distribution and broad range of host species.

Epizootiology

The overall life cycle of *Francisella* spp. is not fully known, but is likely to be similar to Francisella species of mammals. The bacterial agent is present probably in the water column and mechanism of transmission described in tilapia is horizontal. Both fresh and marine fish show the infection, but bacterium strains isolated from these different geographic sites and hosts may vary. The vectors role or vertical transmission of infection is still unknown.

Prevention and Control of Bacterial Diseases

Disease prevention is always preferred and more profitable than disease treatment. Several techniques have been used to prevent and control fish infections.

Conventional Preventive and Control Measures

Standard Hygienic Measure

To overcome disease outbreak, fish farms principally rely on preventative measures by allowing the entry of pathogenic free broodstocks, screening and sterilization of stock and culling of infected populations (Elliott et al. 1989). Many infections like BKD, furunculosis and ERM do not always show themselves in clinical form and transfer the infective agent with the movements of fish. So, it is compulsory to apply transportation restrictions. By quarantine measures and improvement of water quality and feed, the disease can be controlled because dietary supplements like vitamin A, E and C enhance immunity against *A. hydrohila* (Sobhana et al. 2002). It is essential to ensure that no infectious agents can enter the fish farm from any equipment, vehicles, visitors and staff. Better management of hatchery is the foremost strategy to prevent Aeromonads spp. In hatcheries that reuse water, both ozonation and filtration conducted with UV irradiation (Colberg and Lingg 1978) can destroy the *A. hydrophila*.

Disinfectants

Disinfection of fish farms in confined place can be done easily (Toranzo et al. 2005), otherwise it is challenging to prevent and control disease distribution (FAO 2016). Various chemicals are used according to the type of organisms, their life cycle stage, the culture method and intensity of culture (Gomez-Gil et al. 2000). For disinfectation, 5% phenol, $KMNO_4$ (5 mg/l), iodine solutions, 1% sodium hypochlorite, formaldehyde and glutaraldehyde are advised. Moreover, malachite green and $CuSO_4$ are also used but in aquaculture, overdoses of all chemicals may lead to serious toxicity in fish (Bornø and Colquhoun 2009).

Antibiotics

Various antibiotics have been used in aquaculture, but it is not suggested to entirely depend on them because their use has many disadvantages, such as the short period of protection, cost of antibiotics, the necessity for continual treatments during the disease outbreak, the trouble caused by production of resistant strains and increased noxious residual material in carcasses (Miranda and Zemelman 2002). Oxytetracycline, sulfadimethoxine, tetramycine and tetracycline are among the most commonly used antibiotics in fish production. Previous studies have shown that Edwardsiella spp. are susceptible to different antibiotics, such as cephalosporins, aminoglycosides, quinolones, sulphamethoxazole, penicillins, aztreonam, ciprofloxacin, antibiotic beta lactamase inhibitor agents and nitrofurantoin (Jnaneshwara et al. 2016). Chloramphenicol, derivatives of nalidixic acid, ampicillin, sulfonamides and nitrofuran derivatives are usually used to control vibriosis (Aoki et al. 1984). The use of various antibiotics enhanced the rate of antimicrobial resistant strains, as well as presence of residual substances of drugs in the food, which are the main issues that have prompted scientists to investigate for other effective and safe techniques (Pal 2015). Therefore, aquaculturists are focusing on searching alternative techniques to control pathogens (Taoka et al. 2006).

Vaccination

Vaccines are killed bacteria or bacterins acquired from a particular strain of bacteria subjected to formalin deactivation. Vaccinated fish can be a carrier of the disease because the bacteria cannot be eliminated totally from fish body. Conventionally, vaccines are administered mainly by injections, that causes stress to the fish and also stimulates the humoral immune responses, and provides security against the disease for short periods. Thus, the aquaculturists are searching for other eco-friendly and effective methods for treatment and protection of fish against pathogens (Dahiya et al. 2010).

Recent Prevention and Control Measures

Probiotics

Probiotics are characterized as dead or live micro-organisms, or a constituent of the beneficial micro-organism that works through variable modes of action, conferring advantageous effects to the host or its environment. Various probiotics have been used to control fish diseases. Their selection depends mainly on detection of power to kill the pathogens by using tests (agar well diffusion) to analyze the released inhibitory matter. Probiotics are mainly used in dry form as food supplements, or by adding into the drinking water. The liquid form is fast-acting and shows its effects earlier than those of the dry form (Nageswara and Babu 2006). It has been reported that the use of probiotics (*Bacillus subtilis* and *Bacillus licheniform)* defends rainbow trout against infections of *Y. ruckeri* (Raida et al. 2003).

Antagonist of Quorum Sensing (QS)

Quorum sensing (QS) is gene expression regulation as a result of connection between infective cells. Many species of bacteria are using this mechanism to modulate their action. Use of probioticsinduces disturbances of QS, which has used a possible anti-infective strategy in aquaculture and fisheries (Defoirdt et al. 2004). The halogenated furanones can defend rainbow trout from the diseases caused by organisms of genus *Vibrio* (Tinh et al. 2007).

Immunomodulation

Probiotics are also known as powerful immunostimulants that regulate the immunity of the host against diseases by increasing the rate of phagocytosis and leucocytes counts. Probiotics also enhance the antimicrobial peptides formation (Mohapatra et al. 2012).

Improving Water Quality (Bioremediation)

Water quality can be improved through bioremediation or by adding Gram-positive bacteria, because they convert organic matter into CO_2 (carbon dioxide), while the Gram-negatives convert organic matter directly into biomass of bacteria and infections (Balcazar et al. 2006). Nitrite and ammonia toxicity in culture systems can be destroyed by using nitrifying bacteria in culture systems. Maintenance of pathogen-free good water quality,minimizing organic materials and stress by avoiding overcrowding and low dissolved oxygen, and effective cleanliness of fish production tanks by instantly removal of dead fish prevents bacterial cold water disease.

Ozone Nanobubble Treatment

Pathogenic bacteria in water usually enhance bacterial disease outbreaks in cultured fish. Ozone gas nanobubble (NB-O_3) technology is not only a beneficial disinfection method, but also supplies dissolved oxygen (DO) in freshwater aquaculture, and it is not harmful to the fish in low dosage. Jhunkeaw et al. (2021) reported that upon NB-O_3 treatment, the number of bacterial colonies reduced rapidly during 10 min following three times continuous exposure in the treated tanks. Before the treatment, the total bacterial density in the fish-cultured water was $6.93 \times 10^5 \pm 7.81 \times 10^5$ CFU/mL and 42.94% of the bacteria were inactivated after the NB-O_3 exposure for 10 min. When the same method was followed, 84.94 to 99.27% reduction in bacterial loads was observed in treated tanks (Fig. 2).

Prebiotics

Prebiotics are originated from cell wall components of yeast and are non-digestible. They limit the presence of bacterial pathogens in fish farms and improve the intestinal health-boosting bacteria (lactobacillus). Rodrigues-Estrada et al. (2008) reported that diet supplementation with prebiotics improved growth, phagocytic and hemolytic activity, survival of fish in a document with *V. anguillarum*. Rainbow trout (*O. mykiss*) showed better growth rate, antibodies, and activity of lysozyme after diet supplemented with prebiotics (Staykov et al. 2007). Sink et al. 2007 stated golden shiners fed the diets having prebiotic (dairy-yeast) showed lower infection associated with *F. columnare*.

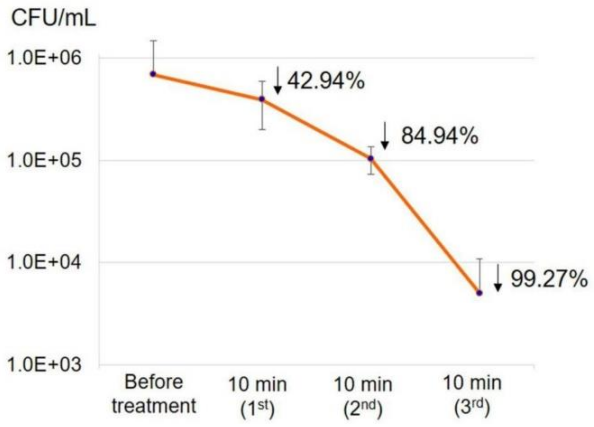

Fig. 2: Total bacterial colony counts from fish-cultured water upon exposure to NB-O_3, for 10 min, three times continuously. Arrows indicate % reduction of bacterial loads compared to the starting bacterial concentration. Bars = standard deviation (SD) from 3 replicates (Jhunkeaw et al. 2021).

Synbiotic

Synbiotic is an assemblage of both probiotics and prebiotics, which increases the survival rate and spreading of live microbiota in the gastro-intestinal tract. The use of *E. faecalis* in fish feed offers a variety of advantages regarding the improvement in immunity and fish survival with *V. anguillarum*. The synbiotic feeding results in significantly good consequences than individual application of prebiotic and probiotic (Gatlin and Peredo 2012).

Bio Vaccines

Living Attenuated Vaccines

The live attenuated vaccine application in aquaculture was started in 1990 (Sun et al. 2010). These vaccines are live and attenuated organisms and establish less infection, resulting in the stimulation of humoral and mucosal immunity. Random and direct strategies can be used to induce certain mutations into bacterial pathogens to achieve better attenuation. Sometime, mutations are reversible, particularly when bacterial pathogens become mutated by passages or by chemicals. This condition poses a serious risk for both environment and the host. To reduce such problems, bacteria should be mutated after genes inactivation involved in biochemical pathways. Examples of targeted metabolic ways to produce attenuated vaccines include: purine biosynthesis, aromatic amino acid biosynthesis, galactose epimerase, adenylate cyclase and capsule biosynthesis. These mutant bacteria were unable to proliferate and increase their abundance to induce infection; they cannot live long enough to cause infections (Roberts et al. 1990). Original live attenuated vaccines have been prepared against *L. anguillarum* and *Y. ruckeri* pathogens by deletion of their *aro*A and *aro*C genes. Vaccinated flounders (*P. olivaceus*) with 107 CFU/ml attenuated strain (ΔaroAΔesrB) showed 100% RPS against 107 CFU/ml *E. tarda* (Li et al. 2015). Mohd-Aris et al. (2019) successfully formulated mutant *V. harveyi* by deletion of protease, as a candidate live-attenuated vaccine against vibriosis in *E. fuscoguttatus*.

Live Feeds Bio-Encapsulated Oral Vaccine

The encapsulation is practically used either to stop the escaping of antigen from the pelleted feed, or for its protection from the acidic medium of the fish stomach. This type of feed releases the immunizing agent into the fish digestive tract, which seems to be the most fascinating method for vaccines treatment. It further lessens the fish handling, that ultimately reduces the stress of fish and is most appropriate for mass immunization against vibriosis (Rombout 1989).

Nano-Bio-Encapsulated Vaccine

The application of nanoparticles as adjuvant and economic delivery systems in vaccine development of fish is increasing due to their nano size. The benefits of nano-vaccines include; site specific delivery of antigens, antigens protection from degradation, enhanced bio-availability and reduced side effects (Zolnik et al. 2010).

Bacteriophages Therapy

The most significant biological control method for bacterial pathogens in fisheries and aqua-farming is through bacteriophages, which are nontoxic for animals and humans and can be used safely as therapeutic agents. In different fields of medical sciences and biotechnology, phages have been applied for prevention of bacterial infection, management, rapid detection and biocontrol of diseases (Haq et al. 2012). Additionally, bacteriophages are more specific and can only infect cells of bacteria that have specific receptors sites (Kutter et al. 2004). Hsu et al. (2000) and Castillo et al. (2011) utilized phages in water of fish pond against *A. hydrophila* and *F. Pshychrophilum,* respectively. Imbeault et al. (2006) suggested that bacteriophage treatments should have multiple phages against *A. salmonicida* to control target pathogens, as well as resistant ones. Proper phage doses and phage characters are essential for efficient therapy.

Conclusion

This chapter summarizes the knowledge about the characteristics, geographical distribution, host range, and epizootiology of common fish pathogenic bacteria, but new etiological agents are being identified every year. The higher disease outbreaks occurred in both larval and juvenile stages of the fish. The most common fish bacterial species that can cause diseases belong to the genera *Vibrio, Flavobacterium, Aeromonas, Yersinia, Edwardsiella, lactococcus, Streptococcus, Renibacterium and Mycobacterium*. There are flourishing indications that different infective bacterial species have broad geographic distribution and host range, causing the emergence of new bacterial pathogens. At last, the conventional and modern disease prevention methods and their control strategies are also addressed. Hence, basic knowledge of pathogen profiles and diseases, in addition to their fundamental economic background of the operational costs, is a primary requisite in the designing of strategies to control most common bacterial diseases. It is strongly recommended that all the possible limitations in control methods must be addressed critically before employing in the aquaculture sectors. Comparative pathogenomics provide important information that how similar bacterial species show different virulence, adapted to various ecological niches and new host species. The determination of main virulence factors in disease-causing strains can assist us to plan effective therapeutic and vaccines strategies to control fish diseases.

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Section C

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VIRAL DISEASES OF ZOONOTIC IMPORTANCE OF DOMESTIC RUMINANTS

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INTRODUCTION

Rudolf Virchow, one of the 19th-century German leading scientists in medicine and pathology, noted a relationship between human and animal diseases and introduced the term "zoonosis" in 1880 (Walter and MikeScott 2017). Later, the World Health Organization (WHO) in 1959 defined zoonoses as the diseases and infections that are naturally transmitted between vertebrate animals and man (Al-Tayib 2019). Zoonotic agents can cause different kinds of diseases, causing major public health issues worldwide (Ermias et al. 2017).

Zoonoses are a group of infectious diseases that are transmitted from animals to humans (zooanthroponoses) or from humans to animals (anthropozoonoses). Ruminant animals such as cattle, buffaloes, sheep and goats are considered as an important source of infections for humans due to their close contact and also due to their importance to the human society (Ganter 2015). Zoonotic diseases can adversely affect public health through reduction in livestock productivity. Zoonotic diseases can be transmitted naturally from animals to humans with or without arthropod intermediates (Al-Salihi 2018). Such diseases include multiple infections like viral, bacterial, fungal, protozoal and parasitic diseases (Al-Tayib 2019). Viral zoonotic pathogens are important in emerging and reemerging virus diseases (McDaniel et al. 2014), and are found in all continents except Antarctica. Some of them are found all over the world, while others in limited ecological areas. There are numerous viral zoonotic species which are considered as important livestock pathogens (Reed 2018).

Viruses are a group of pathogens that are unable to reproduce outside the host cell and always need the host DNA to complete their life cycle, so they are considered as obligate intracellular organisms. A virus consists of DNA or RNA segment and is encircled by a protein coat with or without viral membrane. Virulence factors are essential for viral replication and also for protection against the host immune system. Viruses are excreted by clinically or sub-clinically infected hosts into the environment, where they remain in a dormant state. Survival of viruses in the environment is an important factor concerning their virulence and pathogenicity. Also, their ability to induce genetic changes enables them to have a wide range of hosts. Transmission of viral zoonotic diseases from animals to humans occurs in many ways, such as direct contact (e.g. milker's nodule), airborne transmission (respiratory viruses e.g. H1N1) and mosquito-borne like Rift Valley fever RVF) virus (Garvey 2018).

Rift Valley Fever

Rift Valley fever (RVF) is an acute febrile zoonotic infection that affects sheep, goats, buffaloes, cattle and humans. Its causative agent is an RNA virus, Phlebovirus (family Bunyaviridae). The first record of this disease was noted in 1930, when animal and human affections appeared in a farm in Naivasha Lake, in the Rift Valley after heavy rainfall due to increased number of mosquitos. Many outbreaks of Rift Valley fever were recorded in 1900s. Five large outbreaks of the disease were recorded in Egypt during the last 40 years; in 1977–1978, 1993–1994, 1997, 2000 and 2003, with the largest epizootic was in 1977–1978. The most severe outbreaks were in 1975 in South Africa and during 1977 in Egypt (Helmy et al. 2017). Currently, the disease has economic importance due to the cost of preventive measures, monitoring for introducing the disease to disease-free areas, and restrictions on the import and export of animals (Lichoti et al. 2014).

Epidemiology

Transmission and environmental risk factors

The Rift Valley Fever Virus (RVFV) is a mosquito-borne virus. Once cattle and sheep get infected, they become highly viraemic, allowing efficient viral transmission. Trans-ovarian transmission of Rift Valley fever virus is another feature. Infected eggs of mosquitos can survive for many years in the soil. Under suitable environmental conditions, such as heavy rainfall that creates stagnant water, mosquito eggs will hatch and result in the production of newly infected mosquitos. In the East and South Africa, the main vector for RVF is *Aedes* mosquito. RVFV can withstand environmental temperatures up to 25-30°C for about 80 minutes. Humans can get infected by contact with infected animals and animal products in the abattoir, and the areas of food preparation in endemic areas, but less frequently as a result of a mosquito bite. Human to human transmission has not been recorded (Nakoune et al. 2016).

Geographic distribution

RVFV affects ruminants in Africa, but it can spread to areas other than Africa and can be a worldwide threat for

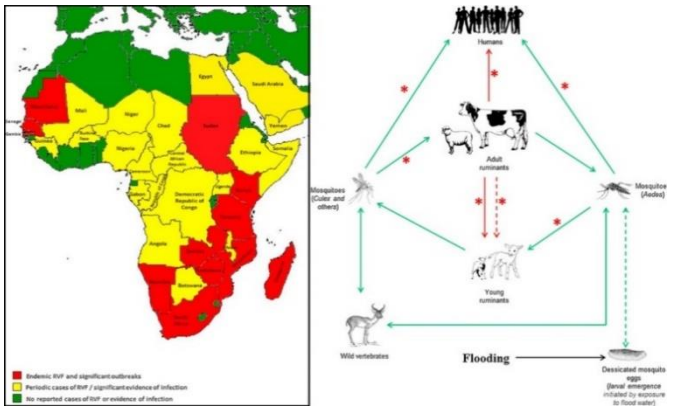

Fig. 1: Geographic distribution of RFV (http://www.cdc.gov/vhf/rvf/distribution-map.html) (left). Diagram of the transmission cycle of RVFV (https://www.pasteur.fr/en/research/virology/units-groups/arboviruses-and-insect-vectors/research) (right).

humans and animals (Fig. 1). The spread of the virus to the Arabian Peninsula in 2000 and Madagascar and Comoros in 2007–2008 shows the possibility for its spreading to many areas. RVF has been reported in four ecologic systems: irrigated areas, Dambo areas, semi-arid areas and temperate and mountainous areas (Kasye et al. 2016).

Host range and susceptibility

RVF virus was reported in antelopes, buffaloes, cattle, goats, camels, monkeys, rodents and sheep, in addition to human. Mortality and morbidity have been recorded in sheep, cattle and humans. Young animals and humans are highly susceptible to RVF infection. However, reptiles and Amphibians are resistant to this virus (Shayif et al. 2018).

Morbidity and mortality

Young animals and humans are highly susceptible to RVFV with a mortality of 70-100%. Adult animals and humans are moderately susceptible with a mortality rate of <10%; case fatality rate in humans is <1%. Equines, dogs, pigs and cats are considered to be resistant (Métrasa et al. 2020).

Source of infection

Tissues of viremic animal, blood, aborted fetus and fomites are considered to be the main sources of RVF virus infection (Petrova et al. 2020).

Pathogenesis

RVFV replicates in mosquitos, invertebrate animals and humans. After an insect bite, RVFV is transported by lymphatics from inoculation site to regional lymph node, where it replicates and is conveyed to the circulation with initiation of viremia and systemic response. Liver, brain and spleen are the main target sites for viral replication. Virus is resistant to alkaline environment and is inactivated by disinfectants such as sodium hypochlorite, acetic acid and calcium hypochlorite. It can be for eight years when stored below 0°C (Javelle et al. 2020).

Clinical Signs in Cattle

Incubation period of RVFV is about 24-72 hours in adult cattle. Calves become feverish (40-41°C), off-feed, weak, depressed, diarrheic with jaundice. Adults often show unapparent infection; although fever lasting for 24 to 96 hours, nasal discharge, lacrimation, dull coat, excessive salivation, weakness, anorexia, low milk production, bloody diarrhea, and abortion in pregnant cows are common signs (Kasye et al. 2016).

Clinical Signs in Small Ruminants

Among small ruminants, the incubation period of RVF virus is 12 to 72 hours in newborn lambs and 24 to 72 hours in adult sheep and goats. Newborn lambs of <2 weeks age show biphasic fever (about 40-41°C), weakness, anorexia, rapid respiration and abdominal pain, with death occurs within 24-36 hours. Lambs aged more than 2 weeks, as well as adult sheep and goats, show fever for 24 to 96 hours, depression, increased respiratory rate, weakness, anorexia, bloody diarrhea, vomiting, mucopurulent nasal discharge, jaundice and abortion in almost 100% cases (Kasye et al. 2016).

Clinical Signs in Humans

Incubation period of RVF virus is 3 to 6 days in humans. RVF in humans is mostly asymptomatic. Sometimes they develop a self-limiting fever with flu-like signs generally characterized by fever, headache, muscular pain, nausea and photophobia. Recovery may occur within 4 to 7 days. Some patients may develop a hemorrhagic fever and jaundice with case fatality about 0.5–2.0%. Neurological disorders and blindness are rare complications (Javelle et al. 2020).

Pathologic Lesions

Primary lesion in RVF infection is liver necrosis. In aborted fetus and neonates, the liver is soft, friable, enlarged and dark in color. Enlarged and edematous peripheral and visceral lymph nodes, hemorrhages and edema in gall bladder, hemorrhagic enteritis, cutaneous hemorrhages, blood-stained fluid accumulated in body cavities with subcutaneous and serosal hemorrhages are also observed (Kasye et al. 2016).

Clinical Diagnosis

Affected animals show fever, depression, mucopurulent nasal discharge, anorexia, vomiting, jaundice, weakness and bloody diarrhea. Clinical pathology reveals severe leucopenia, high serum levels of glutamic dehydrogenase (GLDH) due to liver damage and also thrombocytopenia (Petrova et al. 2020).

Laboratory Diagnosis

Laboratory diagnosis of RVF depends on serology by detecting the antibodies, either IgM or by detecting the

rising titer of IgG. In encephalitis, cerebrospinal fluid can be evaluated for IgM. RVFV is isolated from blood in the acute phase. PCR is also used either in serum or tissue (Petrova et al. 2020).

Treatment

For mild and moderate cases of RVF, analgesics and fluid therapy can be administered; for severe cases as in encephalitis and hemorrhage, critical care like ventilation and blood transfusion is necessary (Kasye et al. 2016).

Prevention and Control

Two vaccines for RVF are available and are commonly used in endemic areas; one is a live attenuated Smith burn vaccine and the other is a formalin-inactivated vaccine. Mosquitoes are the most important vectors for RVF virus. Only female mosquitos feed on blood and lay their eggs near water. The eggs hatch into larvae, which are transformed into pupae. The larvae and the pupae need water to survive. Adult female mosquitoes lay eggs in specific areas (near water sources), so they can be easily managed. Mosquito larvae remain in the same areas, so control of these stages depends on continued management of these areas. Adult mosquitoes can be controlled by the use of insecticides. But it is difficult and expensive way. Also, sterilization of male mosquitoes by radiation may be effective. Ban on the movement of animals from the suspected areas and confinement of animals in covered areas are advised as a preventive measure. Attempts may also be made to prevent the lambing and calving during the rainy season. Use of protective clothes and insect repellents and prevention of outdoor activities during mosquito biting season are essential protective measures. Quarantine practice on entering or leaving the suspected areas is also recommended (Boshra et al. 2011).

Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF) is a fatal tick-borne disease; it is a widespread viral infection. The CCHF virus is maintained in ticks via horizontal and vertical transmission and transmitted to domestic animals, which can convey the disease to humans. In 1944, CCHF disease was first recorded in Crimea. Then in 1969, it was reported from the Congo region, so the disease was named as "Crimean-Congo hemorrhagic fever". The CCHF virus belongs to genus Nairovirus and family Bunyaviridae. It is a single-stranded RNA virus and exists in seven genotypes, namely Africa-1, Africa-2, Africa-3, Europe-1, Europe-2, Asia-1 and Asia-2 (Mostafavia et al. 2017).

Epidemiology

Transmission and environmental risk factors

Person to person transmission of CCHF virus occurs via direct contact with body fluids of infected persons and also via contact with the animal blood and animal products. Nosocomial infection among health care workers, especially during hemorrhagic phase, is common. Hyalomma tick is the vector of the virus. Adult virus obtains its nutrition from cattle, sheep and other large mammals. Migration of the infected livestock from infected area to free area is another factor. Trans-ovarian transmission is essential to maintain a large number of infected ticks. Spread of the virus between ticks and animals is high during summer and spring seasons, when ticks larvae and nymphs develop into adults. Biting results in the transmission of virus from the tick to the host. Human is a dead-end host for the virus. Droplet infection is also considered one of the methods of disease transmission (Aslam et al. 2016).

Geographic distribution

The CCHF virus is characterized by widespread distribution (Fig. 2) and it must be considered as global health threat. The disease is distributed in different areas, including Africa, Asia, central and southern Europe, Eastern Europe, throughout the Mediterranean, north-western China, the Middle East and the Indian subcontinent. Since 2002, the virus has also been found in many countries of the Balkans. From 2000-2008, the disease re-emerged in Albania, Bulgaria, Turkey and Kosovo. It has also been recorded in Greece (Zavitsanou et al. 2009).

Host range and susceptibility

Infected humans can spread the infection through close contacts. Domestic animals including cattle, sheep, goats, horses, ostriches, pigs, donkeys, camels, mice and domestic dogs are considered as the main hosts for the CCHF virus and can transmit it to humans during viraemic phase. Slaughtering and animal handling can facilitate the transmission of the infection. Also, healthcare workers are at risk. Rural area is a risky place for exposure to ticks. Risk is also high among people in urban areas such as animal trading markets (Munibullah et al. 2018).

Morbidity and mortality

The CCHF is highly pathogenic, with a high case fatality rate (10-40%). The prevalence of CCHF in people with history of tick bite can reach 20% in endemic areas (Shayan et al. 2015).

Source of infection

The CCHF disease virus is mainly in hemorrhages from mouth, gums, nose, rectum, urinary bladder, urethra and vagina (Parmar et al. 2017).

Pathogenesis

Hepatocytes and Kupffer cells are major target cells for CCHF virus. Hepatocellular necrosis leads to elevated liver enzymes. Also, elevated myeloperoxidase expression

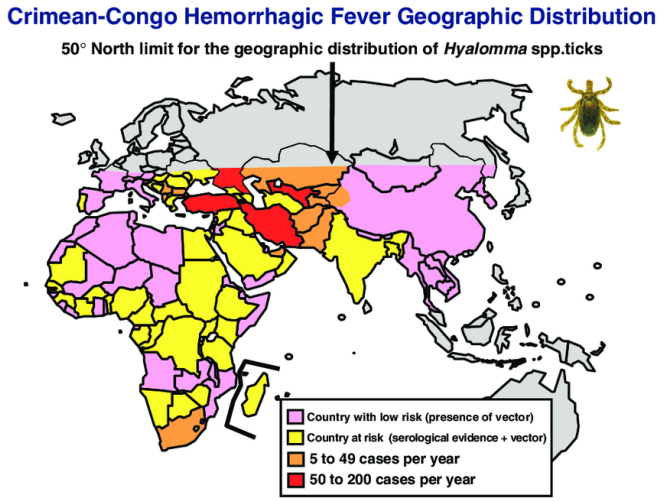

Fig. 2: Geographic distribution of CCHF (https://www.researchgate.net/publication/226967229).

in leukocytes leads to increased leukocytic lysis, leading to leukopenia. Endothelial damage activates the coagulation cascade, resulting in decreased platelets count. Endothelial damage also leads to hemostatic failure and skin rashes. Activation of coagulation may have a role in the development of disseminated intravascular coagulation. Infection by virus or damage by secreted cytokines leads to bleeding (Aslam et al. 2016).

Clinical Signs in Cattle

Incubation period of CCHF virus is 3-7 days. There are four stages of the infection, incubation period (asymptomatic phase), pre-hemorrhagic, hemorrhagic and convalescent (symptomatic phase). Pre-hemorrhagic phase extends from 4-5 days, and is characterized by fever, abdominal pain, headache, hypotension, myalgia, and flushed face. As disease progresses, petechial and ecchymotic hemorrhages, epistaxis, gum bleeding and hematemesis start. Nausea, diarrhea, neuropsychiatric and cardiovascular dysfunction may appear. The convalescent phase starts in survivors about 10-20 days after the disease. Recovery can take around one year period in survivor animals (Zavitsanou et al. 2009).

Clinical Signs in Humans

In human, incubation period of CCHF virus depends on the mode of infection. After insect bite, incubation period is about 1-3 days, with maximum of 9 days. Following contact with infected blood or tissue, the incubation period is usually about 5-6 days, with maximum of 13 days. The pre-hemorrhagic period starts with abrupt fever, dizziness, abdominal and back trouble and harsh head pain. Other signs include vomiting, nausea, diarrhea and cardiovascular and neuropsychiatric alterations. This phase ends after 3 days. Then the characteristics GIT blood loss, respiratory and urinary tract bleeding, blood loss also occurs from skin, ranging from petechiae to ecchymosis. Death occurs due to multiple organ failures (Munibullah et al. 2018).

Pathologic Lesions

Hepatomegaly and splenomegaly are characteristic necropsy findings in CCHF cases. Moreover, cerebral hemorrhage, lung edema and pleural effusion are also seen (Shayan et al. 2015).

Clinical Diagnosis

Besides clinical symptoms, history of insect bite and exposure to tissue or blood of infected animals and humans and also travel to the endemic region are important considerations for diagnosis of CCHF disease. Leucopenia, thrombocytopenia, and increased aspartate aminotransferase, alanine aminotransferase, creatinine phosphokinase and lactate dehydrogenase activity values are helpful. Prolonged prothrombin time, activated partial thromboplastin time, decreased fibrinogen and increased fibrin degradation products are also helpful(Munibullah et al. 2018).

Laboratory Diagnosis

Enzyme-linked immunosorbent assay (ELISA), antigen detection, quantitative polymerase chain reaction (qPCR), serum neutralization and virus isolation are useful tools for laboratory diagnosis of CCHF infection. After first day of viremia, weak IgM is detectable, followed by IgG. Immunohistochemistry is also used to detect the virus (Aslam et al. 2016).

Treatment

There is no drug of choice against CCHF virus infection. However, ribavirin is the only antiviral agent that can be taken orally and intravenously. Supportive therapy like interferons is also used. Immunoglobulin, venin is specific drug for CCHF disease (Zavitsanou et al. 2009).

Prevention and Control

There is no available vaccine for CCHF virus infection. Chances of tick exposure may be reduced by avoiding areas and seasons of high prevalence. High-risk occupation people such as butchers, shepherds and veterinarians should avoid ticks and suspected animal blood and tissue exposure. The use of gloves and protective clothes is also an effective measure. The medical workers should deal with care with suspected patients. Proper processing of milk and food should be done. Treatment of the livestock with acaricide is helpful in reducing the populations of insects. Insect repellents use on skin is also needed. Quarantine measures should be performed when importing animals. Control measures should be taken during slaughtering, butchering and culling. The equipment should be washed after slaughtering. Avoid close contact with the infected persons (Munibullah et al. 2018).

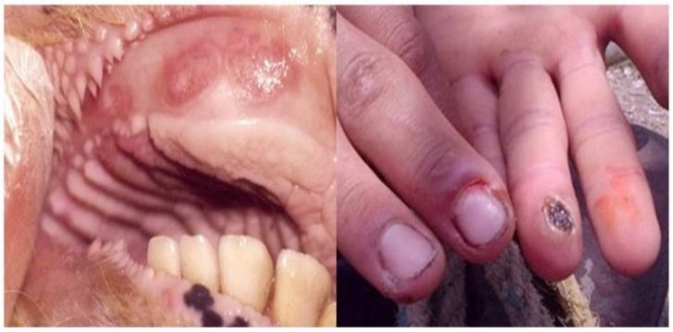

Fig. 3: Bovine Papular Stomatitis in cattle (left), available at https://veteriankey.com/alimentary-disorders/, and in human (right), available at http://coloradodisasterhelp.colostate.edu/prefair/disease/dz/Bovine%20Papular%20Stomatitis.html

Bovine Papular Stomatitis

Bovine Papular Stomatitis (BPS) is known as a granular or proliferative stomatitis. It is an infectious disease, affecting cattle of all ages and has been previously reported in many countries. It is caused by Bovine Pustular Stomatitis Virus (BPSV), belonging to Genus Parapoxvirus and Family Poxviridae. Parapox is an enveloped, oval and double-stranded DNA virus. BPSV has the potential to infect the human; therefore, it is considered as a zoonotic disease (Pal 2020).

Epidemiology

Transmission and environmental risk factors

Virus transmission is by direct contact between infected and susceptible animals. Zoonotic Parapoxvirus infection can cause the disease in milkmen (Fig. 3) and farmers (milker's nodules). Humans get the infection from direct contact with the infected cattle. An incidental bite during examination of diseased cattle can also result in human infection. Virus may also enter the body through a pre-existing skin lesion. The BPS virus mainly causes lesions in immunocompromised cattle or calves with a concurrent disease (Underwood et al. 2015).

Geographic distribution

Bovine Pustular Stomatitis is a widespread disease prevalent in many countries, such as Australia, Canada, Brazil, Europe, Nigeria, Kenya, South Korea and USA. It is responsible for great economic losses in dairy farms and also for the problems related to public health (Gelberg 2017).

Host range and susceptibility

The BPS virus can affect cattle of all ages; although its incidence is higher in cattle less than two years of age. Concerning human beings, BPS is considered as an occupational zoonotic infection (Peek et al. 2018).

Morbidity and mortality

BPS shows a high morbidity rate in severe outbreaks. It has low mortality rate; although, it can cause huge economic losses to the dairy industry (Underwood et al. 2015).

Clinical Signs in Cattle

The infected cattle shows papular and pustular rashes on the skin of muzzle, lips, nose, and buccal mucosa, with profuse salivation, fever, and diarrhea. Occasionally, teats of milking cows are also affected and show painful reddish papule, ulcer and scabby proliferative lesion (Senturk et al. 2016).

Clinical Signs in Humans

After milking of affected cows, milk-men show severe lesions on the fingers, hands, arms and face, besides axillary lymphadenopathy. The lesions of BPS are painful papules and pustules that can progress to ulcers and scabby lesions (de Sant'Ana et al. 2012).

Pathologic Lesions

Necropsy findings usually correspond to the lesions in live animals and are characterized by presence of papules and/or pustules on muzzle, lips, nose, and buccal mucosa. Teats of milking cows also show papules, ulcers and scabby lesions (de Sant'Ana et al. 2012).

Clinical Diagnosis

Clinical signs are not characteristic to give an accurate diagnosis of BPS (Underwood et al. 2015).

Laboratory Diagnosis

Laboratory diagnosis of BPS infectionincludes isolation of the virus in tissue culture, serology, polymerase chain reaction (PCR) and electron microscopy. The disease should be differentiated from the Pseudocowpox (Gelberg 2017).

Treatment

The disease is self-limiting so, no treatment is required. However, antibacterial and antibiotic creams may be applied to the skin lesions to prevent secondary bacterial infection (Peek et al. 2018).

Prevention and Control

Control measures, such as care in handling of infected cattle, wearing the gloves during examination of infected animals, proper attention to the skin lesions, thorough washing of hands with antiseptic solutions, and quarantine of the newly purchased animals help to control BPS infection. Awareness of such zoonotic diseases among the livestock handlers should be created. Moreover, early recognition of infection is necessary to prevent its further spread (Underwood et al. 2015).

Foot and Mouth Disease

Foot and Mouth Disease (FMD), hoof and mouth disease or Aphthae epizooticae, is an infectious or even fatal (contagious) disease of cloven-hoofed animal (wild and domestic bovid). This disease is a severe plague for animal farms, as it is highly infectious. The virus responsible for the disease is picornavirus, a member of the genus Aphthovirus. It is a single-stranded RNA virus having seven serotypes, including O, A, C, SAT1, SAT2, SAT3 and Asia1 (Farsang et al. 2013).

Epidemiology

Transmission and environmental risk factors

FMD is a highly transmissible viral infection, as a limited number of virus particles can initiate the infection. Transmission is by direct contact with contaminated animal products, materials, contaminated clothes and footwear of workers or contaminated equipment. Aerosol spread has also been recorded. Humans can acquire the infection by contact with infected animals or via ingestion of raw milk and dairy products from infected cows (Fig. 3). However, humans rarely acquire FMD infection. The virus enters the body via injured skin and mucous membrane. No person-to-person transmission has been recorded. The atmospheric conditions can either provide a barrier to virus dissemination or can promote its transmission. The virus persists for days or even weeks in organic matters under moist and cold conditions. Also, it can survive in lymph nodes, frozen bone marrow, and cheese during its processing. It can be inactivated by citric acid (0.2%), acetic acid (2%), sodium chloride (2%), sodium carbonate (4%), sodium hypochlorite (3%) and sodium hydroxide (2%). The virus in animal products can be inactivated by heating at 70°C for at least 30 minutes (Elmeligy 2017).

Geographic distribution

FMD is endemic in the Middle East, Africa, Asia and South America. New Zealand, North America, Iceland, most of Europe, Australia and Greenland are free of the disease. The last outbreak in United States occurred in 1929 (Osmani et al. 2019).

Host range and susceptibility

FMD has a wide host range and a rapid spread pattern. Bison, buffalo, alpaca, antelope, chamois, coypu, camel, cattle, deer, elephant, moose, pig, llama, reindeer, elk, giraffe, goat, sheep, and yak are among suspected animals. Laboratory workers, dairy farmers, veterinarians and cattle owners are also at risk of getting the infection (Weaver et al 2013).

Morbidity and mortality

In cattle, morbidity is about 100% but mortality is only about 2%. However, mortality in calves can reach 20%. The death in calves may be due to myocarditis. Carrier state in cattle varies from 15 to 50% (Elmeligy 2017).

Source of infection

FMD virus is found in secretions and excretions of infected animal. The virus persist in milk and semen for 4 days

before appearance of clinical symptoms. Also, contaminated animal products, agricultural tools, vehicles and people can contribute to the mechanical transmission of the virus (Rweyemamu et al. 2008).

Pathogenesis

The FMD virus is taken into the host cell. The cell produces multiple virus copies and then bursts to release the new virus in the blood (Arzt et al. 2011).

Clinical Signs in Cattle

Incubation period of FMD is about 2-14 days. The infection is manifested by fever that declines after 2-3 days, with the appearance of vesicles on feet, in and around mouth, and on mammary glands. Vesicles may also appear on vulva, prepuce or pressure points on legs. Vesicles rupture, leaving erosions. Depression, anorexia, lameness, excessive salivation and difficult to move are also frequent signs. Adult animals may suffer from weight loss. In cows, milk production can be decreased significantly; the disease may lead to myocarditis and death, especially in calves. Some animals remain asymptomatic but act as carriers for the infection and can transmit the disease to other animals (Arzt et al. 2011).

Clinical Signs in Humans

Incubation period FMD virus in humans is about 2-6 days. Signs of the disease include fever, vomiting, malaise, red ulcers on the oral mucosa, and vesicular lesions on the skin. In England, FMD killed two children in 1884 due to ingestion of infected milk (Bauer 1997).

Pathologic Lesions

Necropsy findings of FMD are characterized by presence of vesicles and/or erosions in the mouth, on the tongue, feet and mammary glands. Vesicles may also appear on the external genital organs. Erosions also may be found in rumen with necrotic myocarditis in some cases (Arzt et al. 2011).

Clinical Diagnosis

FMD infection in animals should be differentiated from infectious bovine rhinotracheitis, malignant catarrhal fever, contagious ecthyma, bovine viral diarrhea, bluetongue, bovine papular stomatitis, vesicular exanthema, vesicular stomatitis and mucosal disease.

Also, FMD should be differentiated from infections with many viruses of the Coxsackie A group (hand and mouth disease) (Elmeligy 2017).

Laboratory Diagnosis

The ideal samples for laboratory diagnosis of FMD are vesicular fluid and epithelium, deep swabs or scrapings of erosions, nasal swab and acute and convalescent serum sample. The diagnosis of FMD depends on the isolation of the virus from clinical material. ELISA, complement fixation test (CFT) and serum neutralization test are used for diagnosis of FMD. Detection of antibodies by agar-gel immunodiffusion, antibody detection ELISA and virus neutralization, new technology of nucleic acid detection can also be used with good results (Martinez-Salas et al. 2008).

Treatment

There is no specific treatment for FMD and recovery usually occurs within 15 days. In humans, the disease is often mild and self-limiting, and recovery usually occurs within one week, antibiotic or antiseptic ointment should be applied to the skin lesion to prevent the secondary bacterial infection. Drugs that enhance immunity and systemic antibiotic course may be helpful (Elmeligy 2017).

Prevention and Control

FMD should not be considered a public health threat. Animals with natural or vaccine-mediated immunity can act as a carrier if exposed to the infection; such animals will remain asymptomatic. Certain measures such as quarantine and destruction of infected livestock and use of gloves during examination of the diseased cattle are helpful. Attempts should be made to avoid direct contact with diseased animal, visiting the farms in affected areas and consumption of raw milk and dairy products. Moreover, disinfection of clothes, materials, vehicles premises and equipment, with regular immunization of animals are also advised. However, one of the major difficulties in vaccination is that FMD virus continually evolves and mutates; there is no cross protection between different virus serotypes. Vaccination provides only temporary immunity. Proper disposal of carcasses by incineration or deep burial also helps. Immediate report of an outbreak of FMD in non-endemic areas should be made so that strategies for its control can be undertaken in time (Farsang et al. 2013).

Contagious Ecthyma-ORF

The disease contagious ecthyma-ORF is also named as contagious pustular dermatitis, scabby mouth, sore mouth, infectious labial dermatitis, ecthyma contagiosum, and thistle disease. It is a highly contagious and zoonotic disease which affects small ruminants (sheep and goats) and also other domestic and wild ruminants. It is one of the notifiable viral diseases. The causative agent of contagious ecthyma is a double-stranded DNA virus, belonging to genus Parapoxvirus and family Poxviridae. Other members of this genus are pseudopoxvirus, bovine viral stomatitis virus and Parapoxvirus of red deer in New Zealand. Four strains of the virus have been recognized, OVIA82 and OVSA00 in America, D1701 in Germany and NZ2 in New Zealand (Tedla et al. 2018).

Epidemiology

Transmission and environmental risk factors

Human infection of contagious ecthyma is usually occupational. The virus is conveyed from animals to humans by direct contact or by exposure to fomites. Virus infections in humans occur when injured skin comes in contact with infected animals or contaminated equipment. The virus is highly resistant to adverse environmental conditions and can persist for many years. Human to human transmission has not been recorded. Transmission through aerosol route, ear tagging and contaminated gavage is also possible (Teshale and Alemayehu 2018).

Geographic distribution

ORF is a worldwide infection and it is common in late summer, fall and winter. Its seasonality is related to the increased stress from lambing or cold weather. ORF was firstly reported in 1920 from South West Africa. After that, it has been recorded in all parts of the world that rear sheep and goats. Outbreaks of infection have also been recorded from Norway, Brazil, China, Ethiopia, Indonesia, Spain and Iraq (Kumar et al. 2015).

Host range and susceptibility

ORF is an infection of sheep, goats and other domestic and wild ruminants. Incidences of human infection were reported in many countries, including the United Kingdom. Risk factors include; age, increased orphaned lambs, increased stocking density, immunosuppression, prolonged parturition and thorny plant (Taghipour et al 2015).

Morbidity and mortality

With first exposure of ORF, morbidity can reach 70%, while mortality rates are usually lower than 1%, although higher mortalities (90%) have been recorded in lambs with secondary bacterial infection. The internal organs affection is rare; however, some sheep and goats, which are severely infected with ORF, are found dead. The disease is severe in young animals, as they can die of starvation (Joseph et al. 2015).

Source of infection

Skin lesions and contaminated fomites, such as food and water and contaminated equipment are considered as the main sources of transmission of the infection (Karki et al. 2019).

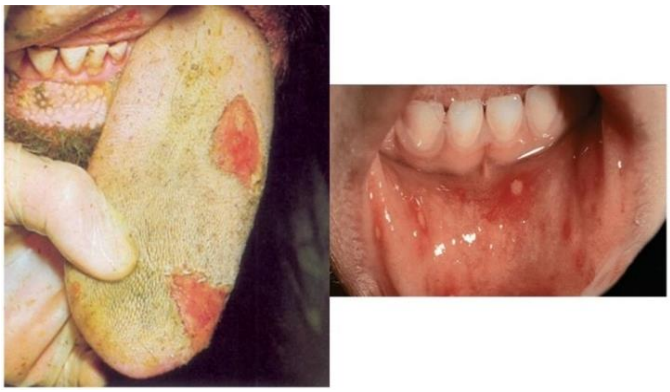

Fig. 4: FMD in cattle tongue (left), available at https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/foot-mouth-info/clinical-signs-cattle, and in human oral cavity (right), available at https://www.medicinenet.com/image-collection/hand-foot-and-mouth_disease_in_mouth_1_picture/picture.htm.

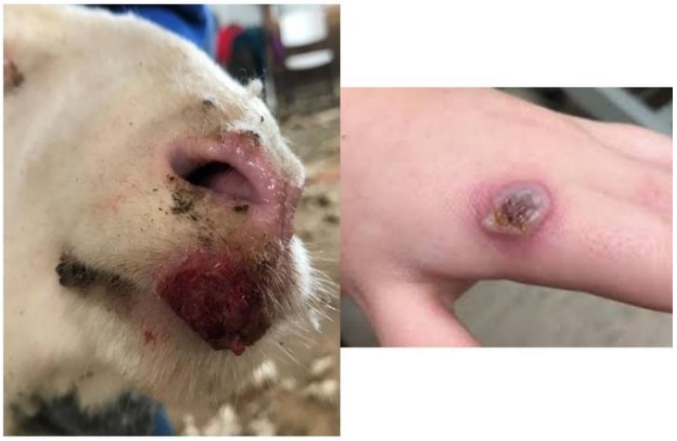

Fig. 5: Orf in sheep (left) (https://u.osu.edu/sheep/2019/04/23/soremouth-orf-in-small-ruminants/), and in human (right) https://dermnetnz.org/topics/orf/.

Pathogenesis

The virus enters the body via injured skin and then replicates in the cytoplasm of epithelial cells. At the portal of entry, the primary skin lesion develops 2-6 days post infection, but there is no viremia. The virus replicates whichleads to edema and granulomatous lesions in the dermal layer. Typical lesions are erythema, papules, vesicles and pustules with yellowish creamy exudate, and scabs that become dry and shed with no scar formation. This pattern occurs in 1-2 months (Kumar et al. 2015).

Clinical Signs in Small Ruminants

In small ruminants, lesions of ORF are seen on the muzzle, lips, nostrils, eyelids, oral mucous membranes, ears and teats of nursing ewes (Fig. 5). Severe pain can interfere with feeding, resulting in weight loss. The lesions of the udder are due to contamination during lactation and cause mastitis. Enlargement of the lymph nodes, pneumonia and arthritis, resulting from the sore mouth have also been recorded. Necrosis of the mucous membranes of the gastrointestinal tract, skin and urogenital tract can also occur (Karki et al. 2019).

Clinical Signs in Humans

Incubation period of ORF virus is 3 to 7 days. Large painful nodules distributed on hands have been recorded (Fig. 5); uncommon locations, including scalp, nose, buttocks, axilla and genitalia, have also been recorded. The infection is usually recovered with minimum scarring 1-2 months later. The disease in humans is self-limiting except for immunocompromised patients, who can develop large tumor-like lesions, progressive disease, erythema, multiform reaction including rashes on mucous membranes and skin. Signs in humans also include painful vesicles or necrotic areas on skin, pruritis, axillary lymphadenitis and lymphangitis (Taghipour et al. 2015).

Pathologic Lesions

Necropsy findings include papules, vesicles, pustules and multifocal-coalescing proliferative and necrotic scabs on hairy skin at the muco-cutaneous junction of lips; these lesions extend to the oral papillae, distal prepuce and medial canthus of the eye. In malignant cases, lesions are congested oral mucosa and upper respiratory tract; in few cases there are esophageal, abomasal, and small intestinal lesions. Lesions are generally proliferative and becomeulcer-like with time (Teshale and Alemayehu 2018).

Clinical Diagnosis

Diagnosis of ORF may depend on histopathology and clinical manifestation but these are less diagnostic tools. The disease should be differentiated from sheep pox, FMD, Bluetongue, facial eczema, ulcerative dermatosis, staphylococcal dermatitis and Dermatophilosis (Kumar et al. 2015).

Laboratory Diagnosis

PCR and qPCR have been developed to diagnose the infection. Serological tests such as agar gel precipitation test (AGPT), agglutination test, CFT, ELISA and serum neutralization test (SNT) may help. Virus isolation is the golden standard detection method, but it is time consuming (Tedla et al. 2018).

Treatment

There is no specific treatment for ORF in animals and humans. Systemic and topical application of antibiotic, antiviral and anti-inflammatory drugs have only limited success. Immune-stimulants are usually indicated. Emaciated animals must be given 10% glucose saline intravenously. The lesion may be washed with 1:100–

1:10,000 potassium permanganate lotion with application of 1:10 boric acid. Supportive treatment of young animals by administering glucose solution through esophageal intubation should be performed (Karki et al. 2019).

Prevention and Control

In animals, the infection can be prevented by quarantine of newcomers. Vaccination with live virus vaccine is important for minimizing the severity of signs in infected herd; vaccines containing a caprine strain are more effective in goats. Disinfection of animal house and incineration of all infected material are also useful. Slaughterhouses should verify that all animals are in a good condition. Among humans, veterinarians and farmers should wear gloves and face masks while dealing with infected animals. Washing of skin injury with soap and water after contact with animals is also necessary (Teshale and Alemayehu 2018).

Vesicular Stomatitis

Vesicular Stomatitis (VS) is an infectious disease of cattle, horses and swines. It is caused by vesicular stomatitis virus (VSV), a member of the Vesiculovirus of family Rhabdoviridae. It is an enveloped single stranded RNA virus. VSV has two serotypes, VSV-New Jersey (NJ) and VSV-Indiana (IND). There is no cross reactivity between the two serotypes. Vesicular Stomatitis is endemic in northern South America, southern Mexico, Central America and eastern Brazil. Major epidemics of the disease in cattle and horses in the United States occurred in 1889, 1906, 1916, 1926, 1937, 1949, 1963, 1982, and 1995. The disease spread to Europe during the first World war. The disease is of great economic importance, as attack rates in dairy cattle can reach 96%. Vesicular Stomatitis in humans is considered as a severe but non-fatal infection and the disease takes the form of influenza-like illness (McCluskey et al. 2013).

Epidemiology

Transmission and environmental risk factors

Cattle can take the VS infection by injection or aerosol route. Intradermal injection of the virus causes skin lesion at inoculation site, intramuscular injection or aerosol route does not cause such lesion. In epidemic areas, human infection occurs by contact with diseased animals. The role of biting insects may also be suggested. Black fly is the most important insect vector in the United States. Disease is common in tropical and subtropical areas at the end of the rainy season or early in dry season. In Colombia, the outbreak in cattle usually reaches the peak in February and August. The virus is susceptible to many disinfectants like 1% sodium hypochlorite, aldehydes (e.g. formaldehyde), 40-70% ethanol, phenolic disinfectants, detergents and 1% cresylic acid. The virus is also susceptible to ultraviolet light, sunlight and heat (Rozo-Lopez et al. 2018).

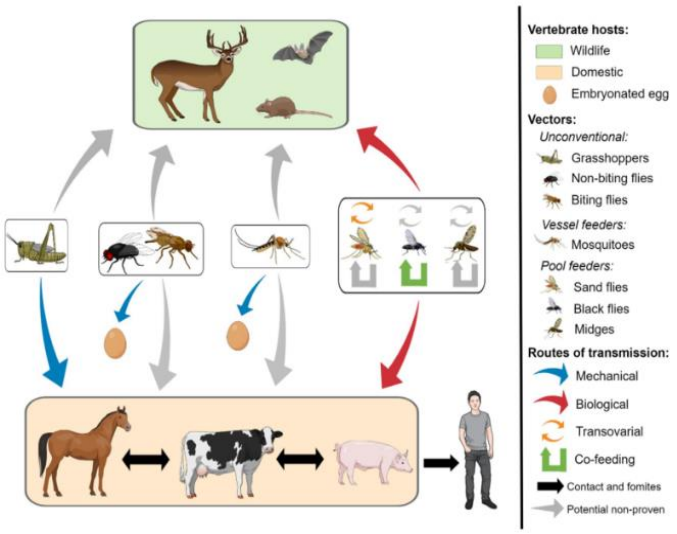

Fig. 6: Transmission networks for vesicular stomatitis (https://www.mdpi.com/2075-4450/9/4/190/htm)

Geographic distribution

Vesicular Stomatitis is endemic in Southern Mexico, South America, Venezuela, Colombia, Central America, Ecuador and Peru (Fig. 6). The infection has also been recorded in South Africa in 1886 and 1897 and in France in 1915 and 1917. The annual outbreak of VS occurs in these areas among susceptible species. Outbreaks of VS occurred in all areas of the United States in 1995, 1997, 1998, 2004, 2005, 2006, 2009, 2010, and 2012, but they were limited to western states. The infection spread to Europe during the First World War and appears periodically in South Africa (Reis et al. 2009).

Host range and susceptibility

Vesicular Stomatitis infection is characterized by a wide host range (cattle, horse and swine). Cattle of less than one year age are asymptomatic. In humans, occupational exposure in laboratory workers and animal handlers, who are exposed to VS, get infected (Smith et al 2012).

Morbidity and mortality

The morbidity rate in VS is highly variable; it ranges from 5-90% and is affected by the previous exposure of animal. Only 10-20% of the animals are usually symptomatic. In non-endemic areas, morbidity rates may reach 40-60% in susceptible species. Mortalities are very rare in cattle and horses but are higher in pigs. The morbidity in human infection is unknown. Although some authors suggest that clinical cases are rare, other human infections may take the form of influenza-like illness. In most cases, illness has no serious consequences (Rozo-Lopez et al. 2018).

Source of infection

VS virus sheds from exudates and epithelium of the lesion. Contaminated fomites are suspected to play an

important role in the virus transmission. The virus survives for about 3-4 days in saliva. Plants and soils are also suspected to be sources of the VS virus infection (Rainwater-Lovett et al 2007).

Pathogenesis

VSV destroys the cells of prickle cell layer; so large vesicle is formed, which quickly ruptures, leaving erosion or necrotic tissue. The lesion heals quickly (Reis et al. 2009).

Clinical Signs in Cattle

Incubation period of VS virus infection is 3–7 days. The lesions of VS resemble those of FMD in cattle. Fever occurs in early stages of the disease. Lesions are found on the palate, lips, gums, tongue, coronary band, and teats. Oral lesions are in the form of raised, blanched and fluid-filled vesicles. Vesicles are short-lived and rupture, leaving ulceration or erosions. Lesions may coalesce together to form large, denuded area of oral mucosa with epithelial tags. Lesions are also present on teats of cows and coronary bands of cattle, horses and pigs (Letchworth et al. 1999).

Clinical Signs in Humans

Incubation period of VS infection in humans is about 1-6 days. Human infection with VSV-NJ and VSV-IND is usually symptomatic. Infection may begin in conjunctiva and is followed by acute, febrile, influenza-like signs as fever, nausea, vomiting, chills, headache, myalgia, malaise, substernal pain, pharyngitis, conjunctivitis, and lymphadenopathy. Encephalitis is a rare sign but may occur in children and may be fatal. Vesicles are also seen in pharynx, tongue or buccal mucosa, or on the skin following direct injection. Signs last 3-6 days and are not associated with complication (Cargnelutti et al. 2014).

Pathologic Lesions

Lesions at necropsy are the same as seenin live animals. Rumen and heart lesions, which may be found in FMD, do not occur in VS (Letchworth et al. 1999).

Clinical Diagnosis

Diagnosis of VS may depend on clinical manifestations but they are not usually accurate. The disease should be differentiated from skin affections like sheep pox, FMD and ORF (Cargnelutti et al. 2014).

Laboratory Diagnosis

The Vesicular Stomatitis virus is isolated from throat swabs, vesicle fluid, saliva and epithelial tissue from the margins of the lesion. Also, electron microscopy, CFT, or FAT can be used. PCR is more sensitive than other methods. Competitive ELISA is used to measure antibodies titer (Letchworth et al. 1999).

Treatment

This disease has no specific treatment. Anti-inflammatory drugs may help to reduce the swelling and pain. Lesion dressing with mild antiseptic is recommended to prevent secondary bacterial infection. In cases accompanied by fever and inflammation, treatment with antibiotics may be recommended (Cargnelutti et al. 2014).

Prevention and Control

During outbreak of VS, normal cattle should be kept away from suspected animals. Quarantine and animal movement restrictions can help in reducing the spread of infection. Isolation of symptomatic animals may be also helpful. Disinfection can also minimize the spread of the infection. Feed and water troughs of dairies should be washed regularly. Milking equipment should be sterilized before each use. Avoid thorny food which may predispose the animal to oral lesions. Moving of animals away from sources of running water during outbreak may minimize the risk of infection, as water sources encourage vector populations. Use of insecticides may be helpful. Vaccines are available in some endemic areas of Central and South America. Wearing of protective clothes and gloves while dealing with suspected animals are necessary. Veterinarian who suspects that the animal is infected, must follow his national and/or local guidelines for disease reporting. In the United States, state and federal veterinarians should be reported for any suspected cases immediately (Letchworth et al. 1999).

Pseudocowpox

Pseudo-cowpox is an infectious disease of cattle worldwide. Infection is caused by member of genus Parapoxvirus, and closely resembles to the viruses of BPS and ORF. Due to the mild signs of the disease in cattle, information about the infection is very little in the literature. The virus has zoonotic importance and farmers are not aware; many times, it remains undiagnosed because of its self-limiting nature. Pseudocowpox virus is one of the two parapoxviruses (PPVs) of cattle, Bovine papular stomatitis virus, previously known as *Parapoxvirus bovis-1*, and Pseudocowpox virus, previously known as *Parapoxvirus bovis-2*. Two other virus species are Orf virus of sheep and goats (ORFV, previously known as *Parapoxvirus ovis*) and Parapoxvirus of red deer in New Zealand (NZPV). Parapoxviruses are epithelia-tropic viruses, identified as causing local vesicles and eruptive skin lesions in wild and domestic animals, especially ruminants (Oguzoglu et al. 2014).

Epidemiology

Transmission and environmental risk factors

Transmission of Pseudo-cowpox disease occurs by direct and indirect ways. Indirect route occurs when a calf suckles multiple cows and inadequate milking management procedure. Infection occurs through injured

skin or occasionally through oral mucosa. The virus infects humans through direct contact. The virus infects the people working with affected cattle with unprotected hands, causing what is called "milker's nodules." (Ouedraogo et al 2020).

Geographic distribution

The Pseudo-cowpox virus is present worldwide, and affects mainly milking cows but with a limited economic importance. However, in some dairies, this infection may cause some economic losses (Chitala et al. 2020).

Host range and susceptibility

Recently introduced adult and freshly calved cattle are the most susceptible animal groups to the Pseudo-cowpox disease. Although, adult cattle including dry cows are affected, cows less than 2 years old appear to be asymptomatic unless they have calved. In humans, the infection is occupational that affects the milkers and other persons in contact with affected cows, the lesion is called Milker's nodule (Craighead 2000).

Morbidity and mortality

Pseudo-cowpox is often benign with no mortalities, the losses occur due to difficulty in milking and increased incidence of mastitis (Olson et al 2019).

Source of infection

Fomites, including calves mouths, hands and milking machines are all considered as source of the Pseudo-cowpox infection (Ouedraogo et al 2020).

Clinical Signs in Cattle

Lesions of the disease are confined to teats, but may also develop on muzzles and in mouths of nursing calves. Lesions are in the form of ring or horseshoe shaped scabs on teats, and usually heal within 6 weeks. Lesions progress to macules, papules, vesicles, pustules, and scabs (Fig. 7A). Vesicles and pustules covered by scabs can also be seen. Scabs detach after few days in the absence of bacterial contamination, and the lesion heals rapidly (Dhillon et al. 2020).

Clinical Signs in Humans

Incubation period of Pseudocowpox in humans is 5-7 days. Lesions include small, erythematous papules that appear on hands (Fig. 7B and 7C); after 4 to 6 weeks they develop into firm nodules of 3-8 mm diameter before resolution. The disease in humans is similar to 'Orf'. No systemic involvement was observed in humans (Dhillon et al. 2020).

Pathologic Lesions

Localized skin lesions start as a pustule, macule or vesicle that will progressively ulcerate, scab and heal after 5-6 weeks (Samemaleki et al. 2014).

Clinical Diagnosis

It is important to differentiate the Pseudo-cowpox disease from other diseases causing vesicles, such as FMD and Vesicular Stomatitis (Craighead 2000).

Laboratory Diagnosis

Samples are taken from scabs of teat lesions. Virus isolation and electron microscopy of lesions are important methods of virus demonstration. Histology and Parapox PCR are also helpful. Clinical and histopathologic characteristics of the disease in humans are indistinguishable and are differentiated based on the history of the initial host exposure (Craighead 2000).

Treatment

Pseudo-cowpox disease is self-limiting and the lesions should be treated symptomatically through the application of topical ointment to teats and other affected parts. The affected animals should be milked after the healthy ones (Dhillon et al. 2020).

Prevention and Control

Milking hygiene is an essential measure for the control of Pseudo-cowpox. Affected animals should be milked last. Application of hygienic measures in milking with the use of teat dip may minimize the risk of virus transmission. Acquired immunity is short-lived and there is no vaccination against this virus. Human infections can be reduced by wearing the gloves, thorough hand washing and general precautions when come in contact with infected cows (Craighead 2000).

Rabies

Rabies is a Latin word for "madness". The Greek name for rabies, lyssa, also means madness. The causative agent of rabies is an RNA virus, belonging to genus lyssavirus and family Rhabdoviridae. This genus has classical rabies virus (RABV, genotype-1) and six rabies-like viruses. Each of them is able to cause rabies-like signs in animals and humans. The virus trans-membrane glycoprotein is important in virus tropism and pathogenicity. It is the main protecting antigen, as it is important in producing the virus neutralizing antibodies. The virus is very sensitive to some environmental factors such as direct sunlight, heat exposure at 60°C for 5 minutes and ultraviolet irradiation; it is also sensitive to lipid solvent (70% alcohol), trypsin, sodium deoxycholate and common detergents (Mtui-Malamsha et al. 2019).

Epidemiology

Transmission and environmental risk factors

Rabies virus can infect warm-blooded animals, including humans. Some birds have been known to produce rabies

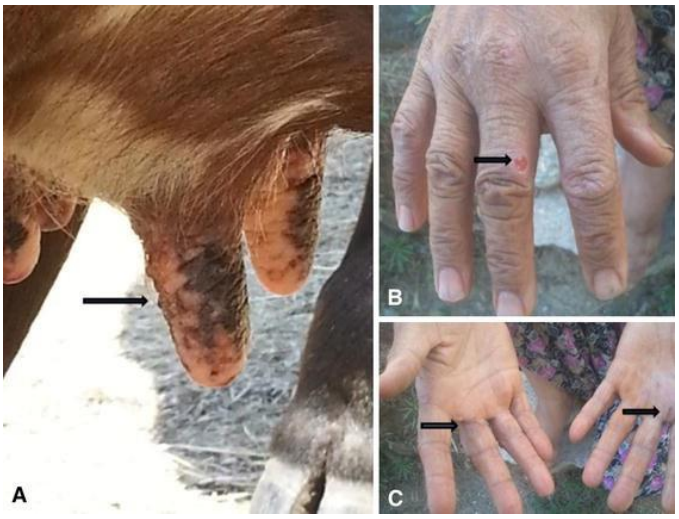

Fig. 7: Pseudocowpox in cattle udder (A) and in human (B and C) (https://link.springer.com/article/10.1007/s13337-014-0214-z).

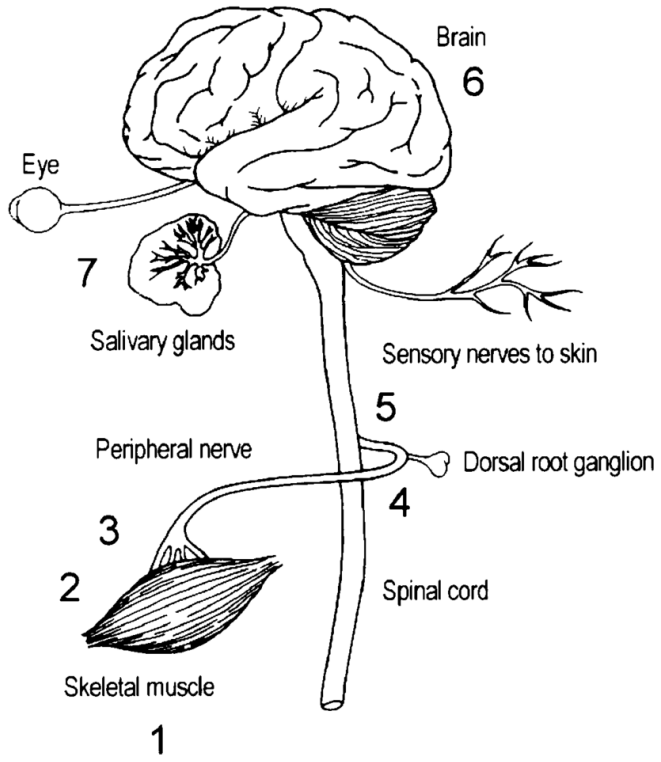

Fig. 8: Pathogenesis of Rabies in man and animals (https://www.researchgate.net/publication/12229458).

antibodies after eating rabid animal. Infected bats, foxes, monkeys, skunks, coyotes, cattle, raccoons, wolves, dogs and cats are considered to be a great risk for humans. Rabies virus can spread via exposure to domestic animals infected with virus. Bites from rodents rarely require rabies prevention, as they are rarely found to be infected. The virus is present in saliva, cerebro-spinal fluid, tears, and nerves of asymptomatic infected animals. The route of infection is mostly through the bite. Transmission from human to human is extremely rare. After infection in humans, the virus is conveyed to peripheral nervous system and is transported along the afferent nerves to central nervous system (Fisher et al. 2018).

Geographic distribution

Reservoir of rabies virus varies throughout the world. Canine rabies is dominant in Latin America, Africa, Middle East and Asia. In North America and Europe, canine rabies has been totally eliminated; rabies virus is maintained in wildlife. Some countries like United Kingdom, Norway, Iceland, Malaysia, Sweden, New Zealand, Ireland, Singapore, Japan, Australia, Pacific Islands, Papua New Guinea and some Indonesian islands have been recorded as rabies-free areas for many years. About 98% of human cases are reported from the developing countries of Latin America, Asia and Africa (Singh et al 2017).

Host range and susceptibility

All warm-blooded animals are susceptible to rabies infection, but limited number of species can act as a reservoir. Cattle with furious form can be dangerous and attack humans and other species. Equines frequently show signs of distress and agitation. Rabid foxes often invade yards and houses and attack dogs and people (Leung et al 2007).

Morbidity and mortality

Before vaccine development, most human cases of rabies were fatal (Fisher et al. 2018).

Source of infection

Sources of rabies infection include members of the families Canidae (wolves, dogs, foxes, jackals and coyotes), Viverridae (e.g., mongooses), Mustelidae (e.g., skunks) and Procyonidae (raccoons) and the order Chiroptera (bats). In Africa, primary rabies virus maintenance cycle occurs among domestic dogs, while other carnivores may act as a non-maintenance population (Sugiyamaa and Ito 2007).

Pathogenesis

Rabies virus enters the body through bite, via injured skin or by contact with mucosa. The virus cannot cross the intact skin. The virus infects local sensory and motor neurons and locally replicates in skeletal muscles or attach directly to the nerve endings, particularly to acetylcholine receptors at motor-end plates. Then the virus migrates in centripetal retrograde axonal transport to CNS (Fig. 8). In the CNS, the virus replicates extensively, and the clinical disease appears. Inflammation is mostly present in the midbrain and medulla in furious stage and the spinal cord in paralytic rabies stage. The virus spreads from the brain to many tissues and organs, replicates in salivary glands and excreted in saliva (Mustafa et al. 2015).

Clinical Signs in Cattle

Period between the infection and first flu-like symptoms is 2-12 weeks. Incubation period of rabies virus is highly

variable, based on location and severity of bite and the amount of inoculated virus. Death occurs 2-10 days after the first symptoms. Once symptoms appear, treatment is not effective and the mortality rate is about 99%. Symptoms in animals include change of behavior, loss of appetite, excitement, aggressiveness, paralysis (especially of the lower jaw), fever, change in phonation (sound of a dog's bark) and excessive salivation. All rabid animals exhibit signs of nervous disturbance, with a little variation among species. The clinical course is divided into three phases. 1: *Prodromal Phase:* This stage usually persists for 1-3 days with minor behavioral changes as aggressiveness, daytime activities and no fear of humans. 2: *Excitement (Furious) Phase* (mad-dog syndrome)*:* There is rare evidence of paralysis and animal becomes irritable, with dilated pupils, infected animal attacks other animals and people. Muscles incoordination and seizures are common symptoms. As the disease progresses, the death may result from paralysis. 3: *Paralytic (Dump) Phase:* In this phase, progressive paralysis occurs, throat and masseter muscles get paralyzed and the animals may be unable to swallow. There may be facial paralysis. Ataxia and incoordination are also typical signs of this phase. Death occurs within 2-6 days due to respiratory failure (Ayele et al. 2018).

Clinical Signs in Humans

Symptoms of rabies appear within 30-60 days in humans, signs include pain and itching at the site of inoculation, also fever, nausea, restlessness, headache, sore throat and loss of appetite may occur. Excessive salivation, muscle stiffness, excitement or convulsions, increased sensitivity to light or loud sounds are also seen (Mustafa et al. 2015).

Pathologic Lesions

There is no pathognomonic lesions for rabies, although there may be fresh or healed bite wounds. There is unusual odor related to reduced hygiene. In CNS, there is congestion of the meningeal vessels, the brain tissue may be congested with mild cerebral edema (Ayele et al. 2018).

Clinical Diagnosis

Clinical diagnosis in this disease is difficult, as Rabies is confused with other diseases with aggressiveness in its early stages. Differential diagnosis includes any infection that causes encephalitis, such as arboviruses, herpesviruses, enteroviruses, tetanus, listeriosis and poisoning (Fisher et al. 2018).

Laboratory Diagnosis

Frequently used diagnostic method is rabies is FRT (fluorescent rabies test) or fluorescent antibody test–FAT. Microscopic examination of tissue helps in identification of virus-specific antigen. RT PCR is the sensitive and specific test (Fisher et al. 2018).

Treatment

After viral exposure, treatment is able to prevent the infection if it is taken within 10 days post-infection. Thorough washing of skin with soap and water is effective; use of povidone-iodine and alcohol is effective in reducing the risk of development of the disease. It is necessary to take one dose of human rabies immunoglobulin and also 4 doses of rabies vaccine over 14 days period. Post exposure prophylaxis should be given without delay (Mustafa et al. 2015).

Prevention and Control

Vaccination before exposure has been done in human and non-human species, with the domestic animals are important to be vaccinated. Control measures should be appliedlike, vaccination of dogs, ferrets and cats, keeping pet animals under good supervision, avoid handling wild and stray animals, contacting the veterinarian when observing wild or stray animals and also washing the site of exposure with soap and water for 10-15 min post exposure (Mtui-Malamsha et al. 2019).

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POSSIBLE ROLE OF PETS, WILD AND DOMESTIC ANIMALS IN THE TRANSMISSION OF COVID-19 TO HUMANS

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INTRODUCTION

Pneumonia due to unknown cause appeared in early December, 2019 in many people residing in Wuhan city, China (Li et al. 2020a). The causal agent of this infection was verified as a novel coronavirus (nCoV), and later the disease was identified as Coronavirus Disease (COVID-19) by the WHO and the causative virus was renamed as SARS-CoV-2 (Lai et al. 2020; Yuen et al. 2020). This viral disease outbreak very rapidly spread all around the globe and WHO declared public health emergency internationally on January 30, 2020, which was afterwards announced as worldwide pandemic on March 11, 2020 (WHO 2020a). As of December 23, 2020, >76.3 million definite cases and >1.7 million deaths have been altogether reported worldwide (WHO 2020b).

This group of Coronaviruses belongs to the subfamily *Orthocoronavirinae* of family *Coronaviridae* (Dhama et al. 2020), and order *Nidovirales*. These viruses are classified on the basis of their genetic, as well as susceptibility to various species, into following four genera i.e., Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV), and Deltacoronavirus (δ-CoV) (Lau et al. 2015; Li et al. 2020a; Gennaro et al. 2020; Kiros et al. 2020; Fig. 1). Generally, viruses belong to α- and β-CoV genera can cause infection in mammalian animals including humans, while δ- and γ-CoVs viruses can cause illness mostly in birds and other animals (Li et al. 2020a; Sharun et al. 2020a).

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has appeared as the 7th member in the family of corona viruses causing infection in humans (Hasoksuz et al. 2020). Before this pandemic, atypical pneumonia due to Middle East respiratory syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) was reported in humans (Li et al. 2020a; Chang et al. 2020). Both the MERS-CoV and SARS had infected humans worldwide in 2002 and 2012 (Wu et al. 2005; Sims et al. 2008; Guillon et al. 2008; Balkhair et al. 2013; Gastañaduy 2013; Su et al. 2015).

As the SARS-CoV-2 virus has a zoonotic, as well as natural, origin, two school of thoughts can probably describe the origin of this virus i.e., i) natural selection of virus in the animal host before to its transmission to the humans; and ii) natural selection of the virus in humans subsequent to zoonotic transfer from the animals (Yin and Wunderink 2018; Lu et al. 2020). Clinical signs and symptoms of the disease are highly variable, which make the clinical severity from asymptomatic to highly severe, leading to death of the affected individuals (Phan 2020).

Though some approved vaccines against SARS-CoV-2 are being marketed but these are beyond the approach of poor nations and even the therapeutic drugs are also not specific and clear. Therefore, it is the best policy that the transmission mechanism of this pathogen be recognized well before its prevention and control. In this context, the possible role of animals in the transmission of this virus to humans must be ruled out so that if involved, it could be curtailed. There are many studies reported (Shi et al. 2020; Kayser and Rottmann 2020; Sia et al. 2020; Kiros et al. 2020; Chan et al. 2020), while others are in progress, but information about the role of pets in spreading this virus to humans is not available. In this chapter, we have tried to review the role of various animal species in the transmission of the COVID-19 to humans.

Zoonotic Importance of SARS-CoV-2

Almost 60% of all known emerging infectious diseases are zoonotic in nature. Among the new pathogens (as SARS, Ebola, Zika fever, MERS and Covid-19 etc.) detected in humans over the period of last three decades, ~75% have originated from animals (Jones et al. 2008). Many of the emerging infectious diseases in the history were actively controlled in time to limit their spread, but COVID-19 has spread all over the world within very limited time duration and causes devastating losses all around the globe. A number of species of CoVs are circulating in mammals and birds, causing illness that leads to huge economic losses (Abdel-Moneim and Abdelwhab 2020; Li et al. 2020a). Previously observed outbreaks of both MERS and SARS-CoV also indicate the zoonotic importance of the pathogen (Kiros et al. 2020). Similarly, indications from the COVID-19 outbreaks pointed out that former cases had been linked in China to the Wholesale Seafood Market (Chen et al. 2020b) and isolation of the SARS-CoV-2 from various samples (structures, soil, people, birds, animals, discharges, etc) advocated the engrossment of some middle hosts (WHO 2020c). Possible animal-human interface, or zoonotic association towards the

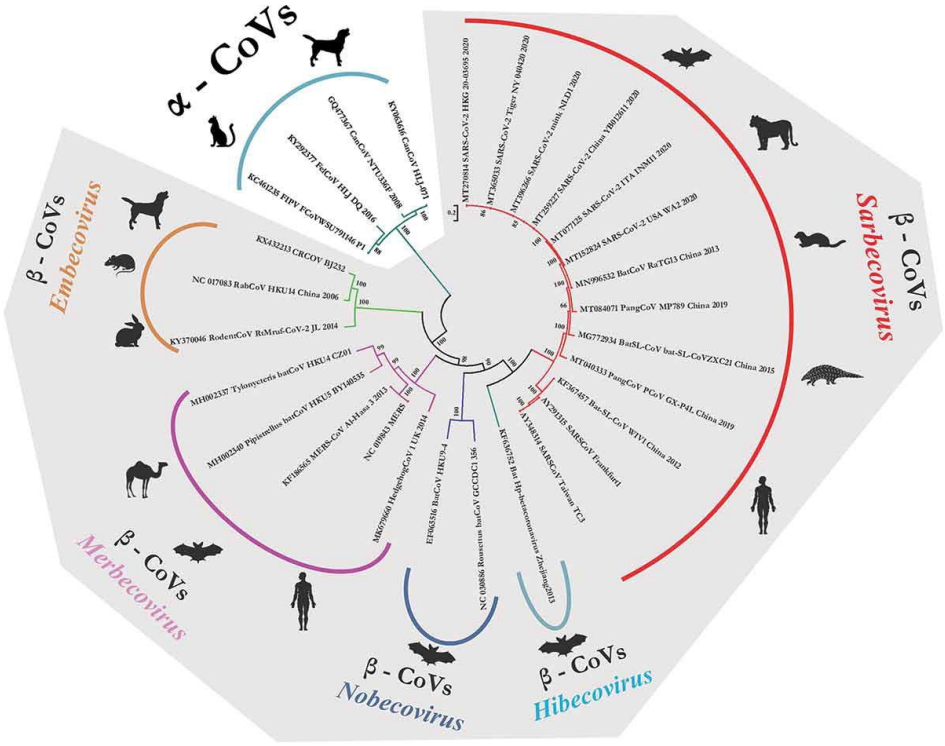

Fig. 1: Grouping of coronaviruses of human and animal origin on the phylogenetic basis (Sharun et al. 2020b).

derivation of the SARS--CoV-2 has also been pointed out recently (Fig. 2; Salata et al. 2020; Ji et al. 2020; Murdoch and French 2020; Tiwari et al. 2020). An outline of coronavirus cross-species transmission from bats to additional faunas prior to spillover to humans as zoonosis, and probable projections for additional spread to mammalian hosts, has been explained in Fig. 3.

Transmission

The transmission and adaptation of coronaviruses infection among different species are based on the existence or absence of certain receptors on the cells of different tissues of the host (e.g. ACE 2 receptors for SARS-CoV, SARS-CoV-2 and HCoV-NL63, 9-O-acetylsialic acids for HCoV-HKU1 and HCoV-OC43, human aminopeptidase N for HCoV-229E and dipeptidyl peptidase-4 for the MERS-CoV), which play an important role in the binding and entrance of the viral particles into the host cells (Tiwari et al. 2020; Salata et al. 2020). These specific receptors exist in several body systems of humans and animals, such as gastrointestinal and respiratory systems (Salata et al. 2020). Reservoir host animals of CoV, including rodents and bats, carry similar receptors as present in intermediate hosts including bovines, camels and masked palm civet (Salata et al. 2020; Wang et al. 2020; Ye et al. 2020). The presence of these receptors (ACE2 or DPP4) in humans makes them susceptible to MERS-CoV and SARS-CoV, causing MERS and SARS infections, respectively (Song et al. 2005; Tiwari et al. 2020; Ali et al. 2021). The spike present on the MERS-CoV has the capability to become accustomed to the disparity in the receptors DPP4 of the host species (Letko et al. 2018). This property of adaptation by MERS-CoV might be present in other coronaviruses. Likewise, spike protein of SARS-CoV interrelates the angiotensin converting enzyme 2 (ACE2) receptors of the host and results in its inter-species, as well as cross-species, transmission (Wan et al. 2020). The variations in the receptors of host species bind the interaction with spike protein of CoV, which results in the formation of a barrier that results in the prevention of spillover infection. Different animal species, including pangolins, civet and snakes, are deliberated as the likely intermediate hosts of COVID-19. Though, this needs to be confirmed by tracing the origin of the virus for the prevention of viral resurgence (Amodio et al. 2020). In addition, detection of susceptible animals and investigation of SARS-CoV-2 would be of significance for the prevention of such outbreaks in the future.

Different reports suggest that the COVID-19 virus can possibly spread during the incubation period, as well as in the convalescent period, of the virus (Rothe et al. 2020). During the COVID-19 infection, the virus is present in respiratory droplets and body fluids of the infected patients with the capability to survive for up-to 9 days on polluted surfaces, leading to human-to-human, as well as nosocomial, transmission (Huang et al. 2020; Kampf et al. 2020; Lee et al. 2020). Similar to SARS-CoV, SARS-CoV-2 can be transferred from one person to another through ocular route, indicating the different ways of spread of the disease apart from respiratory tract (Belser et al. 2013; Lu et al. 2020). Later, transmission of the virus via fecal-oral route was also suggested (Huang et al. 2020). The polymorphism observed in SARS-CoV-2 through metatranscriptome sequencing in the broncho-alveolar lavage fluid has shown intra-hosts variants, proposed *in vivo* evolution, thus affecting the transmissibility, virulence and infectivity of this virus (Shen et al. 2020).

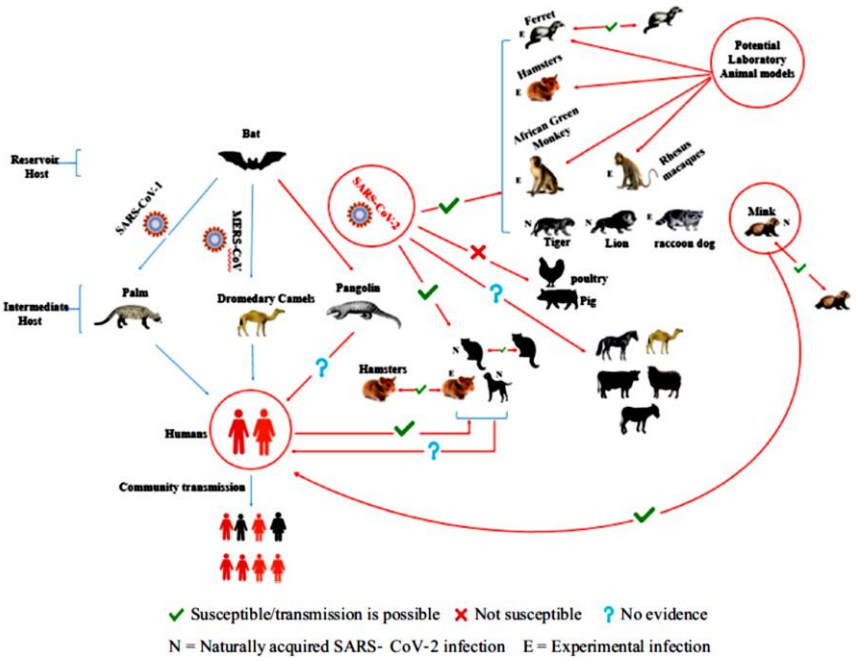

Fig. 2: The origin of the SARS-CoV-2 and its transmission along with the possible role of pets and other animals in the disease transmission. According to this figure, companion animals are prone to SARS-CoV-2 and humans could be origin of infection for the companion animals, however, probable role of companion animals in the disease spread to humans is undetermined (Kiros et al. 2020).

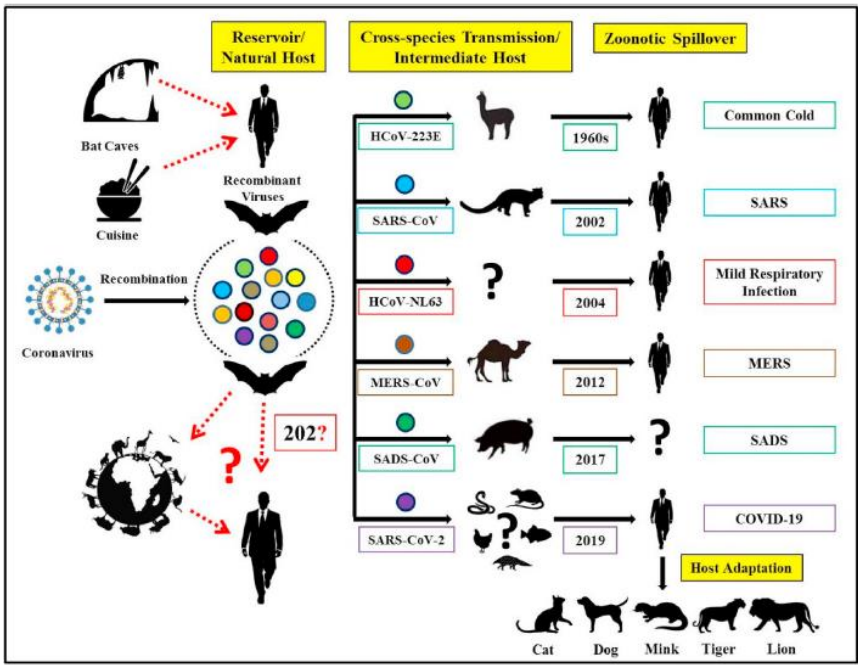

Fig. 3: The possible spread of zoonotic coronaviruses from bats to animals or cross-species spread before spillover to humans. Possible predictions of spread to mammalian hosts are also shown (Dhama et al. 2020).

Dispersion of the SARS-CoV in 2002 to palm civet from bats and afterwards to humans, whereas spread of MERS-CoV to camels from bats and subsequently to humans in 2012 has been documented (Bonilla-Aldana et al. 2020a; Ahmad et al. 2020; Bonilla-Aldana et al. 2020b; Bonilla-Aldana et al. 2020c; Bonilla-Aldana et al. 2021; Li et al. 2020b), indicating the importance of bats as reservoir host and it may have possibly been related to the current SARS-CoV-2 pandemic.

SARS-CoV affected humans (774 deaths) during 2002 in China had close contact with palm civet (Totura and Bavari 2019). Decade after, MERS-CoV, another threat due to coronaviruses, infected humans and showed a very close relationship with camels in Saudi Arabia, where it led to 858 deaths and remained a public health concern in 27 countries (Totura and Bavari 2019). A study detected partial RNA-dependent RNA polymerase (RdRp) sequences in tissues and feces of bareback fruit bats (Anindita et al. 2015). Another study spotted 26.65% positive rate of coronaviruses in bats (Hu et al. 2018). Many other reports have shown that SARS-CoV-2 is identical to coronaviruses naturally present in bats

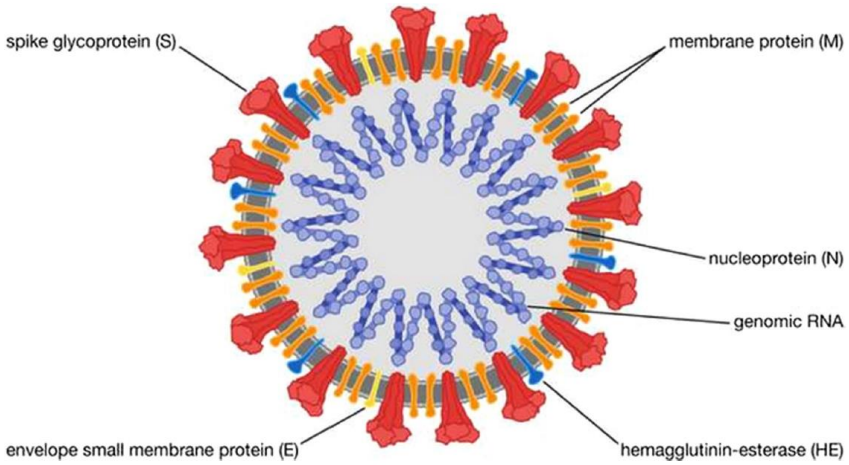

Fig. 4: Coronavirus virion structure shown with structural proteins and hemagglutinin-esterase (Kiros et al. 2020).

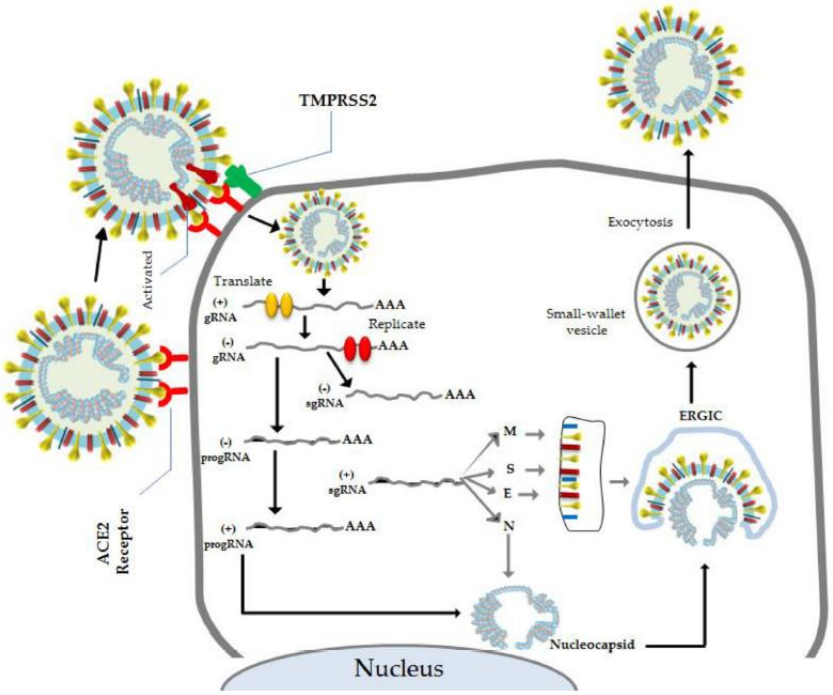

Fig. 5: Mechanism of entry and life cycle of Severe Respiratory Syndrome Coronavirus (Masters 2006; Rabi et al. 2020; Astuti and Ysrafil 2020).

(Morrison et al. 2020; Latinne et al. 2020). However, continuous mutation and evolution make it hard to find out a particular reservoir of SARS-CoV-2 (Bonilla-Aldana et al. 2021). *Rhinolophus*, a genus of bats, is assumed to be carrier host of the SARS-CoV-2. Furthermore, where different studies revealed that SARS-CoV-2 genome is very similar to the genome of coronaviruses from horseshoe bats; the receptors-binding domain of SARS-CoV-2 is found to be similar to that of pangolin corona viruses (Bonilla-Aldana et al. 2021). So, there is a possibility that pangolin CoV may be originated from some unidentified bat viruses, as an outcome of animal mixing. Therefore, SARS-CoV-2 is most likely a recombinant virus originated from bats (Lau et al. 2020). Another viewpoint related to origin of coronaviruses affecting humans, shows that all of these viruses have originated from bats or rodents (Swelum et al. 2020). At present, we have the knowledge of conserved genome sequences of a few coronaviruses naturally found in the bats to draw the phylogenies and until now there is a need to study the reservoirs of coronaviruses in the bats, viral transmission route and the process resulting in the spillover to humans (Bonilla-Aldana et al. 2020d; Bonilla-Aldana et al. 2021).

Several people afflicted with the SARS-CoV-2 owing to exposure in Wuhan City with the seafood market, which might suggest that the virus could have originated from animals and infected to humans, then persisted in the humans for further human-to-human transmission (Rothan and Byrareddy 2020; Ji et al. 2020). Bats could have beeen the initial reservoir of the virus (Temmam et al. 2020). Likewise, several studies have indicated with strong evidence that Malayan pangolins are supposed to be the probable intermediary host for the SARS-CoV-2 spread to humans (Lam et al. 2020).

In humans, primarily the SARS-CoV-2 targets the organs of respiratory system of the individuals, thus aerosol and/or respiratory droplets may act as major source of spread of the pathogen (Sia et al. 2020). It is believed that COVID-19 patients are the principal cause of spreading the pathogen to other individuals (Jahan et al. 2020), where transmission from one person to another is maintained via respiratory droplet within a distance of about 2 meters (Setti et al. 2020). Fomites around the infected person could also be a source of spread of the infection. Moreover, touching contaminated instruments and consecutive transfer to mouth by touching eyes and/or nose with infected hands could be other sources of transfer of the infection (Ong et al. 2020).

Possible role of animals in COVID-19 transmission

Possible role of pets

In February 2020, the first positive COVID-19 case was reported from Hong Kong, China in a Pomeranian dog (HKG 2020a). Later, another case from the same country was reported in a cat in March 2020 (HKG 2020b). In both of these cases, owners of the pets were also reported positive for the COVID-19 infection (HKG 2020a; HKG 2020b). The detailed study showed similarities in the genetic sequence of the owner and the pets SARS-CoV-2, indicating probable human-to-animal transmission. In addition, both serological tests and cultural isolation of the virus were performed for further confirmation. Results of these tests without signs and symptoms in the animals bring about interpretation that the virus was not transmissible to humans and/or another animal from the dog (Almendros 2020).

After the first reported cases of COVID-19 (HKG 2020a), further cases of COVID-19 were also reported from countries like Switzerland (Abdel-Moneim and Abdelwhab 2020), Belgium (ISID 2020a), France (ISID 2020b), Germany (ISID 2020c; ISID, 2020h), The Netherlands (ISID 2020d), South Korea, Canada (AVMA 2020), Russia (OIE 2020a), the United States (AVMA 2020; ISID 2020e; ISID 2020f; OIE 2020b) and India (ISID 2020g). From Switzerland, first case of novel coronavirus was observed on December 3, 2020 in a cat (SWI 2020). Experts believe that transmission of SARS-CoV-2 infection from humans to cats is infrequent but cannot be ruled out, that is why maintenance of fair distance from the pets is recommended by Swiss veterinary affairs (SWI 2020).

According to AVMA (2020), up to June 8, 2020, 7 million worldwide and 1.9 million people in USA were confirmed with COVID-19 infection, however, COVID-19 outbreak during the first five months (January 1 to June 8, 2020) only infected <20 pets throughout the world, which is an indication that pets are liable for SARS-CoV-2 infection (AVMA 2020). Moreover, infection of a few pets with SARS-CoV-2 was typically the result of close contact with COVID-19 positive people. Cats, ferrets and Syrian golden hamsters are also being kept as pets. These animals show potential animal models of human infection in laboratory studies (AVMA 2020; Sia et al. 2020; Chan et al. 2020), however, chickens, ducks, pigs and dogs do not come under potentially affected pets.

Possible role of domestic animals

Similar to the companion pets/animals, domestic animals like ferrets are highly susceptible to SARS-CoV-2 (Shi et al. 2020). A recent study has shown that experimentally infected ferrets can transmit COVID-19 infection proficiently to other healthy ferrets through air and direct contact (Richard et al. 2020). On the other hand, other domestic animals including poultry and pigs do not carry and spread the SARS-CoV-2 infection (Shi et al. 2020; Schlottau et al. 2020). However, no evidence regarding the susceptibility to COVID-19 in livestock animals including horses, donkeys, camels, cows and sheep is observed yet. Raccoon dogs in another contemporary experimental study were found permissive to COVID-19 virus and could have a possibility of pathogen transmission to other animals. Following intranasal inoculation, replication of the virus was recorded in the respiratory tract of these animals (Freuling et al. 2020).

Cats seem to be more susceptible to SARS-CoV-2 with high possibility to spread disease to other un-infected cats, whereas dogs are less prone to the SARS-CoV-2 and have a very minute role in the spread of the virus to other non-infected dogs (Shi et al. 2020). Similarly, golden Syrian hamsters can acquire and spread the SARS-CoV-2 infection to naive hamsters by aerosols, as well as direct, contact (Sia et al. 2020). It can be inferred that under natural condition there is slight to no possibility that pets and other domestic animals can get infested with the SARS-CoV-2. However, people with COVID-19 must protect their pets, as they are at high risk to attain the disease and can further spread the virus. Since no evidence has so far been reported that virus has been transmitted from pets to the people, hence primary type of spread of the COVID-19 in humans is from one person to another (AVMA 2020). Though there is no proof, however, pets could have the probable role in the transmission of the infection to humans, as pets also exhibit identical cell receptors i.e., ACE2 (Schoeman and Fielding 2019; Luan et al. 2020). Hence, the traditional protective measures must always be adopted.

Possible role of wild animals

Other wild animals, such lions and tigers, have also been confirmed earlier to be prone to the COVID-19 infection (ISID 2020e; ISID 2020f). In Oregon State, USA, 2 deaths in minks have been reported due SARS-CoV-2 (OIE 2020c). The problem started on commercial premises on 24th March 2020 and cases of SARS-CoV-2 were confirmed positive at the National Veterinary Services Laboratories, based upon molecular testing results (PCR and sequencing), meeting case definition on 04 April 2020 and these cases were reported to OIE after 2 deaths on 27th November 2020. This is the first confirmed detection of SARS-CoV-2 in mink from Oregon, USA. Reported clinical signs were inappetence, coughing and mild

respiratory signs, including sneezing. Mortality rates on the affected premises remained normal for this time of year and no respiratory or gastrointestinal signs have currently been observed. The colony is reported to be free of Aleutian Disease, making it unique amongst mink colonies in the United States affected to date. The affected premises reported positive COVID-19 humans who were in contact with the mink. State Officials in Oregon are working closely with Federal One Health partners to follow up and monitor the situation (OIE 2020c).

In another study in the Netherlands, minks usually raised for fur are also found vulnerable to the SARS-CoV-2 infection and have the capability to transfer the virus to other animals (van der Poel 2020; Oreshkova et al. 2020). On 23rd April 2020, first ever case of mink infected with the SARS-CoV-2 virus was reported from the Netherlands in highly populated area of mink farms. According to the Dutch government, it is believable that some employees were infected previously with SARS-CoV-2, who transferred the virus to mink farms and created a most likely situation for the infection to farmed animals. Besides that, no other indications were found, showing that these animals served as source of infection for humans (van der Poel 2020; Oreshkova et al. 2020). Since the reporting of the first ever case, countries like France, Spain, Italy, Denmark, and other countries also testified similar COVID-19 cases in the mink as found in Greece (OIE 2021). All of this evidence indicates the possibility of human to animal spread. Thus, the persons who work on wildlife are in close contact with such animals and/or work for wildlife management and are in close contact with wildlife species, should follow stringent preventive measures to avoid the spread of the virus. SARS-CoV-2 has been detected rarely from other animal species including rhesus macaques, African green monkeys and Egyptian fruit bats; this highlights the possibility of cross-species transmission of SARS-CoV-2 (Schlottau et al. 2020; Yu et al. 2020; Munster et al. 2020; Cross et al. 2020).

A latest study from the Netherlands investigated on the spread of SARS-CoV-2 virus from mink back to humans working on the mink farm. This observation was further reinforced by finding similarities in the viral sequence isolated from mink and from an infected employee at that farm, and provided clue to the scientists that the worker was infected with the virus transferred from a mink infected with SARS-CoV-2 virus with no apparent signs of the disease (Oreshkova et al. 2020). Asymptomatic infection of COVID-19 in mink also suggests the possible probability of mink as intermediatory host of the SARS-CoV-2. Since it has been described that various species, excluding rats and mouse, exhibit the ACE2 cell receptors it is reported that animal-to-human and animal-to-animal spread of the disease is conceivable, adding to human-to-human transmission of the pathogen (Schoeman and Fielding 2019).

Pathogenesis of SARS-CoV-2

The structure of CoVs is not so simple. These viruses are ~ 80–220 nm in diameter and enveloped with icosahedral symmetry. These viruses consist of a non-segmented, single-stranded positive sense RNA genome, measuring ~ 26–32 kb (Helmy et al. 2020; Kiros et al. 2020; Wiersinga et al. 2020). Among all RNA viruses, CoVs are the largest viruses. Being spherical enveloped virus, the SARS-CoV-2 has a diameter of 50–200 nm, with a single-strand (30 kb in length), positive-sense RNA genome (Kakodkar et al. 2020; Mousavizadeh et al. 2021). The genome of SARS-CoV-2 shares sequence identification of SARS-CoV (79.6%) and Bat-CoV (96%) (Zhou et al. 2020; Kiros et al. 2020). Four main structural proteins are present in the membrane of SARS-CoV-2 virus, namely, small envelope (E) glycoprotein, spike (S) glycoprotein, nucleocapsid (N) protein and membrane (M) glycoprotein (Tok and Tatar 2017; Fig. 4). The outermost layer of the virus is composed of S glycoprotein that initiates attachment to the ACE2 receptors on the host's target cells (Luan et al. 2020). The M protein, which is the amplest protein, forms the shape of the virus. Along with other structural proteins, it plays a main role in viral get-together (Schoeman and Fielding 2019). The N protein (RNA binding protein) has the main functions of binding of the viral genome (RNA) into a long helical nucleocapsid structure (Kiros et al. 2020; Kang et al. 2020).

Though it is stated that pathogenesis of COVID-19 is not clear, however, in most of the cases, it primarily affects the lungs. Ali et al. (2021) have stated in this regards that the key mechanism for SARS-CoV-2 invasion is the binding of the virus to the ACE2 membrane-bound receptor and the host cell's internalization of the complex (Zhang et al. 2020). ACE2, a glycoprotein and metalloprotease, is present both in membrane-bound, as well as in soluble, forms (Jia et al. 2009). The membrane bound form is comprised of a transmembrane domain, which anchors its extracellular domain to the plasma membrane, whereas it is degraded and secreted in its soluble form, while the circulation of the N-terminal ectodomain is scarcely detectable (Gheblawi et al. 2020). The virus can pass through the mucous membranes of upper respiratory system, especially larynx and nasal mucous membranes, then through respiratory tract, and finally enters the lungs (Gennaro et al. 2020). At this point, the virus targets the organs including lungs, gastrointestinal tract, heart, and renal system, which express ACE2 receptors (Rose-John 2018; Chen et al. 2020a; Bennardo et al. 2020). The virus instigates a second attack, leading the patient's ailment to worsen around 7 to 14 days after onset. Reduction in the number of B lymphocytes may occur in the initial stage of the disease, which may result in the distressed production of antibody in the infected individuals. Besides, IL-6 is also increased in a significant amount, which also performs an important role in worsening the infection due to COVID-19 around 2 to 10 days after the onset of the infection along with other inflammatory factors (Weiss and Leibowitz 2011; Gennaro et al. 2020).

Precisely, it is quite fair to say that SARS-CoV-2 habits the ACE2 receptors. These ACE2 receptors are mostly found on mammalian cells for attachment (Astuti and Ysrafil 2020; Hoffmann et al. 2020; Luan et al. 2020). The binding of S glycoprotein to the ACE2 receptors paves the

admittance of SARSCoV-2. In this regard, two pathways have been proposed (Mahmoud et al. 2020). In the first pathway, endosomes are the target for SARS-CoV-2 entry, followed by endocytosis (receptor-mediated). With upsurge in the H+ influx into the endosome stimulates cathepsin L, resulting in the cleavage of S glycoprotein (Lisi et al. 2020). This proteolytic cleavage within S glycoprotein exposures the interior fusion peptide, that is present close to the cleavage site. Therefore, upon S glycoprotein cleavage, the combing peptide fuses with the host cell membrane and facilitates the virus entrance into the cell (Astuti and Ysrafil 2020; Mahmoud et al. 2020). The other pathway is non-endosomal, the joining of SARS-CoV-2 S glycoprotein to the ACE2 is pursued by cleavage of the viral S glycoprotein through transmembrane protease serine 2 on the surface of the host cell (Fig. 5). This tempts straight combination of the viral and plasma membranes, resulting in the entry of viral particle into the cytoplasm (Lisi et al. 2020; Mahmoud et al. 2020). After the virus entry into the host cells, uncoating of viral genome occurs, then it is transcribed and translated (Mousavizadeh et al. 2021).

Laboratory animal models

The animals which can carry human diseases are of prime importance to develop the understanding of pathogenicity and to investigate effectiveness of vaccines and therapies. Formerly, for the study of MERS-CoV and SARS-CoV different animals including hamsters, mice and non-human primates, which showed viral replication with signs and symptoms of infection similar to human infection, were utilized as animal models (Gretebeck 2015; de Wit et al. 2020). On the basis of previous studies, non-human primates and small laboratory animals are suggested as preferred animal model for the study of SARS-CoV-2. As golden Syrian hamsters exhibited an efficient viral multiplication in epithelial cells of nasal mucosa and lower respiratory system and signs like weight loss, it is proposed as one of the suitable laboratory animal models to study SARS-CoV-2 (Sia et al. 2020; Chen et al. 2020b). Likewise, due to efficient virus replication in their respiratory organs without causing any disease for up-to eight days, ferrets are also proposed as a potent animal model detailed study about this virus (Shi et al. 2020). Other recent studies have suggested rhesus macaque as potential laboratory animal model for carrying out SARS-CoV-2 studies due to viral shedding, signs and symptoms similar to humans (Yu et al. 2020; Munster et al. 2020; Ying et al. 2020). Similar observations of infection after inoculation of virus have been reported in African green monkeys used to study COVID-19 infection (Cross et al. 2020).

COVID-19 Laboratory Diagnosis in Animals

Diagnosis/detection of the SARS-CoV-2 from the wild or domestic animals is akin to the viral diagnosis of this virus in humans. Timely identification of virus is very important to prevent community transmission. Specimens from respiratory tract (soft palate, nasal turbinate and tonsils) are preferred for the detection of SARS-CoV-2 (Shi et al. 2020). Rectal swabs may also be used where sampling from the respiratory tract is not possible owing to risks to the humans or animals (OIE 2020c). The samples should be transported in the triple packaging system to the laboratory (WHO 2020a).

Virus culture can be performed by inoculation of nasopharyngeal samples on vero cell lines (Kim et al. 2020). Culture of the virus is valuable in its isolation and characterization. However, cell culture for isolation of the virus is not recommended for diagnostic purposes due to its high risk of transmission to the laboratory workers.

Serological assays were used extensively to study the coronavirus outbreaks in the past and these serological tests have been played an important role for the diagnosis and understanding of the disease (Chen et al. 2004). The immunological tests can provide the valuable information and aid in the diagnosis of the SARS-CoV-2 by measuring the antibodies generated by host body's, whether the host has shown any symptoms or does not exhibit any signs of the infection. Different reports documented great specificity for the detection of viral IgM and IgG for serological diagnosis of COVID-19. However, sensitivity range of 70-85% for the detection of IgG and IgM makes the testing unsuitable for active cases (Xiang et al. 2020). In general, immunoassay to detect antigen of SARS CoV-2 virus in the form of lateral flow assay are used for rapid detection of COVID-19 (Tang et al. 2020). However, the previous experience for the detection of influenza viruses using lateral flow assay for antigen detection shows that the lateral flow assay can give false negative results due to low viral load in the tested samples. Even though the serological tests have certain restrictions, these immunological tests may play a critical role in the future for the detection of recovered individuals from COVID infection. The serological results also help us in selecting the convalescent plasma used to treat COVID-19 infection (FDA 2020). According to Ali et al. (2021), myoglobin and C-reactive protein (CRP) are specific risk factors related to mortality and highly correlated to organ failure in COVID-19 disease.

Real-time RT-PCR is the widely used technique and is gold standard for the etiologic identification of SARS-CoV-2 in animals (Richard et al. 2020; Zhong et al. 2020). In the recent emergence of corona virus and need of rapid and reliable detection of virus, real time RT-PCR is one of the most reliable laboratory tests for the detection of specific target genome, pursuing and studying the COVID-19 infection in a closed system, which also reduces the chances of false positive results (Sethuraman et al. 2020). Besides real-time RT-PCR assays, many other molecular diagnostic assays are being developed and used for COVID-19 infection worldwide. These include Reverse transcription loop-mediated isothermal amplification (RT-LAMP), multiplex isothermal amplification followed by microarray detection (Tang et al. 2020).

Molecular target has been recognized within the RNA of COVID-19 virus; for example, nucleocapsid (N), helicase (Hel), envelope glycoproteins spike (S), envelope (E) and

transmembrane (M), RNA-dependent RNA polymerase (RdRp), open reading frames ORF1a and ORF1b and Hemagglutinin-esterase (HE) can be used for the SARS-CoV-2 detection through molecular tests (Tang et al. 2020). However, based on numerous studies conducted worldwide for the detection of COVID-19, it is suitable to use a minimum of two genomic targets, one specific region and one conserved region, to avoid the false negative results due to potential genomic drift in the COVID-19 virus (Corman et al. 2020; Chan et al. 2020; Tang et al. 2020).

One health approach

Keeping in view the corona virus infection, first 20 years of this century have appeared to be a nightmare for the mankind, as well as animals, in almost all the countries around the world. After SARS-CoV and MERS-CoV infections, the SARS-CoV-2 is re-emerged as third zoonotic CoV infection in December 2019, which has caused global crisis due to widespread COVID-19 infection (Anonymous 2020; Charf 2020). The emerging and re-emerging of infectious diseases capable of jumping the species barrier evolved from animal reservoirs to infect humans has increased over the past three decades. The risk of emergence with rapid spread of novel infectious agents is increasing due to increased travel and trade around the globe (Jones et al. 2008). The examples of these pathogens include highly pathogenic avian influenza (HPAI) viruses, Ebola virus, MERS, and SARS-CoV-2. The later has affected almost all the sectors regardless of underdeveloped or developed countries, including economics, healthcare system, infrastructure service, production sectors and trade of the countries (Qamar 2020). The worldwide crisis due to SARS-CoV-2 infection has strengthened the significance of principles of One Health in the global governance of infectious diseases to prevent and control the zoonotic diseases. These infections can impose vast impacts on health of the communities, as well as cause social and economic crises (Gatiso and Bureau 2020). The spread of such infections can result due to numerous limitations in domestic and global governance arrangements (Salata et al. 2020). Since COVID-19 is a novel zoonotic pathogen with many unrevealed features including modes of transmission, intermediate host, pathogenesis and ecological aspects, there is an immediate need of creation of collaborated setting to control the disease. The applied execution of the One Health approach is much difficult and challenging for many of the countries, as well as worldwide, due to certain limitations in the work structures (Zowalaty and Järhult 2020; Lee and Hsueh 2020). However, association of various authorities was sought after the widespread COVID-19 infection (Tiwari et al. 2020; Ahmad et al. 2020). The concept of One Health has been accepted by different countries during this outbreak and coordinated methodologies have been opted between public health, medical, environmental, food safety, veterinary, wildlife departments and many others for better control of the COVID-19 infection (Zowalaty and Arhult 2020). This association involved development of infrastructure for disease surveillance in both humans and animals for the diagnosis, treatment and prevention of the COVID-19 infection. As the human, animal, and environmental health are interlinked, the prime and imminent efforts focused on finding the source of the disease, its modes of transmission among different species and humans, screening as well as monitoring of infection, contact tracing, diagnosis, treatment, proper infection control and prevention, isolation of the pathogen, quarantine and cure of infected persons, public awareness related to prevention and control of the disease and facilitation of infrastructure are required by all concerned sectors of medical, veterinary and environmental sciences and specialized persons of various departments for the effective management of current crises. COVID-19 cases in humans are increasing owing to a very efficient transmission of disease from one person to another, leading to a consequent increase in the infection among the wild, as well as companion, animal species. This could be due to some particular features of the coronaviruses that enable them to cross inter-species barriers (Leroy et al. 2020). Though the evidence of animal-to-human transmission is not available, still collective insights from environmental, social, animal, and human health sciences are required to control this pandemic virus.

Prevention and control of the COVID-19

In the early phases of the SARS-CoV-2 infection, it takes some time to implement the stringent control and preventive measurements. Early detection/recognition, isolation, and management of disease-ridden persons, as well as awareness about the essential prevention and control measures, needs to be implemented for a long-term achievement of control of the disease. Development of the specific therapeutic drugs and vaccines can play a vital role to prevent this emerging pathogen. Nevertheless, completely depending on health measures will not control the disease. So, effective restrain of this fatal pathogen can be achieved through enforcement of One Health approach. Preclusion and the control of the COVID-19 is primarily based on mitigation of person-to-person transmission of the pathogen through use of personal protective equipment like facemask, following personnel hygiene protocols, temperature screening, social distancing, timely testing, surveillance measures and quarantine of infected persons, as well as individuals with recent travel history (Gasmi et al. 2020; Lipsitch et al. 2020; Hellewell et al. 2020). As different experimental results have indicated that origin of COVID-19 virus is linked to a seafood market in Wuhan, the prevention and control of COVID-19 virus can be attained by reducing the transmission of the pathogen through identification of the susceptible animals (Xiao and Torok 2020). Different studies have shown that pets and other animals are susceptible to COVID-19 virus. So, it is highly advisable that infected and suspected individuals must have limited contact with the animals to reduce the chances of infections to the pets and other animals (WOAH 2020).

Conversely, basic hygienic procedures/measures should always be opted while restraining/handling of animals and inappropriate consumption of animal products should be avoided (Kiros et al. 2020; WOAH 2020). In addition, animals should also be kept isolated at the home, specifically the pets belonging to SARS-CoV-2 infected persons to avoid animal-to-animal spread (WOAH 2020). Keeping in view both public health and ecological reasons, many agencies have proposed a ban on legal and illegal trade of wild animals for their meat and other products to thwart the advent of newly emerging viruses having zoonotic importance (Singh et al. 2020). Overall, restraint and control of the COVID-19 in both animals and humans can be achieved through application of one-health approach. As per recommendations of USDA and OIE, food safety and hygiene measures should be implemented (OIE 2020c). The spread of the virus can further be minimized by using disinfectants on different surfaces. Keeping in view the contagious nature of the SARS-CoV-2, besides public health hygiene measures and isolation of diseased individuals, implementation of environmental cleanliness and hygiene strategies are also very essential for the prevention of the COVID-19 infection (WHO 2020c; EPA 2020).

Conclusion and future perspectives

Control of coronaviruses is a matter of concern due to emerging zoonotic infections caused by these viruses over last few decades. Coronavirus is a large family of viruses which cause infection in several animals including cattle, camels and bats, as well as in humans. Viruses of this family usually cause cold-like illnesses. Some of coronaviruses only cause infection in the animals, like feline and canine coronaviruses. However, COVID-19 appeared as a newly emerged zoonosis, which has not been understood properly and requires careful handling in both humans and animals. International efforts depend greatly on the research carried at the infection location to detect/isolate viral characteristics of COVID-19. Earliest studies related to determination of origin, source and root cause of the infection revealed cross-species jumping of COVID-19 virus from animals to humans. In the future, serological studies of domestic, as well as wild, animals living in the close surroundings to humans are required to know and prevent expected spillover of other bat-related coronaviruses. The early detection of probable spillover can be achieved by a robust surveillance system for the detection and understanding the circulating viruses in animals with possible risk of zoonotic infection to humans. This may also be helpful in the prevention of human to-human transmission of a possible epidemic or pandemic. At present, around 200 viruses of different families have been identified and there are a lot of more viruses still to be discovered in the bats (Moratelli and Calisher 2015). Due to enormous reservoirs of viruses, bats are proposed to cause transmission of pathogens. However, intricacy of spread in the natural systems, variations observed in experimental designs and understanding of results is rarely definite due to occasional evidence of transmission of virus from bats to humans and indicates need of more extensive studies. Global spread of COVID-19 infection in a very limited duration of time suggests that pets can play a vital role in the disease transmission. A recent study has suggested that mink could cause animal to human viral spill. In the light of these evidences and rapidly evolving virus, human population could be hypothetically disease-ridden with the SARS-CoV-2 via animals such as pets, domesticated species and wild animals. Thus, precautionary actions should be taken while dealing with pets and other animals. For the prevention of resurgence of COVID-19, detection of virus specifically in pets of owners who are positive for corona virus is very important. Undesirable abandonment of pets should be evaded. Side by side, prevention of further transmission of virus can be achieved through tracing and surveillance in the animals. Moreover, zoonotic threats due to COVID-19 need to be evaluated in more details to prevent the re-emergence of SARSCoV-2 in the future.

Different technologies for the diagnosis of the pathogen are available for the community. Among these, real time RT-PCR assay is the test of choice for the diagnosis of the SARS-CoV-2. Moreover, serological tests work as supplementary tools for the screening of individuals and community. Both assays in combination can help us to confront against this outbreak, which imposes huge impact on global economy and life of the people. For the proper diagnosis of the case, it is necessary to take the correct specimen sample at correct time. In summary, through appropriate use of these tests, we can identify the SARS-CoV-2 at the earliest to save the lives and prevent the further spread of the disease.

During the initial period of disease spread, public health measures are implemented for the effective control of the COVID-19 outbreak, which may play a vital role in the long-term prevention and control in the predisposed group of people. However, crises due to COVID-19 infection cannot be resolved by depending on public health measures alone. Therefore, efforts need to be imposed under One Health approach for effective control of COVID-19. The rapid spread of this emerging zoonosis worldwide potentiates environmental health approach to understand drivers and control elements of the disease. Furthermore, implementation of One Health approach including public health, wildlife, veterinary and other associated professionals could be helpful in tracing/detecting the infection, identification of associated risk factors, reducing threat to susceptible and formulating improved prevention and control strategies. So, we recommend that all countries improve their strategy through key concept, with human, animal and environmental health be considered in a unified way to control the crises due to COVID-19.

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SOCIO-ECONOMIC IMPACT OF COVID-19 ON LIVESTOCK

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INTRODUCTION

On 11th of March 2020, World Health Organization (WHO) stated that COVID-19 had become a pandemic disease. In last week of December 2019, the disease started from a report of human beings having pneumonia from an unknown source that was epidemiologically connected to a seafood marketplace in Wuhan city in Hubei Area of China. After research on the causative virus, the disease was named as corona virus disease 2019 (COVID-19). The causative agent is narrative human CoV. It was first named Novel Corona Virus 2019, abbreviated as nCoV-2019, then International Committee for the Taxonomy of Viruses, Corona Virus Study Group, named the novel virus as SARS Corona Virus 2 and abbreviated as SARS CoV-2 (Gorbalenya *et al.* 2020). Corona Virus pandemic disease (COVID-19) is now being considered as a public health disaster for the whole world. The disease, caused by corona virus specifically named as severe acute respiratory syndrome corona virus type 2 (COVID-19), appeared in China on 22nd of December, 2019 and was stated a pandemic by the World Health Organization (WHO) on 11th of March in 2020 due to its global reach. This disease has now been reported in almost 213 counties and territories, with more than 7.8 million definite patients and above 0.49 million humans died internationally.

COVID-19 is transmitted from humans to humans by inhaling aerosol, droplets or through getting in touch with infected surfaces, while its transmission by fecal and oral route is still under consideration. Before this, the world had also seen the appearance of severe acute respiratory syndrome, also called as SARS, outreached in China; and also the outburst of Middle East Respiratory Syndrome (MERS) in the Kingdom Saudi Arabia. These diseases were also caused by Corona virus strains. Corona viruses (CoVs) are divided into four different classifications, named as Alpha-CoV, Beta-CoV, Gamma-CoV and Delta-CoV. Each has its own host, such as humans, swines, dogs, birds, and other animals. To date, there are several human CoV. The alpha-coronaviruses are named as HCoV-229E and HCoV-NL63. The HCoV-229E transmits to humans by alpacas and is coming from bats, while HCoV-OC43 originated from rodents and was transmitted to humans via cattle. The beta-corona viruses are named as SARS-CoV that causes SARS, while MERS-CoV that causes MERS and the latest is SARS-CoV-2 that causes COVID-19 (Corman *et al.* 2014).

Zoonotic Perspective

The incidence of three extremely pathogenic CoVs outbreaks with a zoonotic origin was recorded in just two decades, enforcing the animal role in creating CoVs with augmented virulence that can acclimatize to human beings, resulting into epidemics and finally pandemic. Thus, the understanding of how zoonotic aspects can affect the human diseases outbreak is important due to the unpredictable nature of these family viruses, evolving and generating strains with different biological properties. This chapter aims to present comprehensive current literature of the three pathogenic CoVs that have already become epidemics and pandemics by showing the structural differences and the zoonotic perspective, such as the animal reservoirs and potential intermediate hosts or the susceptible animals. When the World Health Organization (WHO) has declared COVID-19 as a pandemic disease, it was discussed by all researchers at each level. That's why veterinarians are also considering such pandemics more from Zoonotic point of view to save animals and humans. In the past, MERS and SARS outbreaks affected humans, as well as food, animals. Comparison of SARS, MERS and COVID-19 causative agents structure, their mode of transmission and effects on community are being studied. It is need of time to set policies worldwide to manage the COVID-19 pandemic. By learning from previous outbreaks, some plans must be made and studies should be conducted to overcome this pandemic. The chapter also aims to present and compare the animal reservoirs of these three diseases by collecting information from various sources. With this information, a better understanding about zoonotic aspect of these diseases will be clear. Moreover, better surveillance and vaccine development are expected to be obtained in the near future (Cavanagh 2007; Pedersen 2014).

Source of Transmissions

A wet and humid environment is most suitable for the survival of this COVID-19 virus. During the outbreak of COVID-19, paper documents, paper money and couriers were also considered as its source of spread, because of its

highly infectious nature. The virus remains on different inanimate objects from 2 hours to 9 days. It can persist on inanimate objects for up-to 30 days. Transmission risk is low via contacting the contaminated paper, while it is high via respiratory and fecal specimens at room temperature. Poorly ventilated areas increase the chances of virus survival, and absorbent material like cotton have low risk of virus comparative to non-absorbent material used for covering and protection from viral protection (Fletcher *et al*. 2020).

Deactivation of Virus

Corona virus (COVID-19/SARS-CoV-2) is ahighly contagious and zoonotic virus. It is transmitted through respiratory droplets, aerosols or contacts. Its frequent route of transmission is touching of infected inanimate public and laboratories surfaces, while due to the highly contagious nature nosocomial infection is also very common. Because of its highly infectious nature, a broad range of disinfectants are used for the inactivation of this virus. In histology and histo-pathological laboratories, a routine tissue processing protocol including the heating of samples in liquid paraffin, inactivates the virus. Use of 80% ethanol is highly effective for deactivation of the virus. Moreover, tissue preservation in formalin for 24 hours and in glutaraldehyde for 24–48 hours can also inactivate the virus (Luqman *et al*. 2020).

Presence of Virus in Raw Water

There is a vital need to give crystal clear information related to the competence of the water disinfection treatments that is being practiced now-a-days to stop the spread of this virus. The health of workers dealing with hospitals and quarantine centers raw waste water should also be confined by subsequent finest protection practice. This emphasizes the call for the consideration of the possible hazard to developing societies from ecological spread in a different way than those who are provided by sufficient disinfection of raw waste water. It has therefore, been requested to the officials of developing and under developed countries to incorporate safe drinking water supply, leisure water environments, as well as management of raw waste water in the fight against the Corona virus. A final step of disinfection ought to be enforced immediately if existing raw waste water treating centers are not boosted to get rid of viruses like COVID-19 (Wang *et al*. 2020).

Presence of Virus in Waste

Since the presence of COVID-19 virus in feces sample has been reported, it has been obvious that this virus is also widespread in human waste water, specifically to the societies near to the hospitals and quarantine centers (Wang *et al.* 2020). COVID-19 virus has been recognized in sewage water in Australia (Ahmed *et al*. 2020), Italy (La Rosa *et al.* 2020); Netherlands (Medem *et al.* 2020) and France (Wurtzer *et al.* 2020). The first ever report of COVID-19 finding in sewage appeared from Netherlands shows its scientific possibility (Medem *et al.* 2020). A study in some areas of France indicated the continuation of COVID-19 in all type of raw, as well as waste, samples of water and almost 6 out of total 8 treated waste water samples were positive for the virus (Wurtzer *et al.* 2020). Likewise, almost 6 out of about 12 raw waste water samples coming from the hospitals and quarantine centers in Italy were positive for COVID-19 infection (La Rosa *et al*. 2020). It has been expected that approximately 5% of total samples of feces were tested positive for COVID-19 in the testing phase, while the figure of clinically definite cases was about less than 1%. The total number of affected patients in Australia that has been expected from unprocessed waste water was parallel with the clinical observations gained from tested positive patients (Ahmed *et al.* 2020). On 16th of April 2020, Reuter's news revealed that Government of Australia was by then planning to observe its sewage and raw waste water for the occurrence of COVID-19. A study in the United States depicted those advanced levels of COVID-19 in waste water than what would have been predictable from confirmed clinical cases in different localities (Wo *et al.* 2020). Studying raw waste water for COVID-19 has, thus been planned as a caution and corresponding move to study the occurrence of COVID-19 infection (Medem *et al.* 2020; Ahmed *et al.* 2020; La Rosa *et al.* 2020, Wo *et al.* 2020; Wurtzer i 2020). As per report of World Water Development in 2017, it can be depicted that almost 80% of raw waste water worldwide has been spread in the environment devoid of sufficient treatment. Raw waste water inspection for COVID-19 could offer an impartial chance to find out its epidemiology in different countries that have restricted measures for clinical diagnosis of the disease. Though there is at present no proof of COVID-19 spread by contact to raw waste water coming from aerosols, this spread route was recognized during the severe acute respiratory syndrome occurrence back in 2003 (Hung 2003). Raw waste water aerosols, produced by a faulty raw waste water plumbing organization, were recognized as a possible way of spread the infection inside an accommodation block in the Hong Kong (Hung 2003). The incidence of COVID-19 in raw waste water may, in addition have after effects for human health in the developing countries that are deprived of sewage and water technical disinfection potential, infrastructure, inadequate institutional facilities and low investment for this sudden outbreak. Raw waste water related exposure to COVID-19 remains an important option in such susceptible societies (Wu *et al.* 2020). Similarly, it is an ordinary observation to deposit hospital raw waste water with improper and insufficient action into lakes and canals, which downstream has been utilized for drinking purpose in different areas for human, as well as animals in under developed countries. Cases coming from unintentionally polluted drinking water with unprocessed sewage are common in developing and under developed countries and have also been reported in some developed countries around the globe (Kujansuu *et al.* 2019). Lastly, the use of raw waste water coming from contaminated

irrigation water source has the possible effect on the quality of soil and possible boost in crops or the contagion of ground water assets coming from hospitals and quarantine centers by the virus if there.

In the developing countries of the world, a supplementary spread way may be through faecal and oral contact. Theoretically on this phase, the transmission by fecal and oral route has been accounted for spread of many viral diseases like Hepatitis A, Hepatitis E, Hemorrhagic fever and Ebola Virus Disease (Heller 2020). It turns out to be a critical reflection when almost 5 billion people worldwide are lacking right to safely use managed cleanliness, as reported by UNICEF/WHO in 2019. Reserves into raw waste water transportation would also be necessary to add in scheming outbreaks of water borne diseases like Hepatitis. Effective raw waste water disinfection treatment and reduce ecological spread of contaminated raw waste water is also crucial at this stage (Wu *et al.* 2020). A major study gap stay alive concerned with the persistence, spread and destiny of COVID-19 in raw waste water coming from hospitals and quarantine centers. Moreover, the environment requires better perception of the ecological persistence, dynamics and transmission of this virus as well. Research is need of the hour to examine relations of the virus with the environment surface and its mode of spread, persistence, and fate in soils, as well as in the aquatic environment, and most importantly in the food chain of under developed and developing countries.

Structure of SARS-CoV-1, MERS-CoV and SARS-CoV-2

SARS-CoV is an enveloped positive-strand RNA virus, having about 30 kb nucleotides (Marra 2003). Genome of SARS-CoV-2 also contains positive-strand RNA. SARS-CoV-2 can encode a minimum of 4 chief structure proteins, named as envelope protein (E), membrane protein (M), nucleocapsid protein (N) and spike protein (S) (Wu 2020). S protein, which is type 1 glycoprotein in nature, lies on the exterior of this virus and it first gets in touch with the host cell. Because of its role in receptor binding, S protein is significant in attachment of virus. Like SARS-CoV and SARS-CoV-2, MERS-CoV also contains four main structural proteins, as have been previously mentioned. Angiotensin-converting enzyme-II (ACE II) is attached to the receptor-binding motif, abbreviated as RBM, in the receptor-binding domain which is denoted as RBD of SARS Corona virus. It serves as a receptor for the virus SARS-CoV (Li *et al.* 2005; Li *et al.* 2003). ACE2 is extensively scattered in heart, liver, kidney, testis and intestine. Its basic role is in functions of kidney and heart and also in regulating blood pressure (Anguiano *et al.* 2017). In recent times, it has been suggested that the entry of SARS-CoV-2 in human cells is promoted by ACE2 (Letko *et al.* 2020; Zhou *et al.* 2020). SARS-CoV-2 has receptor-binding domain, known as RBD, which interacts with human ACE2. Therefore, this enzyme is known as the SARS-CoV-2 receptor (Luan *et al.* 2020). The cellular receptor of both SARS-CoV and SARS-CoV-2 is ACE2, while the MERS-CoV recognizes the dipeptyl peptidase 4 (DPP4) (Raj 2013).

The Natural Reservoir and Intermediate Host

Wild animals, including bats, are considered natural resources and have an important role in transmitting highly pathogenic viruses, such as Ebola, Coronavirus, and others. Animal markets feared the outbreak of the 2002-2003 SARS-CoV, and are also linked to the sea food and wildlife market for SARS-CoV-2 infections. Wildlife was also thought to be involved in the emergence of SARS-CoV-2. However, it is not confirmed from which species and under what conditions the virus crossed the barrier to infect humans. SARS-COV-2 is the seventh member of the family coronavirus to infect humans, a beta-CoV that has more than 70% similarity in the genetic sequence of SARS-CoV-2. SARS-COV-2 is more likely to occur in bats, but further confirmation is needed as to whether pneumonia is directly transmitted by SARS-COV-2. Current researches have proved that the novel virus is similar to the bat corona virus throughout the genome level, indicating that bats can be potential hosts of SARS-CoV-2 (Zhou *et al.* 2020).

Due to their comparable biological characters, SARS-CoV and SARS-CoV-2 are two closely related groups. Moreover, both have animal origin that indicates their ability to infect inter-species. SARS-CoV-2 is evidently from one of the species which are presumed as the zoonotic in origin. This hypothesis is supported by the discovery of several viral sequences that are related to SARS-CoV-2 in several animal species, such as Rhinolopus bats and Malayan pangolins (*Manis javanica*) (Zhou *et al.* 2020; Zhang *et al.* 2020). Nevertheless, the coronavirus of the bat *Rhinolophus affinis* cannot bind sound to the cellular receptor of humans due to its major difference of the RBD of spike protein in RaTG13, coronavirus in bat (Anderson *et al.* 2020). On the other hand, the spike protein (S) of SARS-CoV-2, can attach sound to the ACE2 receptors of humans, cats, ferrets and other species with high receptor homology (Wu *et al.* 2020). Some studies have shown that Rhinolophus bats (which include specimen bats in Europe) have coronaviruses closely related viruses found in raccoon dogs (*Nyctereutes procyonoides)* and masked-palm civets (*Pagumalarvata*) (Guan *et al.* 2003; Gouilh *et al.* 2018). Furthermore, it cannot be concluded that bats are a likely reservoir of MERS-CoV, although MERS-CoV bats were determined to be genetically related to coronaviruses. Some animals, such as the dromedary camels, the alpaca, and the non-human primate are susceptible to MERS-CoV. To face the COVID-19 pandemic, WHO has advised people to avoid any contact with wild animals, to process animal products properly, and keep a distance (6 feet distance) with anyone with or without symptoms (Peeri *et al.* 2020).

CoV can infect the respiratory tract, gastrointestinal tract, hepatic system and central nervous system of bats, humans, birds, cattle, rodents and various other wild animals (Chen *et al.* 2020) The origin of severe acute respiratory syndrome corona virus type II is related to bats but the intermediate host has not yet been identified. Due to zoonotic aspects, the susceptibility of ferrets, as

well as other animals that have close contact with humans, to severe acute respiratory syndrome corona virus type II is very necessary. This virus multiplies poorly in pigs, ducks, chickens and dogs, but cats and ferrets are more vulnerable (Chen and Hualan 2020).

Beside these 2, cats also tested positive for SARS-CoV-2, with mild clinical signs in New York in late April, as reported by the United states Department of Agriculture and Federal Centers for Disease Control and Prevention. The diseases were transmitted by mildly ill or asymptomatic contacts or household members that might have COVID-19. Therefore, USDA recommends routine testing of animals, as the occurrence is rare (CDC, 2020; Almendros 2020).

Virus in Laboratory Animals

Macacamullata has limited utility to become SARS-CoV animal model in showing SARS pathogenesis and the evaluation of therapies. Because of its high titer in the air tract of mice and findings in mice after SARS-CoV inoculation, it makes mice a good choice to be the animal model for the vaccine, antiviral and immune prophylaxis researches (Du *et al*. 2009). The mice also can be the animal model of SARS pathogenesis. Hamsters also can be the ideal animal model because of the high titer in the respiratory tract, while other susceptible animals are ferrets, macaques and African green monkeys. Squirrel monkeys and mustached tamarin are not susceptible to SARS-CoV (Subbarao *et al*. 2006). The macaquesis are one of the MERS-CoV susceptible animals. Therefore, the macaques can be the suitable model for the study of MERS-CoV pathogenesis and vaccine development. However, ferrets, mice and hamsters are not infected by MERS-CoV. The dromedary camel and alpaca can be the animal model because of their susceptibility. Based on Jianzhong Shi's research, ferrets and cats show high susceptibility. The dogs have low susceptibility and the livestock such as pigs, chicken, and ducks are not susceptible to the infection (Shi *et al*. 2020).

Effects of Coronavirus on Global Food System

Coronavirus disease (COVID-19) has adversely affected the current food system globally. Like the health system, the stability of the food system should be a priority during a lockdown. The pandemic severely affects food safety and security. The livestock sector is a major source of animal food products. The economics of the processed milk and meat industries are being severely affected during the lockdown. Due to the lack of transportation facilities, livestock products are not available to everyone. The importance of incorporating new technologies into the livestock sector to lessen the undesirable effects of the global epidemic on the animal-based food industry cannot be over-looked. Animal production and disease resistance must be improved through breeding and genetics. The role of veterinarians in maintaining animal health and preventing zoonotic diseases during infectious diseases outbreaks should also be highlighted. Implementation of new policies in the livestock sector regarding the telemedicine, online sale and purchase of animals and their products is necessary. Such guidelines could help the state to overcome crises related to food, livestock and human health. Countries around the world have policies to prevent the spread of the pandemic, such as lockdowns, inter-regional traffic restrictions, prohibition of various activities involving gatherings and large-scale COVID-19 testing (Tesso 2020). Government has also established a community health emergency in the framework of accelerating the handling of COVID-19 through Presidential Decree No. 11 of 2020 and Government Regulation No. 21 of 2020 (Djalante *et al*. 2020). The pandemic not only has a negative impact on the health sector but also threatens various sectors, such as the food, economy, society, education and many others (Viner *et al*. 2020). Faced with the current situation, scientists and stakeholders have predicted that the current pandemic will have both short and long-term effects. Livestock is an important sector for preparation. The sector that is predicted to attain the Sustainable Development Goals (SDGs) in 2030 cannot escape the negative effects of this global crisis (Rasul 2016). Not only the impacts mentioned earlier, but also the lack of animal-derived food, is one of the challenges and risks of the current COVID-19 pandemic and post-pandemic rehabilitation period (Galanakis 2020). Numerous fields related to animal-based food supplementation have been suspended during the pandemic, so that production processes, distribution and consumption are not functioning normally (Thornton 2010; Deaton and Brady 2020).

Importance of Livestock Sector during Pandemics

Food security is primary task of every state; and like agriculture sector, livestock sector also has great importance in providing food to us all. The importance of agro-industrial complex can never be ignored, especially during pandemics. Balance between food sources and population requirements is necessary to provide sufficient food supplies during current COVID-19 lockdown situations (Monchatre 2017). As urbanization is increasing, the consumption of livestock products is also increasing continually. Farming and processing units are present only in big cities but not in every city. People of many cities face great shortage of livestock products, like milk and meat, during pandemics and lockdowns (Grace *et al*. 2020; Mhlanga and Emmanuel 2020).

Numerous fields related to animal-based food supplementation have been suspended during the pandemic, so that production processes, distribution and consumption are not functioning normally (Thornton 2010, Henchion 2017). In addition to the effects of COVID-19, the ability to produce many animal-derived products that have not yet met the requirements, the risk factor for antibiotic resistance in meat and milk, causes a shortage of food for humans. According to One Health concept, animal health is very important for keeping human healthy (Boland *et al*. 2013; Osterhaus *et al*. 2020).

Optimization of Animal Products during Pandemics

The data related to previous scientific studies about veterinary hygiene and veterinary medicine can help a lot in optimizing the supply of livestock products. By such scientific data, we want the minimum cost of livestock products by optimum parameters of the climate and maintain production mechanisms up to the optimum level (Bishwajit *et al*. 2014). *In-ovo* feeding of L-arginine amino acid increased the meat quality and final weight of birds (Luqman *et al.* 2020). Effective solutions of systems during pandemics save time, money and provide food sources to everyone (Dalvit and Cassandro 2007). Technology is the most needed tool to optimize production of animals, their feed consumption, and environmental system of animal farms and livestock products (Samarin *et al*. 2018).

Improved Genetics and Modern Breeding Technologies

Modern breeding technologies can improve genetic potential of food animals, and animal products can be increased by these procedures. Current pandemic has shed light on importance of such advances in all livestock fields (Xu *et al.* 2020) An effective breeding strategy in food animals is one of the ways in which cattle breeders can be successful. In many under developed countries, cattle selection is usually traditionally performed with phenotypic observations. This choice is less efficient. This is evidenced by the self-sufficiency of meat, which has not been announced since 2010. The mapping of Quantitative Treat Loci - encoding the quantitative role of genetic loci in food animals - is expected to provide a more accurate selection process. Animal selection through genome selection begins with an understanding of genome mapping. Genome mapping is used to identify the location of genes, locus on chromosomes and mutations (Xiong 2006). Quantitative Trait Loci or QTL is the locus that is mapped. Many of these characteristics are important, both economically and medically, such as resistance to diseases, meat and milk production. The basic concept in genomic selection is that single nucleotide polymorphisms (SNPs) are considered as a marker of the relationship between loci. Next, identification or marking of SNPs can be done by identifying QTL locations in the genome for genetic mapping of species (Mrode *et al.* 2019). However, some simple markers can be used to understand the advantages and disadvantages of these markers. The precision of genomic selection depends on the distance of the marker with QTL, the process identification of phenotype, and the number of genes to be identified (Semagn 2006). *In-ovo* technique can meet the embryonic requirement during the incubation period by increasing the viability of embryo (Luqman *et al*. 2019).

The Flow of Genetic Mapping Can Easily be Described as:

- To use SNPs as markers, the genome needs to be read using microarrays. Unfortunately, the use of micro-arrays is less common in underdeveloped countries than the use of electrophoresis.
- Using a marker other than SNP would mean an easier flow, namely: DNA extraction – amplification of DNA by utilization of PCR (polymerase chain reaction), electrophoresis using markers, Readout of results (Gutiérrez-Gil *et al*. 2007).
- Due to the limited supply of feed, animal feed management also needs to be improved to deal with food safety. Safe production of animal feed is one of the most important methods for the health and management of livestock. Genome mapping can also be done in animal feed plants. Selection through this genome can improve the accuracy of phenotypic selection, which has been done extensively in Pakistan. Several parties involved in the development of the livestock sector in Pakistan have been able to work together to obtain selective livestock genomes through the collection of integrated livestock genome data (Xiong 2006).

Branding and Digitalization of Livestock Products to Overcome Sales Decline

A previous study has shown that consumers between the ages of 15 and 25 years buy food and ingredients prepared during pandemics online (Kirtiş and Filiz 2011). This can be done through various platforms and communities that are formed on social media. Promotion through media can be done by many people with a large number of followers or paid promotion on accounts with access to large media (Salomonsson 2003). Promotional material in the form of a campaign to use nutritious livestock products during the current pandemic can be an interesting selling point. Processed food made from livestock products or local culinary products can be sold by this tricky way (Heath 2018).

The second step is delivery services, which are very important in this situation. Delivery services can be made through form employee courier or using campaign services, or it can also use e-commerce which provides delivery services and even free shipping coupons (Pueyo 2020).

Easy access to product information, payment and product delivery can help propel the market forward to keep the business going during the lockdown. Small-scale farms can encourage each other through collaboration to maximize their impact on improving livestock product development methods and marketing processes. It is expected that these two solutions will solve the problem of low market for livestock products and help in distribution of livestock products (Shaner 2019).

Processing Techniques and Marketing Facilities for Animal Products

Improving the processing techniques and marketing facilities of animal products by increasing their distribution facilities and increasing the marketing force are necessary steps to prevent food shortage during lockdown and pandemics (Galanakis 2020). If a lockdown lasts longer, then people will need animal products and by-products that are long-term stable, durable and have quality packaging (Khan *et al.* 2020). Processed livestock products like meat, which has high nutritional and water level, is the right medium for growing micro-organisms, thus making it easier to rot the meat. Therefore, proper processing of such products is much necessary. Because the meat might have to be stored for longer durations in stores or at homes in lockdown situations through freezing, the meat is cooked with selected herbs and then packaged and frozen. Freezing techniques are also used mostly in indoor areas (Rahman 1999). In addition, thermal processing and smoking products are used in other scales to avoid the damage caused by decaying conditions. Processing techniques, flavoring skills and preservation techniques are key to improving the quality of livestock products (Robinson *et al.* 2002; Chelule *et al.* 2010). The creative ability to make coffee flavor with milk can be an attractive product to increase sales during such pandemics. Some other preparations for milk processing, such as doodle milk, may be other options (Dixon *et al.* 2001).

Telehealth Facility

This situation is causing difficulties and delay in providing treatment to animals. However, this problem can be solved by using telehealth facility. Barriers in the use of telehealth at each community level should be solved at the earliest. Cases other than emergency situation can be tackled easily from the farm. In this way, animals can remain safe and food resources will not decline. Government should prepare telehealth doctors force for livestock, and related courses should be included in curriculum of veterinary education. Telehealth is safe for doctors and it will also decrease the stress for farmers and their families (Zhou *et al.* 2020).

The Future of Animal Farming

The complexity of these food issues requires a multifaceted and appropriate strategy for mutual cooperation, so that we can fight malnutrition during COVID-19 epidemics. After recovering from the current pandemic, we must be better prepared for the future crisis (Galli *et al.* 2020; Holmes *et al.* 2020) The malnutrition warning alarm starts when one-third of the world's population experiences limited mobility (Galanakis 2020). The pandemic of COVID-19 will definitely reduce the attention of different countries on livestock health and production. The Food and Agriculture Organization (FAO) recommends that countries pay special attention to food and develop strategies to deal with it in order to create food safety during coronavirus emergencies. The long-term measures are needed to be taken seriously. The livestock sector also supports success in meeting food security (Rosegrant and Sarah 2003). To improve the health of food animals, especially during pandemics and during communicable health issues, telehealth technologies should be utilized proactively in livestock sector. During the current coronavirus pandemic lockdown, farmers are also facing transport issues (Malhotra 2020).

It is responsibility of political leaders and educational institutes to make necessary policies regarding food resources management for the whole nation during pandemics (Moon *et al.* 2015) One can't say anything about the occurrence of such pandemics in future. Because epidemics and pandemics are occurring from hundreds of years and we can't say anything even about COVID-19 reoccurrence in future (Sun *et al.* 2020). But we can prepare public for such future crisis by improving our health facilities, especially those related to food animals. Since theCOVID-19 also has some origin from wet food market, direct involvement of animal health should be the first concern (Yang *et al.* 2020).

Covid-19 Vaccination Preparation and Use of Animals

Till new advance in developing vaccines against pandemic causing viruses, scientists are using laboratory animals for vaccination trials (Ahmed *et al.* 2020) Therefore, new policies and guidelines are very necessary for laboratory or experimental animal health and production management. Industry and government co-ordination can solve such problems by collaborating with academic institutes (Malhotra 2020).

Role of Veterinarians in Meat Markets

Meat markets, where animals are of different kinds (wild and domestic) are sold, are a main source of origin of zoonotic disease. Many researchers have suggested that bats are reservoir hosts for many coronaviruses, but they are asymptomatic to coronaviruses (Daszak *et al.* 2020; Tiwari *et al.* 2020). The SARS-CoV-2 was also transmitted between different species in wet markets in Wuhan city, China. Therefore, these markets should be considered by veterinarians from a zoonotic point of view (Leach and Ian 2013). Such markets cannot be banned but can be regulated by new rules and regulations. It is now imperative to reduce the likelihood of this pandemic occurring in the future through a collaborative process between veterinarians, doctors and environmental scientists, nationally and internationally. Because there is a direct relationship between humans, animals and their common environment, therefore, it is important for everyone's health to protect themselves from future outbreaks and pandemics, such as from SARS, MERS and COVID-19 like pandemics (Almendros 2020).

Conclusion

Based on the above explanation, the effects of COVID-19 have affected the livestock sector. This is evidenced by the

poor condition of the livestock market. In addition, the risk of future malnutrition and food shortage is also predicted. To solve these issues farmers should increase the immunity of their domestic animals by adding micronutrients to their rations. It is important to avoid feed shortages in the future, especially during pandemics. Healthy eating and drinking food animals are a source of healthy meat and milk. Animal food shortages can be overcome by improving the genetics of animals and animal feed plants and incorporating unconventional feed resources into their diets. Tele-health or telemedicine facilities should be improved to provide proper treatment to the farmers in case of any kind of lockdown. Increase the number of meat and milk processing plants in all cities to provide permanent fresh food to the people during any future crisis. Increasing the value addition of livestock products can ensure an increase in income. Digitization in the livestock business will make it easier, faster and safer to sell and buy livestock products. This will be especially helpful during infectious and contagious diseases. Online buying and selling, such as the use of information technology and computer science, can create new markets for livestock products and services by implementing the COVID-19 security protocol. Farm biosafety and biosecurity are essential to prevent infectious and zoonotic diseases. These suggestions can help academic researchers, food sector scientist and livestock sector to reduce losses and waste of food. New research is needed to identify alternative safe sources of protein that can meet all kinds of nutritional needs of people in under-developed countries like Pakistan.

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CURRENT UPDATES ON THERAPEUTIC AND VACCINE APPROACHES FOR COVID-19 DISEASE

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INTRODUCTION

Several infectious viruses are rapidly emerging and evolving for the last two decades, because of their rapid mechanism of mutation (Zolnikova *et al.* 2018). The viruses associated with respiratory infections are especially considered as major etiological agents of death in both developing and developed countries. It has been reported that acute respiratory infections such as influenza, pneumonia, adenovirus, respiratory syncytial virus and enterovirus infections are responsible for millions of deaths globally (Olaimat *et al.* 2020).

Coronaviruses are most commonly involved in causing respiratory, neurologic and gastrointestinal (GI) disorders (Jiang *et al.* 2020). These viruses are called so because of their crown-shaped structure attached with long surface spikes (Zu *et al.* 2020). These are highly diverse enveloped viruses with single strand, large (25-32 kb), and positive sense RNA genome (Zhu *et al.* 2020). The host organisms of coronaviruses include humans and several other vertebrates like bats, camels, mice, cats, masked palm civets and dogs (Jiang *et al.* 2020). Among these infectious coronaviruses, SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 are highly pathogenic and are linked with serious infections and mortalities in human beings (Lu *et al.* 2020; Meo *et al.* 2020). SARS-CoV-1 was first reported in 2002 in China and an outbreak of severe acute respiratory syndrome (SARS) appeared across the world, involving 37 countries with 10.87% causalities (Zhai *et al.* 2005). Later, MERS-CoV was first identified in 2012 in Saudi Arabia (Jiang *et al.* 2020; Rodriguez-Morales *et al.* 2020), causing 30% or more mortality in Middle East countries (Luo and Gao 2020). Recently, severe acute respiratory syndrome coronavirus-2 (previously known as 2019 novel coronavirus, 2019-nCoV) rapidly emerged from an epidemic in Wuhan city of China in December, 2019 to Public Health Emergency of International Concern on 30th January, 2020 and a Global Pandemic on March 11, 2020. The virus is described as successor to SARS CoV-1, the strain that caused an outbreak on SARS in 2002-2004 (Gorbalenya *et al.* 2020). Moreover, it is more transmissible and contagious than both SARS-CoV-1 and MERS-CoV. It is an enveloped, positive sense, single stranded RNA virus with a helical nucleocapsid. At the time of writing this review (17th March, 2020), a total of 121,423,007 confirmed cases of COVID-19 have been reported from around the globe, out of which 2,684,813 people have died (Worldometer 2020). It is worth mentioning that actual positive cases are much higher than these numbers, the reasons being subclinical infections, under-reporting and lack of proper diagnostic facilities in some parts of the world (Krantz and Srinivasa Rao 2020). The scale of humanitarian and economic impact drives the exploration of conventional and novel strategies for effective disease control.

Treatment Strategies

Potential non-vaccine therapy, including protease inhibitors, monoclonal antibodies and antiviral drugs is being developed or clinically tested cases. One example of antiviral drug is remdesivir, which is a nucleoside analogue prodrug under clinical investigation. Some combinations have also been shown to be of superior value to the drugs used separately. A combination thereof, is interferon beta-1b, oral ribavirin (nucleoside analogue) and oral lopinavir and ritonavir (protease inhibitors) (Wang *et al.* 2020a). Several therapeutic agents undergoing different phases of clinical trial have been considered and summarized in Table 1.

Anti-Malarial Drugs

Chloroquine and Hydroxychloroquine: Chloroquine has been considered as a drug of choice for malaria treatment and various immune disorders like rheumatoid arthritis for many years (Rynes 1997). Its anti-COVID-19 activity was found to be very low, with EC50 of 1.13 µM (Wang *et al.* 2020b). Due to these appropriate *in vitro* results of low EC50 and higher cytotoxic concentrations (CC50), chloroquine has been introduced into human clinical studies. Chloroquine was administered to COVID-19 patients in more than 10 hospitals in China. The results were promising in terms of decreasing viral load, controlling the exacerbation of pneumonia and reducing the course of disease (Gao *et al.* 2020a). Chloroquine actually inhibits the fusion and entry of the virus into the host cells and reduces the production of cytokines (Rabi *et al.* 2020). The potential effect of chloroquine against SARS-CoV-2 infection was identified in a report received during the outbreak in China (Gao *et al.* 2020a). Prior to the outbreak of COVID-19, many *in vitro* studies on chloroquine revealed its ability to inhibit viral replication of another coronavirus (SARS-CoV) responsible for causing severe acute respiratory syndrome (Keyaerts *et al.* 2004; Vincent *et al.* 2005).

Table 1: Summary of potential therapeutic candidates undergoing clinical trials for COVID -19

Drug	Trial Registration No.	Phase	Action	Posology	Reference
Antiviral drugs					
Remdesivir	NCT04292899	III	Inhibits RdRp polymerase inhibiting RNA synthesis	200 mg, OD, IV for 1 day followed by 100 mg, OD, IV for next 4-9 days	(Goldman *et al.* 2020; Sheahan *et al.* 2020)
Lopinavir + Ritonavir	ChiCTR2000029539 NCT04455958	II	Lopinavir: Inhibits 3CL protease activity, Blockage of protein processing Ritonavir: same as lopinavir	Lopinavir: 400 mg oral Ritonavir: 100 mg BD oral for 14 days	(Cao *et al.* 2020)
Favipiravir	ChiCTR2000030254 NCT04600999	Completed and recommended	Antiviral, RNA-dependent RNA polymerase inhibitor	1600 mg Oral BD for day 1, followed by 600 mg oral BD till the trial end	(Agrawal *et al.* 2020)
Ivermectin + Nitazoxanide	NCT04360356	II	Ivermectin: Antiviral/ Antiparasitic inhibits viral replication and assembly of new virions. Nitazoxanide: Antiviral, Antiparasitic, interferes with 3CL protease activity	Ivermectin 200 mcg/kg OD oral, empty stomach + Nitazoxanide 500 mg BD oral during meal for 6 days	(Patra *et al.* 2018)
Darunavir/ Cobicistat	NCT04252274	III	800mg/150 mg –one tablet oral for 5days	Protease inhibitor Darunavir: Cytochrome P-450 CYP3A Inhibitors Cobicistat: Cytochrome P-450 Enzyme Inhibitors	(Chen *et al.* 2020)
Arbidol (Umifenovir)	NCT04260594	IV	14-20 days course of 2 tablets TID	hinders trimerization of viral spike glycoprotein and inhibits host cell adhesion.	(Gao *et al.* 2020c)
Ribavirin + Interferon beta-1B	NCT04494399	II	S/C injection of interferon beta-1B 16 million IU for 5 days 400 mg BD oral for 5 days	Ribavirin: inhibit capping of viral transcripts and viral polymerase Interferon beta-1B: Enhance activity of suppressor T cell and reduce proinflammatory cytokines	(Brzoska *et al.* 2020; Rahmani *et al.* 2020)
Oseltamivir	NCT04516915	II	Oseltamivir alone 75mg twice daily for 14 days in hospitalized pts. Or IMU-838, 22.5mg BD for 14 days +75mg BD for 14 days	Oseltamivir: inhibits the neuraminidase enzyme, expressed on surface of virus. This enzyme facilitates release of virus from the infected cells and aids in movement of virus in the respiratory tract.	(Tan *et al.* 2020)
Antimalarial drugs					
Chloroquine	NCT04353336 NCT04286503	II	500 mg/dose orally BID for 7-14 days	Increase the endosomal pH and changes ACE-2 glycosylation, interfere with interaction of virus and receptor.	(Fantini *et al.* 2020; Snawerdt *et al.* 2020)
Hydroxy-chloroquine	NCT04261517 NCT04328272	III	400 mg OD for 5 days	Enhance endosomal pH and changes glycosylation of ACE-2, interfere with interaction of virus and receptor.	(Hashem *et al.* 2020)
Anti-interleukin drugs					
Tocilizumab	NCT04317092 NCT04320615 NCT04356937	II III III	1 IV infusion, dose 8 mg/kg, up to max dose of 800 mg. 1 additional dose could be administered if clinical condition worsen with no improvement.	monoclonal antibody which competitively impedes the binding of interleukin-6 to its receptor.	(Gotera 2020; Sahebnasagh *et al.* 2020; Ucciferri *et al.* 2020)
Sarilumab	NCT04315298 NCT04327388	II III	Single dose IV on day 1 and second dose may be given after 24-48 hrs of first dose.	Human recombinant monoclonal antibody which blocks IL-6 receptors, inhibiting IL-6–mediated signaling.	(Fala 2018)

Bevacizumab	NCT04275414	II	Bevacizumab 500mg + normal saline (NS) 100ml, ivdrip ≥ 90min	It inhibits angiogenesis by targeting and blocking vascular endothelial growth factor A (VEGF-A).	(Pang *et al.* 2021)
Anakinra	NCT04341584	II	At day 1, day 2 and day 3; Two IV infusions (200 mg) / day At Day 4, two IV infusions (100mg)/ day. At day 5, one IV infusion (100 mg) /day.	Interleukin 1 receptor antagonist, inhibits the production of inflammatory cytokines	(King *et al.* 2020)
Janus Kinase inhibitors					
Ruxolitinib	NCT04348071 NCT04338958	III II	2 x 10mg with defined response adapted dose increase to 2 x 20mg for 7 days	Inhibit competitively ATP-binding catalytic site of the kinase domain. Inhibit of the JAK-STAT pathway	(Yeleswaram *et al.* 2020)
Baricitinib	NCT04373044	II	Daily dose by mouth for 14 days in combination with antivirals	reversibly inhibits JAK1 and JAK2 , block phosphorylation and activation of signal transducers and activators of transcription (STATs)	(Favalli *et al.* 2020)
Convalescent anti-SARS-CoV-2 plasma	NCT04345289	III	Reported dosage varied depending upon the amount of transfused plasma and antibody titer	Binding of the transfused antibodies to the pathogenic organism, leading to phagocytosis, cellular cytotoxicity, or direct pathogen neutralization	(Bloch *et al.* 2020)

Hydroxychloroquine is a chloroquine derived drug and differs only by one hydroxyl group. It is considered to be an appropriate alternative to chloroquine with less toxic effects for the treatment of COVID-19 and is undergoing phase III clinical trial (NCT04261517) (Effectiveness of Hydroxychloroquine in Covid-19 Patients - Full Text View - ClinicalTrials.gov, n.d.). In a previous study, hydroxychloroquine was found to have more potent antiviral activity *in vitro* than that of chloroquine (Yao *et al.* 2020). Three mechanisms have been proposed for the antiviral activity of hydroxychloroquine and chloroquine. The first mechanism involves the interference of the drug with terminal glycosylation of the cellular receptor ACE2, hence inhibiting virus-receptor binding; the second mechanism involves the increase in pH induced by the drug, leading to acid cellular organelles, hindering endocytosis of virion transport and potentially affecting post-translational modification of newly synthesized molecules of virus; and third mechanism comprises the drug induced disturbance in the process of assembling of virion and viral protein molecules synthesis (Savarino *et al.* 2003; Cortegiani *et al.* 2020).

A clinical trial was performed in France with 36 confirmed COVID-19 patients. The end point was the absence or presence of virus within six days from inclusion in the protocol. The results showed a significant decrease or absence of viral load and this effect was summed up by azithromycin. An important point to be noted is that the patients included in this study ranged from asymptomatic to those with pneumonia and none was critically ill (Gautret *et al.* 2020).

Despite the lack of strong and reliable evidence of efficacy, due to the pressure exerted by COVID-19 worldwide, many health authorities have implemented official guidelines for the use of hydroxychloroquine and chloroquine for the treatment of COVID-19 patients (Liqian and Zheng 2020). Both hydroxychloroquine and chloroquine have been in clinical use for many years, and the safety profile of both the drugs is well established (Devaux *et al.* 2020). Reported side effects include gastrointestinal upset with hydroxychloroquine and retinal toxicity with long term use of both drugs (Mavrikakis *et al.* 1996; Srinivasa *et al.* 2017). In addition, heart arrhythmia and cardiomyopathy have also been reported (Costedoat-Chalumeau *et al.* 2007; Pieroni *et al.* 2011; Sabato *et al.* 2017).

Antiviral Drugs

Favipiravir: Favipiravir is a protease inhibitor drug, and was used for the first time in Wuhan, China against the SARS-CoV-2 pandemic (Agrawal *et al.* 2020). It is a purine nucleoside analogue and is included in class of pyrazines. This drug was found to demonstrate *in-vitro* antiviral activity against SARS-CoV2 with relatively high concentrations (EC_{50}: 61.88 µM) compared to remdesivir and chloroquine (Coomes and Haghbayan 2020). Another study comprising non-randomized clinical trial on favipiravir showed that the drug significantly decreased the treatment duration of SAR-CoV-2 infection and showed better therapeutic results than lopinavir/ritonavir (Cai *et al.* 2020). Its protein binding is 54%, bioavailability ~94% and small apparent volume of distribution is 10–20 L. After a single dose, it reaches Cmax within 2 hours and due to rapid renal eradication, it has a low half-life of 2.5-5.0 hours (Agrawal *et al.* 2020).

Lopinavir/Ritonavir

Lopinavir (LPV) is an antiviral agent most commonly used in combination with ritonavir for HIV disease treatment

(Meini *et al.* 2020). Lopinavir is a protease inhibitor drug that is used for the treatment of SARS-CoV-2 and suppresses 3-chymotrypsin-like protease. Lopinavir/ Ritonavir shows no statistical difference in fatality rates and no reduction in duration for medical development (Giovane *et al.* 2020).

Ivermectin/Nitazoxanide

Ivermectin is an FDA-approved broad spectrum anti-parasitic agent that also shows anti-viral activity. The causative agent of COVID-19 epidemic, SARS-CoV-2, is a single stranded positive sense RNA virus that shows severe acute respiratory syndrome (Caly *et al.* 2020). Nitazoxanide is a broad-spectrum antiparasitic drug that shows antiviral activity against influenza, hepatitis B&C, and coronaviruses. It suppresses SARS-CoV-2 reproduction at low micromolar concentrations in Vero CCL81 units and is available in oral form (Rocco *et al.* 2020).

Darunavir/Cobicistat

Darunavir is a protease inhibitor that is used for the treatment of HIV-1 disease. It is highly compatible with bone and renal profile development but has fewer positive effects on different lipoids (Deeks 2018). Cobicistat is another agent used to enhance the plasma levels of darunavir among other antiretroviral drugs and a specific cytochrome P450 3A inhibitor (Kakuda *et al.* 2015).

Oseltamivir

Oseltamivir is an FDA-approved neuraminidase inhibitor with an antiviral activity against influenza A and B, and pneumonia caused by severe acute respiratory syndrome coronavirus (SARS-CoV) (Tan *et al.* 2020). It was not effective in the treatment of COVID-19 disease with hypoxia or dyspnea in Wuhan because COVID-19 pneumonia developed resistance at the onset of therapy (Chiba 2020).

Ribavirin/Interferone beta-1B

Ribavirin is a non-interferon agent with broad spectrum of antiviral activity against extensive RNA and DNA viruses throughout the epidemic of severe acute respiratory syndrome and Middle East respiratory syndrome (Wang *et al.* 2020c). Interferons (IFNs) have a resistance against viral diseases, such as a constituent of intrinsic immunity and also against Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV. While IFN α is less commonly used than IFN β, and also essential for the treatment of MERS-CoV, IFN β is considered to be an effective agent for the treatment of SARS-CoV-2 (Rahmani *et al.* 2020).

Arbidol (Umifenovir)

Arbidol (ARB) is also called as Umifenovir, and is used for the treatment of infections caused by certain viruses, including hepatitis C virus (HCV) and influenza A and B viruses. ARB may suppress COVID-19 disease by intrusive relief of SARS-CoV-2 from receptor-mediated follicles (Nojomi *et al.* 2020). It may also show a suppressive effect on a wide range of diseases for example RNA viruses. However, there is an inadequate indication to promote the use of this medicine for improvement of infected persons with COVID-19 (Teimury and Khaledi 2020).

Given all the benefits of therapeutic management of disease, the need for prophylactic management does not diminish. A safe vaccine has yet to be developed and evaluated to provide effective strategy for controlling the pandemic. Therefore, there is an ultimate need to vaccinate the entire population against SARS-CoV-2.

Convalescent Plasma Therapy

Currently, convalescent plasma has been approved by the FDA under Emergency IND (eIND) for severe and life-threatening condition of COVID-19. Clinical trials are underway, as the convalescent plasma contains antibodies donated by confirmed COVID-19 recovered patients, and its administration may theoretically assist to clear virus. Convalescent plasma has already been administered against viral infections of MERS, H1N1 influenza and SARS (Soo *et al.* 2004; Arabi *et al.* 2015).

Limited data are available regarding the efficacy of convalescent plasma against SARS-CoV-2. In a case study, the clinical symptoms and status was improved with the administration of convalescent plasma to 5 severely ill COVID-19 patients (Shen *et al.* 2020). In another study, 10 critically ill COVID-19 patients showed improvement in laboratory reports within 3 days and lung lesions within 7 days following administration of convalescent plasma (Duan *et al.* 2020). However, further clinical studies are needed to determine the advantages and limitations of current therapeutic protocol. Many clinical trials are going on and mentioned on clinicaltrials.gov (NCT04333251, NCT04323800, and NCT04345523). Due to scarcity of clinical trial data, NIH neither recommend for nor against convalescent plasma administration (COVID-19 Treatment Guidelines 2020).

Adjunctive Treatments

Multiple cohort studies in patients with COVID-19 showed that most patients died due to increased interleukin-6 (IL-6) levels and serum ferritin levels. Moreover, in another cohort study, the ICU patients were observed with higher serum levels of IL-2, IL-7, interferon-γ inducible protein 10 (IP10), tumor necrosis factor α (TNF-α), granulocyte colony-stimulating factor, monocyte chemoattractant protein 1 (MCP1) and macrophage inflammatory protein 1-α (MIP1A) (Grasselli *et al.* 2020; Ruan *et al.* 2020; Zhou *et al.* 2020a). These hyperinflammatory reactions in patients with COVID-19 suggested a cytokine release syndrome (CRS) (Chiang et al. 2008; Ramos-Casals et al. 2014; Huang et al. 2020; Mehta et al. 2020). Cytokine release syndrome in the most critical cases leads to intravascular coagulation, circulatory shock, and multiorgan failure

(Shimabukuro-Vornhagen et al. 2018). These observations have sparked the importance of immunosuppression therapy, including anticytokine agents, corticosteroids and immune-modulatory agents. These active agents not only assist to reduce mortality rate but also improve the clinical outcomes.

Corticosteroids

A classical immunosuppressive drug, methylprednisolone, has been used against the progress of pneumonia and proved to be effective for the treatment of acute respiratory distress syndrome (ARDs). Patients with COVID-19 pneumonia have been observed to develop acute respiratory failure (Huang *et al.* 2020). Methylprednisolone is, therefore considered to be a candidate drug for use in critically ill cases. In a clinical study with 201 COVID-19 patients having ARD, administration of methylprednisolone (1–2 mg/kg/daily IV dose for a week) reduced the mortality rate (Wu *et al.* 2020). In another study with 46 severely ill COVID-19 patients, early administration of low-dose of methylprednisolone improved the clinical status and shortened the disease course (Wang *et al.* 2020d). Another short and limited study with 15 patients and no control group indicated the usefulness of low dose corticosteroids treatment in severe COVID-19 pneumonia patients (Zhou *et al.* 2020).

A fluorinated corticosteroid, dexamethasone has also been considered earlier to be used in the treatment of ARD. In a clinical trial, it was found that the use of dexamethasone in patients with moderate to severe ARDS significantly reduced the duration of mechanical ventilation, ICU mortality and all-cause mortality at 60 days (Villar *et al.* 2020). However, the use of corticosteroids in ARDS due to COVID-19 remains more complicated and controversial than historical ARDS literature might suggest. Clinical trials are currently going on or are expected to carry out to investigate the role of hydrocortisone or methylprednisolone in COVID 19 with severe hypoxia and ARDS, either administered alone or in combination with anti-interleukin agents and can be seen on clinicaltrials.gov (NCT04244591, NCT04330638, NCT04348305 and NCT04323592).

Interleukin-6 Receptor Antagonists

The use of Interleukin-6 receptor antagonist is currently suggested only in the context of a clinical trial (Alhazzani *et al.* 2020).

Tocilizumab

Tocilizumab is a humanized monoclonal antibody which blocks both soluble and membrane-bound IL-6 receptors. Interleukin-6 is the major component of cytokine release syndrome and hyperinflammatory response.

Tocilizumab was recently approved by FDA for CRS (Sanders *et al.* 2020). Many clinical studies are going on by using several dosing strategies on hospitalized patients with either critically ill condition or non-critically ill patient populations, which can be found on clinicaltrials.gov (NCT04345445, NCT04332094, NCT04331795, NCT04332913, NCT04346355, NCT04339712, NCT04320615, NCT04335071, NCT04322773, NCT04317092, NCT04310228, NCT04306705, NCT04335305, NCT04315480, NCT04330638, NCT02735707, NCT04331808, NCT04347031 and NCT04349410).

Sarilumab

It is another IL-6 receptor inhibitor. Theoretically, based on the mechanism of action, it would be considered beneficial in the COVID-19 related CRS (Stebbing *et al.* 2020). Several clinical trials are conducted or still ongoing and can be found on clinicaltrials.gov (NCT04321993, NCT04341870, NCT04327388, NCT04345289, NCT04324073, NCT04315298 and NCT02735707).

Anakinra

It is an antagonist of the interleukin-1 (IL-1) receptor. This potent drug has a theoretical place in the treatment due to increased IL-1 levels in COVID-19 and therefore may cause a significant reduction in cytokine storm (Mehta *et al.* 2020). Anakinra has also been administered for the treatment of hemophagocytic lymphohistiocytosis and CAR-T–associated CRS. Several clinical trials are going on to indicate the use of anakinra alone or in combination with other therapeutic agents. These can be found on clinicaltrials.gov (NCT04324021, NCT04330638, NCT04339712 and NCT04341584).

Janus Kinase Inhibitors

Fedratinib, baricitinib, and ruxolitinib are Janus kinase inhibitors which have been approved by FDA for the treatment of myelofibrosis and rheumatoid arthritis. Theoretically, these therapeutic agents are considered to have an effect on cytokine levels (including interferon γ), which appear to be increased in COVID-19 patients (Ramos-Casals *et al.* 2014; Huang *et al.* 2020; Mehta *et al.* 2020; Stebbing *et al.* 2020). Ruxolitinib is being investigated for both the prevention and treatment of COVID-19 and trials can be found on clinicaltrials.gov (NCT04348695, NCT04334044, NCT043 31665, NCT04348071, NCT04337359 and NCT04338958).

Other Agents of Interest

Vitamin C: Vitamin C (ascorbic acid) is an antioxidant vitamin, needed to boost the immune system (Ang *et al.* 2018). Studies have shown that several high-dose vitamin C infusions (e.g., 200 mg/kg per day through IV route, divided into 4 doses) reduced the duration of the intensive care unit (ICU) by 7.8% (Hemilä and Chalker 2019).

Vitamin C is also involved in the synthesis of endogenous catecholamines as a cofactor and maintains immune function by helping with neutrophil action and lymphocyte proliferation (Marik 2020). These properties,

along with low level of endogenous vitamin C during infection, improved interest in its clinical use in COVID-19.

Coronaviruses enhance oxidative stress that stimulates cellular malfunction and ultimately causes organ failure (Boretti and Banik 2020). However, high intravenous dose of vitamin C could have a beneficial effect by inhibiting the phenomenon of cytokines storm production due to COVID-19. Several Chinese physicians have shown positive results by using this approach for treatment of COVID-19 (Carr 2020; Cheng 2020). The successful use of high-dose intravenous vitamin C has been reported in a study on 50 moderate to severe COVID-19 patients in China. The dose range was 10-20 g/day. The oxygenation index was improved and all the patients eventually recovered and were discharged (Cheng 2020).

Probiotics

Probiotics are the living microorganisms involved in generating valuable physiological effects on the host. Several bacteria present in various fermented foods like pickle, cheese and yogurt are recognized as probiotics due to their health benefits (Kok and Hutkins 2018; Rezac *et al.* 2018). It has been suggested that human beings should consume 10^8 to 10^{10} CFU dose of probiotics on daily basis to have beneficial effects on health. Many health benefits of probiotics have been proved, including inhibition of the initiation of allergic diseases, treatment of ischemic heart disorder, decreasing blood cholesterol content, producing vitamins B, improving the bioavailability of dietary calcium, and boosting immune activity (Bustamante *et al.* 2020).

Probiotics include bacteria as well as yeast. The probiotic bacteria are *Lactobacillus acidophilus, L. brevis, L. amylovorus, L. bulgaricus, L. cellobiosus, L. curvatus, L. gallinarum, L. casei, L. crispatus, L. helveticus, L. delbrueckii spp. bulgaris, L. fermentum, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus; Lactococcus lactis; Streptococcus thermophilus, Leuconostoc mesenteroides, Pediococcus pentosaceus, P. acidilactici, Sporolactobacillus inulinus, Bifidobacterium adolescentis, B. animalis, B. bifidum, B. breve, B. essensis, B. infantis, B. laterosporum, B. thermophilum, B. longum, Propionibacterium acidipropionici, P. freudenreichii, P. jensenii, P. thoenii, Enterococcus faecium, E. fecalis, Bacillus subtilis, B. alcolophilus, B. cereus, B. coagulans, B. clausii and Escherichia coli. The* probiotic yeast include *Saccharomyces cerevisiae* and *S. boulardii* (Bron *et al.* 2012; Saad *et al.* 2013). Probiotics have been mostly considered as antibacterial agents however, anti-viral activities are also reported recently for some probiotic strains (Al Kassaa 2016).

Probiotics are involved in controlling drug-linked diarrhea, sepsis, gastrointestinal infection and respiratory tract infection (Li *et al.* 2020). An organized, randomized and controlled study on more than 5,000 infants treated with *lactobacillus plantarum* strain linked with prebiotics showed a reduction in severity of lower respiratory tract infections and sepsis (Gao *et al.* 2020). Viruses are the main causing agents for upper respiratory tract infections. The beneficial and protective effect of probiotics have been confirmed in prevention of upper respiratory tract infections. Multiple randomized controlled studies, involving the administration of probiotics to 4,230 youngsters and kids, have shown a 2-fold decrease in occurrence of respiratory tract infections and a considerable decrease in the severity of the disease in infected patients (Sencio *et al.* 2020). Furthermore, a double-blind randomized study was conducted in 523 youngsters, who received *Bifidobacterium longum* SP 07/3, *Bifidobacterium bifidum* MF 20/5 and *Lactobacillus gasseri* PA 16/8 along with some vitamins and minerals. The probiotics administration decreased the flu period and also a decrease in fever days (Pullano *et al.* 2020). A randomized, placebo controlled trial with *Lactobacillus brevis* also indicated promising results in 1,692 school kids, as the probiotic strongly reduced the risk of influenza respiratory infection (Bell *et al.* 2004). In healthy persons, many lactic acid bacteria commonly present in the upper respiratory tract are considered for probiotics (Wan *et al.* 2020). Studies have also indicated that probiotics could have a valid therapeutic and preventive contribution in the incidents of coronavirus outbreak. However, all probiotics are not involved in reducing the risk of respiratory tract infections (Turner et al. 2017). Examples of probiotics that might be useful to reduce the load of viral COVID-19 include *Lactobacillus gasseri, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium longum, Pediococcus pentosaceus, Leuconostic plantarum, L. paracasei ssp. Paracasei, L mesenteroides* (Lehtoranta et al., 2014; Zelaya *et al.* 2016) . These probiotics are involved in reducing the occurrence and severity of respiratory tract infection, as well as in boosting the immune system of the body (Zafar *et al.* 2020). A study to determine beneficial effect of *Lactobacillus coryniformis* K8 along with dietary supplements to protect healthcare workers from contracting COVID-19 was carried out and has been registered at ClinicalTrials.gov (NCT04366180) (Tahir et al. 2020).

Challenges and Progress on Vaccine Development for COVID-19

After the gene sequence of SARS CoV-2 was published on 11th January, 2020, intensive research was focused on the development of vaccines (Yadav *et al.* 2020). Some of the challenges relating to the development of a COVID-19 vaccine are as follows:

Vaccine development takes time, as the vaccine should not only be protective but also safe, because it is administered to healthy populations. The fastest development was the mumps vaccine, which took nearly 5 years (Sharma *et al.* 2020). Accelerated development involves trials to be done in smaller groups. There is considerable concern about the safety of a vaccine. If such vaccine is approved for public use globally, adverse effects may arise which might not have been observed in small groups.

Table 2: A summary of vaccine candidates undergoing clinical trials for COVID-19

Sr.	Vaccine Name	Type	NCT Ref No.	Route	Target	Principal Developer	Comments	Reference
1	ZF2001	Protein subunit	NCT04646590	IM	RBD protein and virus neutralizing antibodies	Anhui Zhifei Longcom Biologic Pharmacy Co, China	Phase III trials in China, Uzbekistan, Indonesia, Pakistan and Ecuador, 3 doses	(Yang *et al.* 2020)
2	ChAdOx1 nCov-19 (AZD-1222)	Adenovirus vector	NCT04516746	IM	S protein	Collaboration of Oxford University and Astra Zaneca, UK	Under Phase III trials	(Jeyanathan *et al.* 2020; Wang *et al.* 2020a)
3	Ad5-nCoV	Adenovirus vector	NCT04341389	IM	S protein	CanSino Biologics. Beijing, China	Phase II trials, for adults aged 18 years and above, single dose	(Wu *et al.* 2020b)
4	mRNA-1273	LNP, Lipid-mRNA nanoparticle	NCT04283461 NCT04470427 NCT04405076	IM	S protein	Moderna, NIAID, USA	Phase I, II, III, trials, fully synthetic, no risk of disease transmission, 2 doses, 2-year immunity	(Baden *et al.* 2020)
5	CoronaVac (PiCoVacc)	Inactivated virus	NCT04352608 NCT04383574 NCT04456595	IM	Multiple surface antigens	Sinovac Biotech, Beijing China	Phase I, II, III trials, for adults 18-59 years, 2 doses	(Palacios *et al.* 2020)
6	NVX-CoV2373	Recombinant protein nanoparticles using Matrix-M adjuvant	NCT04368988	IM	S protein and virus neutralization	Novavax, USA	Phase I, II trials, for adults aged 18-84 years, 2 doses	(Keech *et al.* 2020a; b)
7	BNT162b1	LNP, Lipid-mRNA nanoparticle	NCT04523571	IM	RBD of S protein	BioNTech (Germany), Pfizer, Fosun (China)	Phase II, III trials, after Phase I, approved for emergency use in UK, USA and Singapore, 2 doses	(Mulligan *et al.*, 2020)
8	BBIBP-CorV	Inactivated virus	NCT04560881	IM	Multiple neutralizing antibodies	Sinopharm, Beijing, China	Large scale Phase III trials in China and UAE, 2 doses	(Xia *et al.* 2020)
9	INO-4800	Plasmid DNA	NCT04642638 NCT04447781 NCT04336410	Intradermal	S protein	Inovio and Advaccine, China	Phase I, II, III trials, 2 doses with electroporation	(Smith *et al.* 2020; Tebas *et al.* 2020)
10	Inactivated SARS-CoV-2 Vaccine (Vero Cell)	Inactivated virus	NCT04510207	IM	Multiple neutralizing antibodies	China National Biotec, China	Phase III trials in China, 2 doses	(Jeyanathan *et al.* 2020)
11	Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine	LNP, Lipid-saRNA nanoparticle	N/A	IM	S protein	Imperial College London, UK and Morningside Ventures, China	Phase I and II trials in UK, 2 doses	(Jeyanathan *et al.* 2020)
12	Inactivated SARS-CoV-2 Vaccine	Inactivated virus	NCT04470609 NCT04412538	IM	Multiple neutralizing antibodies	Qihan Li, Chinese Academy of Medical Sciences, China	Phase I and II trials in China, 2 doses	(Clinical Trials, n.d.)
13	CVnCoV	LNP, Lipid-mRNA nanoparticle	NCT04652102	IM	S protein	CureVac, Germany	Phase III trials, for adults aged 18 years and older, 2 doses	(Kremsner *et al.* 2020)
14	GamCOVID-Vac Lyo	Adenovirus vector, 2 viruses (rAd26, rAd5) heterologous	NCT04437875 NCT04436471	IM	S protein	Gamaleya Research Institute of Epidemiology and Microbiology Russia	Phase II trials, approved for distribution in Russia, single dose and prime boost dose	(Logunov *et al.* 2020)
15	GX-19	Plasmid DNA	NCT04445389	IM	S protein	Genexine Consortium, Korea	Phase II trials in South Korea, 2 doses	(Kaur and Gupta 2020; Seo *et al.* 2020)
16	SCB-2019	Recombinant trimeric S protein	NCT04405908	IM	S protein	Clover Biopharmaceuticals (China), GSK (UK) and Dynavax (USA)	Phase I trials, 2 doses	(Richmond *et al.* 2020)

17	COVID-19 vaccine	Protein subunit	NCT04445194 NCT04466085	IM	Dimeric RBD	Anhui Zhifei Longcom Biologic Pharmacy Co, China	Phase I, II trials in China, 2 or 3 doses under trials	(Jeyanathan *et al.* 2020)
18	ARCoV	mRNA	N/A	IM	S protein	Suzhou Abogen Biosciences, Walvax Biotechnology and Academy of Military Medical Sciences, China	Phase I trials in China, 2 doses	(Jeyanathan *et al.* 2020)
19	AG0301-COVID19	Plasmid DNA	NCT04463472	IM	S protein	AnGes, Inc., Japan	Phase I, II trials in Japan, 2 doses	(Rego *et al.* 2020)
20	VIR-7831 (recombinant coronavirus-like particle covid-19 vaccine)	Plant-based virus-like particle	NCT04450004	IM	Multiple viral antigens	Medicago and Laval University, Canada	Phase I trials in Canada, for adults aged 18-55 years, 2 doses	(Craven 2020)
21	Lunar-COV19 (ARCT-021)	mRNA	NCT04480957	IM	S protein	Arcturus Therapeutics and Duke-NUS Medical School, Singapore	Phase I, II trials in Singapore, for adults aged 21-80 years, single dose	(Baviskar *et al.* 2021)
22	Covaxin (BBV152A, BBV152B and BBV152C)	Inactivated whole virion	NCT04471519	IM	Multiple viral antigens	Bharat Biotech and Indian Council for Medical Research, India	Phase I, II trials in India, 2 doses	(Rego *et al.* 2020)
23	ZyCov-D	Plasmid DNA	N/A	Intradermal	S protein	Zydus Cadila Healthcare, India	Phase I, II trials in India, 3 doses	(Jeyanathan *et al.* 2020; Kaur and Gupta 2020)
24	SARS-CoV-2 Sclamp (COVID-19) Vaccine	Protein subunit	NCT04495933	IM	Molecular clamp-stabilized S protein	University of Queensland, Australia	Phase I trials, 2 doses, development halted after unintended results in Phase I	(Normile 2020)
25	Ad26.COV2.S (Janssen COVID-19)	Adenovirus vector (Ad26)	NCT04436276	IM	S protein	Janssen, Belgium	Phase I, II trials, 2 doses	(Sadoff *et al.* 2021)
26	KBP-201 COVID-19	Protein subunit	NCT04473690	IM	RBD-based protein	Kentucky BioProcessing, USA	Phase I, II trials, 2 doses	(Mathew *et al.* 2021)
27	IAVI-Merck COVID-19	VSV vectored	N/A	IM and oral	S protein	Merck and IAVI, USA	Phase I, II, single dose	(Mahalingam *et al.* 2020)
28	COVAX19 (Monovalent Recombinant COVID19)	Protein subunit	NCT04453852	IM with Advax-SM adjuvant	S protein	Vaxine (Australia) and Medytox (South Korea)	Phase I in Australia, single dose, development halted due to lack of funds	(Jeyanathan *et al.* 2020)
29	MVC-COV1901	Protein subunit	NCT04487210	IM	S protein	Medigen Vaccine Biologics (Taiwan)	Phase I in Taiwan, 2 doses	(Mathew *et al.* 2021)
30	Covigenix VAX-001	Plasmid DNA	NCT04591184	IM	Multiple epitopes	Entos Pharmaceuticals, Canada	Phase I trials in Canada, 2 doses	(Ashraf *et al.* 2021)
31	bacTRL-Spike	Bacterial vector	NCT04334980	Oral	S protein	Symvivo Corporation, Canada	Phase I trials,	(Alturki *et al.* 2021)

There are indications that respiratory viruses are especially difficult to protect against with vaccines. This is because the mucous membranes of respiratory tract are protected by IgA antibodies, whereas vaccine response is determined taking IgG and IgM or total immunoglobulin in focus. Most vaccines are inoculated as intramuscular injection with minimal mucosal immunity or IgA secretion (Chung *et al.* 2020).

In the past, recombinant nucleic acid has not resulted in the development of a successful vaccine for human use (Han 2015). Furthermore, the dependence of DNA vaccines on an injection device or an electroporator is a potential issue.

The pre-existing immunity to adenoviruses results in reduced immune response in individuals receiving adenovirus vector-based vaccines. Single stranded RNA viruses are capable of highly efficient self-amplification of RNA in host cells. Virus mutation may result in lack of efficacy of the vaccine (Lundstrom 2020).

There is risk of vaccine-enhanced disease for inactivated virus-based vaccines (Graham 2020). Moreover, fast-tracked large-scale production of vaccine stills remains a challenge to meet the demands of pandemic.

Phases of Vaccine Trials

The first phase of vaccine development is an exploratory phase, involving identification of antigens and computational modeling of whether a vaccine can help to treat or prevent a disease. The second phase is preclinical stage, which involves testing of vaccines on cell cultures and/or animal models to check for efficacy, immunogenicity and safety. Once immunogenicity and safety are verified by animal studies, progress is made for testing on human population, first in small groups and then in large groups in three phases.

Phase I (Safety): The vaccine is administered to healthy immunocompetent human subjects in small groups. Vaccine is primarily checked for safety. Appropriate dosage adjustments are made, and immunity production is checked as secondary effect.

Phase II (Expanded safety): The vaccine is given to hundreds of people (split in small groups according to demographic features). This phase again is a test for safety, while immunization is taken as secondary effect. This phase determines dosage, interval between doses and other requirement to be accorded during Phase III trials.

Phase III (Efficacy): The vaccine is given to thousands of people to evaluate its efficacy. Vaccine efficacy (VE) is defined as percent reduction in the incidence of disease in the vaccinated group as compared to placebo. In case of low disease incidence in the population, sample size should be sufficiently large to determine reliable vaccine efficacy in the population (Mahase 2020). After successful completion of Phase III trials, vaccine can be moved for Review and Approval and then to Marketing and Post-Marketing Surveillance (Sharma *et al.* 2020). Normally, regulatory bodies must review results of clinical trials and decide whether a vaccine can be approved or rejected. Under ordinary circumstances, this can take 1-2 years but, during a pandemic, vaccine can be approved on emergency basis. After marketing, effectiveness and adverse effects of vaccine are still monitored during widespread use in general public.

Vaccines Candidates

In Table 2, vaccines undergoing different phases of clinical trials are summarized. It is pertinent to mention that live vaccines are not being attempted for human use due to safety reasons (Caddy 2020).

Conclusion

The whole world is going through the deadly challenge to deal with lethal coronavirus infection in humans. Scientists and researchers from all over the world are working day and night to discover potential preventive moieties and therapeutic agents against this deadly disease. Different protocols and strategies, such as preventing the viral binding to host cells, inhibition of viral replication, use of drugs and compounds to enhance both innate as well as passive immunity, are under consideration to treat and control COVID-19. Up till now, not a single therapeutic agent has been approved against SARS-CoV-2. Several types of vaccines and pharmacological drugs are under clinical research trials and this will take several months to years to be commercially available in the market. The major challenge of COVID-19 is the development of effective therapeutic strategies against SARS-CoV-2. Some of the antiviral drugs and adjunctive therapeutic agents have shown substantial effects *in vitro*, however there is an ultimate requirement to confirm their safety and efficacy in the clinical trials. It is expected that scientific strategies will assist in developing new, effective, cheap and safe antiviral agents against SARS-CoV-2.

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VIRAL AND BACTERIAL ZOONOTIC DISEASES

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INTRODUCTION

Zoonotic diseases are infectious diseases that can spread from animals to humans. In this chapter, description about zoonotic diseases in different animals, including companion animals (Section 1), ruminants (Section 2), poultry (Section 3), equines (Section 4) and swine (Section 5) has been given. For each disease, available information about its characteristics, etiology, epidemiology, pathology, diagnosis, histology, treatment, and prevention has been discussed.

Zoonotic Diseases in Companion Animals

Companion animals are animals living with us in our homes. These are mostly cats and dogs. These animals satisfy our emotional needs and help us to enjoy a happy and healthy life. It is important to take care of these animals and protect them from different diseases. Therefore, it is especially crucial to prevent and treat zoonotic diseases to protect the family with animals and humans. In this section, description about three zoonotic diseases in companion animals: rabies, leptospirosis and tetanus, has been given.

Rabies

Rabies is caused by lyssaviruses (rabies virus) and can affect any mammalian species. Dogs are major reservoirs of this disease. It is a fatal disease and can spread to people and companion animals after a potential rabies exposure, such as a bite or scratch by an animal with rabies. The virus can cause brain lesions, leading to death of a person without appropriate care after rabies exposure. It is fatal once the symptoms show up. However, it is preventable by vaccinating dogs and treating patients appropriately after rabies exposure.

Etiology

Lyssaviruses, such as rabies virus and Australian bat lyssaviruses, are the cause of rabies. The virus has helical symmetry so that viral particles are approximately cylindrical in shape. It is an RNA genome, with negative sense and single-strand. It encodes five conserved genes: These genes are nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and the viral RNA polymerase (Finke and Conzelmann 2005).

Epidemiology

Rabies virus causes approximately 59,000 deaths worldwide each year, and almost all due to dog bites and mostly in Asia and Africa (Hampson et al. 2015). Although rabies virus can live in a range of animals, dogs are the principal host. Although strategies to control rabies, including vaccination of cats and dogs and elimination of stray dogs, have proven to be successful especially in developed countries, dog rabies is still commonly found in many countries especially in developing ones (Wallace et al. 2020).
Elimination of rabies in developed countries has proved to be successful. Most rabies deaths are from countries with poor resources in public health, limited surveillance and preventive treatment (Taylor and Nel 2015). Vaccination of domestic dog is less effective in rural regions compared with urban regions.

Symptoms

Animals infected with rabies typically show symptoms of central nervous system disturbance. Common symptoms of rabies are behavioral changes (irritability, hyperexcitability, loss of appetite). Unexplained paralysis appears and worsens as the disease progresses.
Rabies has two forms. The first form is called as “mad-dog” or furious form. In this form,the animal is irritable, and uses its teeth and claws in an aggressive way with loss of fear of other animals and dilated pupils. Lack of muscle coordination and seizure may be observed. Progressive paralysis finally results in death (Beeler and Ehnert 2020). The second form is called as “dumb rabies” or paralytic form, in which affected dogs show paralysis of the jaw or throat, are unable to swallow and have excess salivation. Dogs seldom attempt to bite and do not show viciousness. Death typically occurs within a few hours after progressive paralysis of the whole body (Alello et al. 2018).

Diagnosis

Animals affected with rabies may show a range of clinical signs, including aggression, fearfulness, excessive drooling, seizures and paralysis. Multiple tests can be applied to diagnose the disease. Testing methods include reverse transcription (RT) after PCR, testing for antibodies to rabies virus, and examination of rabies

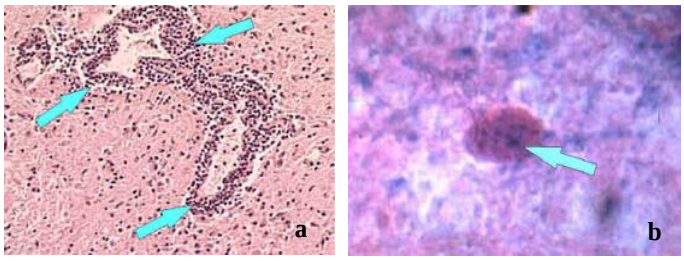

Figure 1: Histopathologic evidence of rabies in brain tissue. Perivascular cuffing or inflammation around a blood vessel in brain tissue with hematoxylin & eosin staining under 100x magnification (1a). A Negri body in brain tissue with Sellers staining (1b). Courtesy of Centers for Disease Control and Prevention (2021).

antigen in the nerves. Detection of rabies virus from the brain tissue of affected animals confirms the diagnosis (Centers for Disease Control and Prevention 2021).

Histology

Samples of brain tissues of the infected animals are stained with hematoxylin and eosin (H&E). Histologic examination can be performed after the staining. Histological findings in the brain tissue supporting rabies are mononuclear infiltration, perivascular cuffing of lymphocytes or polymorphonuclear cells, Negri bodies, babes nodules consisting of glial cells, and lymphocytic foci (Centers for Disease Control and Prevention 2021). In Fig. 1a, perivascular cuffing around a blood cell is observed in brain tissue with H&E staining. In Fig. 1b, Negri body in infected neuron is observed in brain tissue with Sellers staining (Centers for Disease Control and Prevention 2021).

Prevention and control

Despite nearly 100% fatality rate after the appearance of symptoms, rabies is still preventable. Large-scale vaccination of dogs is the most effective strategy in controlling rabies. Other strategies include, elimination of stray dogs, and registration of dog licensing (Banyard and Fooks 2020). Extensive wound cleansing (washing for at least 15 minutes with water, soap and substances that can kill the virus) and injecting effective rabies vaccine after rabies exposure is crucial and can prevent rabies onset and death (World Health Organization 2018).

Leptospirosis

Leptospirosis is a zoonotic disease, caused by the bacterium Leptospira. Patients affected with leptospirosis can show mild symptoms like headaches and muscle pains, and severe symptoms like lung bleeding or meningitis.

Etiology

Leptospirosis is caused by Gram-negative bacterium Leptospira. It is an aerobic, 6-20 mm long and right-handed helical (Karpagam and Ganesh 2020). Leptospira can be classified according to their serovar. More than 250 pathogenic serovars and 24 subgroups of Leptospira have been recognized (Cerqueira and Picardeau 2009). Leptospira lives in the kidneys of affected animals. After being infested, Leptospira enters and moves in the bloodstream and lodges in kidneys. The bacteria are then shed in the urine, without inducing serious ill effect to the animal. Leptospira can be killed at a temperature of 50°C, or inactivated by ethanal (70%), detergents, formaldehyde, and 1% sodium hypochlorite.

Epidemiology

Leptospirosis is a worldwide zoonotic infection, with the organisms are found in ponds, rivers and moist soil (Karpagam and Ganesh 2020). Leptospira are found mostly in mammals. There are a range of transmission mechanisms. Leptospira can spread through the urine of the infected animals. It can enter into soil and water and survive for months. Animals in contact with contaminated urine, water or soil can be infected. The bacteria enter the body of the host through mucous membranes (eyes, nose or mouth) or skin. Infected animals shed the bacteria in their urine for years. Leptospirosis is associated with rainfall amount, so the disease spread is seasonal and becomes widespread after heavy rains (Karpagam and Ganesh 2020).

Symptoms

Patients infected with Leptospirosis show a range of nonspecific symptoms, including fever, vomiting, depression, diarrhea and stiffness. In general, younger animals exhibit more severe symptoms than adults. Cats are less susceptible to Leptospirosis compared with other animals. Cats infected with leptospira may have antibodies detected but show no or mild symptoms (Murillo et al. 2020).

Diagnosis

Patients infected with Leptospirosis may show increased white blood cell count, blood urea and creatinine, and decreased platelet count and sodium level in the blood (Haake and Levett 2015). ELISA can be used to quantify the IgM antibodies of Leptospira for quick detection of the disease by using L biflexa antigen. ELISA test is useful for rapid detection in the early stage (Rosa et al. 2017). Microscopic agglutination test (MAT) can be used to help diagnose Leptospirosis (Guedes et al. 2021). In this test, patient sera (after serial dilutions) are mixed with different serovars of Leptospira and examined the mixture for evidence of agglutination under a microscope (Bennett, Dolin and Blaser 2014). Polymerase chain reaction (PCR) can be used to replicate the leptospira DNA from the samples of serum and urine (Bennett et al. 2014).

Treatment

Leptospirosis can be treated with antibiotics. Dogs affected with Leptospirosis have been recommended to be treated with penicillin derivatives and doxycycline.

Initial path should be use of doxycycline parenterally because dogs often show symptoms of vomiting. After these symptoms resolve, oral doxycycline can be administered for two weeks to clear leptospires from the renal tubules. Supportive care can be provided for dogs with Leptospirosis, depending on the symptoms and severity of the disease. Supportive care includes maintaining adequate hydration with intravenous fluid therapy, nutrition support, and pain relievers.

Prevention and control.

Prevention and control measures of Leptospirosis include (1) vaccination of pets, (2) control of rodents (rates, mice, etc), and (3) keeping pets away from contaminated water, animal tissues and urine.

Tetanus

Tetanus toxemia is a bacterial infection, characterized by muscle spasms. It is caused by the neurotoxin emitted by *Clostridium tetani* in necrotic tissues. Most mammals (domestic and wild animals) are susceptible to the disease. Cats and dogs are more resistance to the toxin compared with other mammals (Acke et al. 2004).
Clostridium tetani enters the body through wounds, particularly wounds with deep puncture. The bacteria replicate and produce the toxin whichcauses convulsions (Kahn 2007).

Etiology

Clostridium tetani is an anaerobic bacterium, and can thrive in the environment lacking in oxygen. Potent neurotoxin produced by the bacteria can be absorbed by the motor nerves. It can spread along the nerve tract to reach the spinal cord (Alello et al. 2018). *Clostridium tetani* is found in soil. It is more common in soil with more organic matter in damp climates. It is commonly found in the feces of many animals including cats and dogs, so that *C. tetani* spores have been reported in manure-treated soils (Acke et al. 2004).

Symptoms

Incubation period of the disease usually averages 10-14 days but can vary from 1 to several weeks (Alello et al. 2018). Localized stiffness near the wound is firstly observed. One day later, general stiffness shows up with more apparent spasms (Acke et al. 2004). Animals infected with tetanus show increased intensity in reflexes and more violent general spasms. Lockjaw (difficulty in eating food due to spasms of head muscles) is a characteristic symptom. General spasms can hamper respiration and circulation. In dogs and cats, more localized symptoms are seen, because they have high resistance to the toxin (Alello et al. 2018).

Diagnosis

Diagnosis of tetanus is based on symptoms and history of recent trauma. The diagnosis is confirmed by identifying characteristic spastic paralytic signs. Presence of a wound provides evidence supporting tetanus. Electromyography can also be used to support the diagnosis (Popoff 2020). Evidence of tetanus toxin in serum would confirm the diagnosis (Alello et al. 2018).

Treatment and control

Patient in the early stages of tetanus may be treated with tetanus antitoxin and tranquilizers. Supporting therapy of tetanus includes cleaning the wounds and administering broad-spectrum antibiotics, including penicillin (Alello et al. 2018). Vaccination with tetanus toxoid can control tetanus. Animals should be injected with another dose of toxoid to increase antibody titer after a dangerous wound is found. Animals surviving tetanus should still be vaccinated with tetanus toxoid, since immunity built may not be adequate (Kahn 2007).

Zoonotic Diseases in Ruminants

Bovine tuberculosis

Tuberculosis (TB) is an infectious disease, caused by bacilli of the genus Mycobacterium. In most cases, TB is a chronic debilitating infectious disease, though the progression of the disease may occasionally be very rapid. All species of vertebrates can be infected with the disease (Mahmood et al. 2014). Bovine tuberculosis (Bovine TB) is a zoonotic disease caused by *Mycobacterium bovis* (Khan and Khan 2007). Bovine TB is especially significant in countries which are not well industrialized and remains a serious problem in many developing countries (Azami and Zinsstag 2018). Cattle are the major reservoir of *Mycobacterium bovis*. However, this organism has also been isolated from many other species, such as bison, buffaloes, goats, sheep, pigs, deer, dogs, cats, and equines. Cattle-human infection route is the major source of infection to humans. The usual clinical signs of Bovine TB include weakness, fluctuating fever, diarrhea, weight loss, appetite loss, enlarged lymph nodes, and pneumonia (Bapat et al. 2017), which eventually leads to death. Bovine TB is characterized by nodular granulomas and caseous calcified necrotic lesions in tissues and organs (Moustakas et al. 2018).

Etiology

There are three main types of *Mycobacterium tuberculosis*: *Mycobacterium bovis* (bovine), *Mycobacterium tuberculosis* (human) and *Mycobacterium avium* (avian). The morphology of *Mycobacterium tuberculosis* is slightly different in different types. Human tuberculous bacilli are straight or slightly curved elongated bacilli, single or parallel together, mostly stick-like, between branching (Moustakas and Evans 2016). Bovine tuberculosis bacterium is shorter and thicker than that of human tuberculosis, and the pigmentation is uneven. Avian tuberculosis bacterium is short and small, and shows pleomorphic features. This bacterium does not

produce spores and capsules, nor can it move. It is a gram positive bacterium. *Mycobacterium tuberculosis* is a strict aerobic bacterium, and the optimum pH for growth is 5.9 to 6.9 for bovine Mycobacterium, 7.4 to 8.0 for the human Mycobacterium and 7.2 for avian Mycobacterium. The optimum temperature for growth is 37-38°C (Gilbert et al. 2005).

Epidemiology

Tuberculosis is the main source of infection. The disease spreads by the pollution of the surrounding environment because the causative organisms can be excreted in feces, milk, urine, and tracheal secretions. It is mainly transmitted through the respiratory and digestive tracts (Khan et al. 2019). Darkness, dampness, dirty environment, excessive labor and milking, and poor feeding can promote the occurrence and transmission of the disease.

Clinical symptoms

The incubation period of Tuberculosis is generally 10-15 days, but can be up to several months. The disease is chronic, showing progressive wasting, cough and dyspnea. The symptoms are different when the bacteria invade different organs. In cattle, the bacteria mainly invade the lungs, breast, intestine and lymph nodes. The invasion of the bacteria into lungs leads to tuberculosis. The disease is responsible for progressive emaciation of cattle. A short dry cough occurs in the beginning, and it gradually develops into wet cough. Grinding sounds could be heard in the pleural tuberculosis. The invasion of bacteria into the breast leads to mammary tuberculosis. In this condition, milk production decreases gradually with pus sometimes found in the milk. The invasion of organisms into lymph nodes leads to lymphatic tuberculosis, in which lymph nodes are enlarged. It is commonly found in the mandible, pharyngeal, neck and inguinal lymph nodes. The invasion of bacteria into the intestine is most common in calves and characterized by alternating constipation and dysentery or by intractable dysentery.

Pathological changes

The lesions of tuberculosis are characterized by the formation of white tuberculous nodules in the lungs and other affected tissues. The nodules are off-white, translucent and hard. The pearly-like nodules in the pleura and peritoneum are commonly known as "pearl disease". In late stages of the disease, there is caseous necrosis or calcification in the center of the nodule, or the formation of pus cavity (Dallenga et al. 2017). Histopathological examination shows a large number of *Mycobacterium tuberculosis* in the nodules. Microscopic examination of lungs usually reveals macro and micro tubercles, with both caseating and non-caseating centers (Mahmood et al. 2014). Extensive caseous material arranged in concentric lamellar fashion is observed in mature tubercles (Figs. 2 and 3). These tubercles are further enclosed within the fibroblastic rim punctuated by lymphocytes, plasma cells, macrophages and massive population of multinucleated giant cells (Figs. 4 and 5). Eosinophilic homogeneous cellular fluid can be seen in the alveoli. Abundant rod shaped bacilli can also be visualized (Mahmood et al. 2014).

Diagnosis

Tuberculosis can be diagnosed on the basis of clinical symptoms, blood tests, X-ray, and tuberculin test (Al-Zamel 2012). Tuberculin test is a convenient and reliable method.

Prevention and control

Healthy cattle without tuberculosis should be quarantined once a year in spring and autumn. People with tuberculosis should not be allowed to raise livestock. Feeding and management practices may be improved to ensure environmental hygiene.

Bovine Spongiform Encephalopathy

Bovine Spongiform Encephalopathy (BSE) is a progressive, fatal neurological disease of adult cattle. It is characterized by mental disorder, ataxia, hypersensitivity to vision, touch and hearing. The histopathological features are spongy vesicular degeneration of the brain (Prusiner 1991).

Etiology

The pathogen is prion, which is an abnormal protein formed by the normal glycoprotein on the surface of the host nerve cell and mainly composed of β folding (Bolton et al., 1982). Prions can survive in the soil for up to three years. Formalin, hydrogen peroxide and phenol cannot deactivate them.

Clinical symptoms

The incubation period for BSE is normally 4-5 years. The disease is characterized by long incubation period and slow progression. The development of BSE can be divided into four stages: biological onset stage, preclinical stage, clinical stage and transition stage. Cattle infected with BSE can show mental disorders, like fear or become aggressive when approached by persons (Bruce et al. 1994), motor disorders such as ataxia, tremor or collapse and sensory disorders including hypersensitivity to touch, sound, and light. The infected cows may have a gait of "goose step" with limbs over-extended.

Pathological changes

Histo-pathological changes in BSE are mainly limited to the central nervous system. The main features are: vacuoles degeneration, loss of neurons and glial cells hypertrophy, nerve cells swelling, and cytoplasm shrinkage.

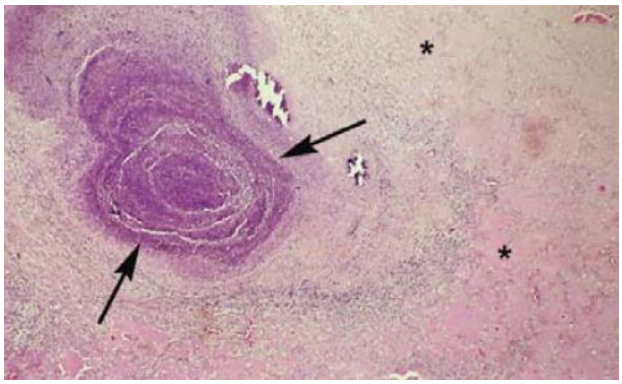

Figure 2: Lungs of a cow died of bovine tuberculosis exhibiting lamellated arrangement of caseous material (arrows) in mature tubercle and edema in alveoli (asterisk). H&E stain, 100X. Courtesy of Mahmood et al. (2014).

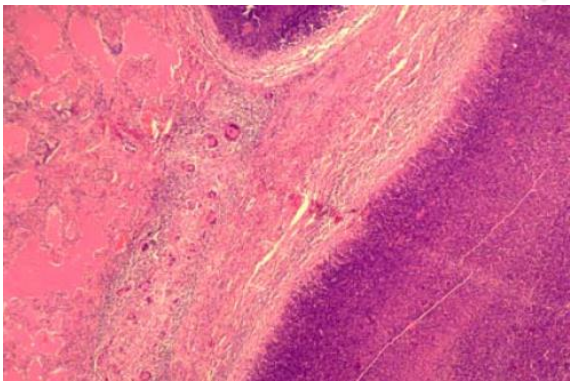

Figure 4: Mature tubercle of lungs exhibiting granular caseous center with punctuation of brim with fibroblasts and massive population of giant cells. H&E stain, 100X. Courtesy of Mahmood et al. (2014).

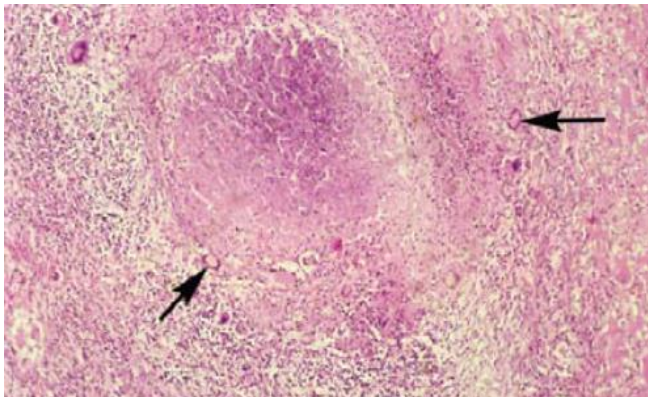

Figure 3: Immature tubercle with large number of multinucleated giant cells (arrows). H&E stain, 200X. Courtesy of Mahmood et al. (2014).

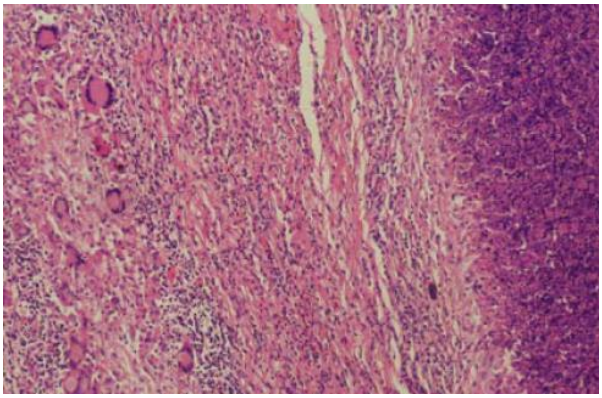

Figure 5: Granular caseation and punctuation of fibroblasts and massive population of giant cells in lungs of a bovine tuberculosis died cow. H&E stain, 200X. Courtesy of Mahmood et al. (2014).

In addition, there is obvious degeneration and necrosis of nerve cells. Apoptosis of nerve cells and the formation of vacuolar structures lead to disorders in signal transduction, so that the animals can show autonomic movement disorders, fear, biological clock disorders and other neurological symptoms.

Diagnosis

The primary diagnostic method of BSE is histopathological examination. Brain tissue sections are inspected under a microscope. The characteristic pathological changes in neurons are more common in the transverse sections of the medulla oblongata, the collateral cerebellar angle, the pons and the midbrain. The occurrence frequency is very high, especially in the nucleus of the solitary tract of the medulla oblongata and the nucleus of the spinal trigeminal nerve. Therefore, the examination of degenerative changes, such as vacuolar degeneration of neurons in the cross section of the brain, is a routine method to diagnose the early onset of BSE (Ducrot et al. 2008).

Prevention and control

To control BSE, it is important to identify animals infected with BSE. Good feeding management and BSE epidemic prevention can help control BSE.

Zoonotic Diseases in Poultry

Avian influenza

Avian flu is a viral zoonosis caused by avian influenza viruses. Avian flu can cause multiple symptoms, especially those of the respiratory tract.

Etiology

Avian influenza viruses belong to influenza A group of viruses of the orthomyxoviridae family, which in turn belongs to RNA viruses that are easily mutated. Most of the influenza A viruses infect only birds, and a few infect humans and mammals, such as pigs. Influenza B and C viruses have been found in seals and pigs, respectively. Influenza A viruses are pleomorphic, encase with the diameter ranging from 80 mm to 120 mm. The genome of the virus is a segmented, negative-sense, single-stranded RNA. Because of the different antigenicity of the outer membrane hemagglutinin (H) and neuraminidase (N) proteins, they are divided into 16 H subtypes (H1-H16) and 9 N subtypes (N1-N9). The avian influenza virus subtypes that infect humans are mainly H5N1, H9N2 and

H7N7, with H5N1 being the most virulent, the sickest and the most fatal (Wu et al. 2017). Studies have shown that highly pathogenic strain (H5N1) can be derived from low pathogenic strains of avian influenza (H5N2, H7N7, H9N2) after 6 to 9 months of rapid mutation among birds (Quan et al. 2019).

Epidemiology

Depending on the virulence of the strain, the transmission rate and fatality of the virus vary (Bui et al. 2017). The disease mainly occurs between winter and spring, or earlier between autumn and winter. Sick birds and the birds that carry the virus are the main source of infection of avian flu. In addition, wild birds and pigs can also become the carriers of this disease. Many poultry birds can be infected including turkeys, pigeons, guinea fowl, chickens, ducks, geese and parrots (Peng et al. 2018). Chickens and turkeys are the most susceptible, with high morbidity and mortality rates.

Symptoms

The symptoms of avian influenza include loss of appetite, head and facial edema, and nervous symptoms. Respiratory symptoms are mainly cough, canthus tears, and sneezing. Sick poultry can show diarrhea, with discharge of yellow green thin feces. There are numerous necrotic nodules. Dark red blood patches can be seen in the hairless parts of sick chickens. The virus can also affect the laying performance of chickens, and decrease the egg production.

Pathological changes

The sick chickens show head swelling, hairless bleeding patches, oral bleeding and necrotizing lesions. Surface of the heart shows bleeding spots, accompanied by fibrinous pericarditis. Bleeding spots are also seen in adipose tissue around the muscles of stomach and on the surface of the small intestine. The pancreas is enlarged and shows signs of bleeding. There is obvious grayish white exudate under the skin of air bag and oviduct.

Diagnosis

A preliminary diagnosis of avian influenza can be made on the basis of clinical symptoms of the disease and the characteristics of meteors. To make an accurate diagnosis, laboratory testing methods such as over-complement binding and hemagglutination inhibition are needed.

Treatment and prevention

Multi-electrolyte or weak saline is added in the drinking water of birds, and 1% potassium permanganate can be offered once a week to improve the resistance of poultry for the prevention and control of the disease. Quinarene of sick birds is necessary.

Salmonellosis

Salmonellosis is caused by the bacterium Salmonella. Every year, a large number of people and livestock suffer from salmonella infection and even death.

Etiology

Salmonella, belonging to the genus Salmonella, is a gram-negative bacterium with size between 0.6×3.0 microns. It has no buds. In general, it also has no capsule, except the *Salmonella pullorum* and *Salmonella typhimurium*. It mainly survives in the intestinal tract of the host, with weak resistance to heat and can survive for less than 15 minutes at temperatures above 60°C.

Epidemiology

Diseased animals and carriers are the main source of Salmonella infection. The causative bacteria can be transmitted through host metabolites and excreta, and infect healthy animals through the digestive tract. It is infectious to all species of poultry and domestic animals. Young animals are more susceptible to the infection than adult animals. The disease has no obvious seasonality, and is generally sporadic or epidemic after its occurrence in herds. In addition, feeding conditions and poor environment, as well as climate and childbirth, can increase the occurrence of this disease.

Symptoms

There are three manifestations of infection in chicks, namely acute septic type, arthritis type and nervous type. The main characteristics of acute septic type are pullorum and dyspnea, with the mortality rate can reach 100%. Arthritis type is mainly characterized by swelling of the hocks, and the fatality rate is about 30%. The main symptoms of neurologic type are motor dysfunction, including head tilt, head back, beak touch and turning, with the fatality rate is about 10%.

The disease of adult chickens is usually subacute in nature and results in high morbidity rates. The main characteristic is swelling of the synovial sac of the hock and sternum; the fatality rate is about 10%. In adult chickens, symptoms are not obvious, and the infection is usually inconspicuous, sometimes with dysentery and severe stress that results in sepsis and a significant decrease in egg production. The fatality rate is about 20% (Kariuki et al. 2015).

Pathological changes

There are necrotic foci in myocardium, lungs, cecum and large intestine, and the liver becomes brittle and swollen. The gall bladder is enlarged with white urate deposits in the ureter. The pathological changes of laying hens are mild, and the main manifestations include liver and kidney swelling, salpingitis and ovaritis.

Diagnosis

The clinical symptoms of salmonellosis are similar to those of swine fever, so it is impossible to draw a direct conclusion based on symptoms. A preliminary diagnosis can be made on the basis of clinical symptoms observed in sick animal, and then the feces or secretions are sent for inspection. After collection, samples are inoculated on the SS Agar medium and cultured at 37ºC for 24 hrs. The characteristics of the bacterial colonies are basically the same as those of Salmonella. The extracted and isolated pathogenic bacteria are further purified, isolated and cultured. Drug sensitivity test has revealed that the isolated pathogens were highly sensitive to cefuroxime, cefotaxime and gentamicin.

Prevention and control

Combined with drug sensitivity test, the infected chickens can be treated with cefotaxime and gentamicin. Salmonella agglutination test can be used for the diagnosis of the disease. To control the spread of this bacterial disease, it is necessary to guide farmers to strengthen daily quarantine and formulate strict sanitation and disinfection strategies. Vehicles and personnel entering and leaving the farm need to carry out strict sanitation and environmental disinfection. Farms also need to develop strict quarantine inspection system to phase out flocks of chickens.

Zoonotic Diseases in Equines

Glanders

Glanders is an infectious disease of equines caused by *Burkholderia mallei*. It is a highly contagious disease of hoofed animals including horses, mules and donkeys. People can be infected upon contact with sick animals or pathogenic bacteria. The main clinical symptoms are acute fever, cellulitis, necrosis, abscesses in the respiratory tract, skin, muscles and other places, formation of melirax nodules, ulcers and scars in the nasal cavity, larynx, tracheal mucosa or skin, and the occurrence of melirax nodules in the lungs, lymph nodes or other important organs.

Etiology

The pathogen of glanders is *Burkholderia mallei*. In 1993, Burkholderia was listed in the genus Burkholderia on the basis of its biological characteristics (Khan et al. 2013). The bacterium is *Corynebacterium microcurvae*, with different sizes, ranging from 2 to 5 μm in length and 0.5 to 1.0 μm in width. It is mostly isolated and sometimes arranged in pairs. It has no flagella and cannot move. It has no capsule or spores. It is negative for gram stain. The optimal temperature for growth is 37–38ºC and pH is 6.8-7.0. There are two antigens for melioidosis; one is specific antigen, and the other is common with melioidosis antigen. There is cross reaction with melioidoid in agglutination test, complement binding test and allergic reaction. This bacterium does not produce exotoxin. The protein part of its toxin *in vivo*, mallein, can cause allergic reaction in infected animals, which is used as skin test antigen for diagnosis. The bacteria show strong resistance and can survive for 1 hour in feces and urine, 70 days in water, and 6 months in sterilized tap water. However, it can survive for only 10-15 days in dry environment. Exposure of the organism to direct sunlight for 24 hours and a temperature of 56ºC for 15 min can kill it. It can also be killed in 3% kerosene soap solution, 10% lime milk and 2% formaldehyde in 1 hour.

Epidemiology

Equine gangrene (Glanders) can be transmitted to healthy horses from sick horses. Natural infection is transmitted through nasal secretions, wet cough and pus from ulcers of sick animals. The infection can also occur through the digestive tract when feeding in the same tank, drinking in the same bucket, biting each other and with the intake of feed and drinking water contaminated by anthrax (Van et al. 2013). Infection caused by skin or mucous membrane trauma is rare. The infection of human gangrene is mainly through traumatic skin and mucous membrane, but rarely through food and drinking water. Humans and different animals are susceptible to this disease. Donkeys are the most susceptible animals, followed by mules. Cattle, goats, dogs, sheep and goats are occasionally infected with the disease. The captured wild lions and tigers can die of the disease due to eating the meat of infected animals.

The new disease area often presents this disease as an explosive acute epidemic. In the frequently-affected areas, the horse population shows slow and continuous transmission. Once the disease appears in the absence of timely eradication measures, it can exist for a long time, and most of the cases are chronic or recessive. Stress factors, such as poor management and feeding, overwork, disease or long-distance transportation, can cause an explosive epidemic with a large number of deaths.

Symptoms

The incubation period of the disease varies from several hours to several months. Clinically, there are two types; acute and chronic. Acute glanders is fatal within a few days to weeks, and begins with chill and high fever, depression, anorexia and emaciation. It is clinically characterized by bouts of acute cellulitis, local swelling, necrosis and ulceration at the site of skin infection, forming an ulcer with irregular edge and gray base, and covered with grayish yellow exudate (D'Elia et al. 2019). The adjacent lymph nodes are swollen, and there are many muscular and subcutaneous nodular abscesses in the lymphatics. When the abscess is opened, the red or gray pus is discharged, the mouth does not heal, and usually forms a fistula. Abscesses also appear in skin or soft tissue, and nearby lymph nodes become swollen. Sometimes, abscesses burst and a large amount of pus

flows out. Joints, bone marrow, liver, spleen, lungs, eyes and central nervous system can also be involved (Samy et al. 2017). The disease progresses slowly and can last for several months to several years. The patients gradually become thin with cachexia and often die gradually.

Diagnosis

The causative organism, *Burkholderia mallei*, penetrates the mucosa and reaches the lymphatic system. As the disease progresses, hematogenous spread occurs. The characteristic lesions of Glanders in equids include granulomata and ulcers in different tissues, nodules and fulminating ulcers on the mucous membranes of the nasal passages, larynx and upper lips (Samy et al. 2017). Bone lesions are observed in mules and humans. Modular foci underneath the pleura and diffuse granulomatous nodules may be found in the lungs. Histologic lesions are observed with granulomatosis in the lungs accompanied by inflammatory elements including macrophages, epithelioid cells and small foci of hemorrhage, edema and aggregation of interlobular and intra-alveolar fibrin (Estes et al. 2010).

Treatment and control

Horses suffering from Glanders should be isolated and thoroughly disinfected. The abscesses must be opened and drained, but care should be taken to avoid infection spread. Animals serological testing can be conducted prior to cross-regional transport to reduce disease spread risks.
Because *B. mallei* is sensitive to sulfadiazine, sulphathiazole, sulphamethazine, sulphamdimidine, tetracyclines, neomycin, oleandomycin, polymyxin B, erythromycin, kanamycin, sigmamycin and nystatin, they can be used to treat the disease. For example, as shown in Figs. 6A and 6B, horses suffering from Glanders show improved symptoms after intravenous injection (Khan et al. 2013). Figs. 7A and 7B show the effect of treatment follow-up for six months in a horse (Khan et al. 2013).

Zoonotic Diseases in Swine

Japanese encephalitis

Porcine Japanese encephalitis is a mosquito-borne porcine reproductive disorder, caused by the Flaviviridae Japanese encephalitis virus (Leake 1990). It is manifested by miscarriage, stillbirth, mummified fetus in sows, and orchitis in boars. Pigs are the main amplification host and the source of infection of Japanese encephalitis virus. The outbreak of Japanese encephalitis in humans is related to the outbreak of this disease in pigs. Therefore, Japanese encephalitis in pigs also has public health significance. The structure of Japanese encephalitis virus (JEV) is shown in Fig. 8 with the viral particle (A), the E protein structure (B) and the cross-section profile of JEV (C) (Luca et al. 2012).
Japanese encephalitis virus belongs to the Flaviviridae family. It is an RNA virus. The virus particles are spherical

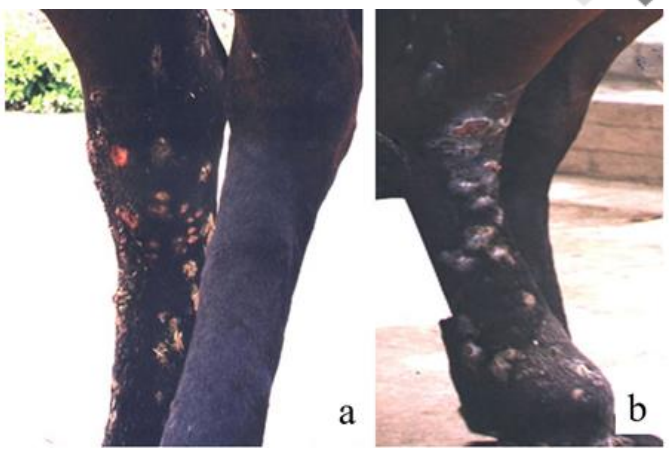

Figure 6: The reaction of experimental treatment to intravenous injection in a horse with Glanders. Ulcer appeared in right hind leg before treatment (a), and healed ulcer and scar after intravenous treatment in the second week (b). Courtesy of Khan et al. (2013).

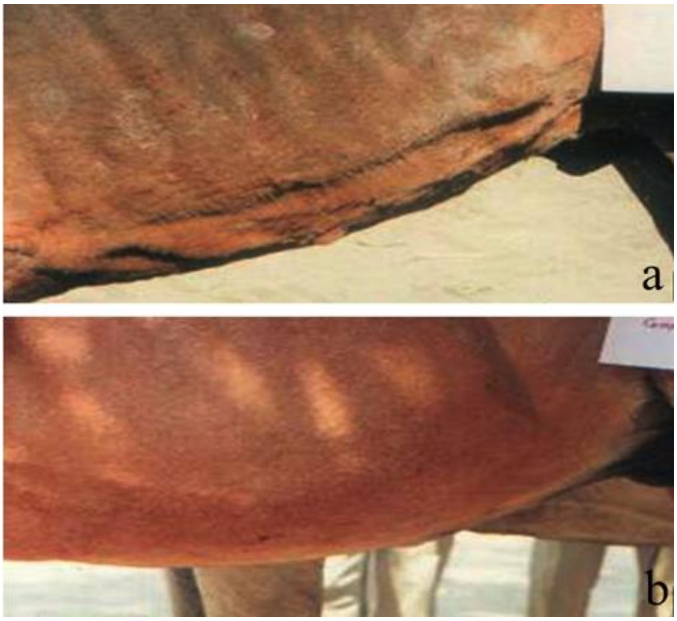

Figure 7: The experimental treatment in the horse with Glanders was followed up for 6 months. Body hair and lateral medial abdominal lymphangitis (a). The lesion (lymphangitis) disappeared completely, the physical condition improved significantly, and the rib boundary disappeared (b). Courtesy of Khan et al. (2013).

in shape with a diameter of about 40 nm. It has a capsule and is sensitive to lipid solvents. It can agglutinate the red blood cells of chickens, pigeons, geese and sheep. A variety of cell monolayers can be used for culture and proliferation of the virus. Chicken embryo culture can also be used. The virus has poor resistance to external environmental factors. Commonly used disinfectants can kill the virus. It can be inactivated by exposure for 30 minutes at 56°C.

Epidemiology

Pigs are the main propagation host and source of infection of this virus. Other domestic animals, wild

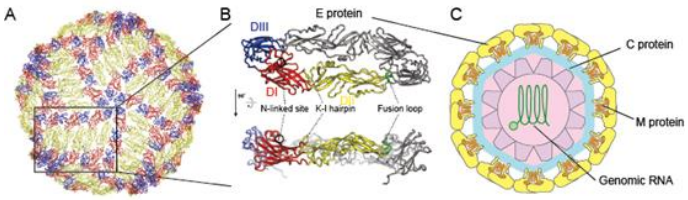

Figure 8: The viral particle (A), the E protein structure (B) and the cross-section profile of Japanese Encephalitis Virus (C). Courtesy of Luca et al (2012).

vertebrates, birds, mosquitoes, amphibians, reptiles, and bats can all serve as the host of Japanese encephalitis virus. Pigs are susceptible regardless of breed and sex, and the age of onset is mostly consistent with sexual maturity. Pigs have a high infection rate, but morbidity rate is low. In addition, humans, equines, cattle and sheep are all susceptible to the infection (Ladreyt et al. 2020).

There is a correlation between porcine Japanese encephalitis and human Japanese encephalitis. The peak of natural infection with Japanese encephalitis virus in pigs occurs 3 to 4 weeks earlier than the peak of infection with human Japanese encephalitis. It spreads through the circulation route of pig-mosquito-human etc. Transmission of the virus can occur between pigs and humans.

The occurrence of this disease is obviously related to the active season of mosquitoes. The virus can often be isolated from blood-sucking insects in endemic areas, especially Culex and Aedes, which are typical source of mosquito-borne infectious diseases. About 90% of the cases are recorded between July and September, and almost no cases occur from December to April of the following year.

Symptoms

The incubation period of Japanese encephalitis virus is generally 3 to 4 days. Affected pigs are depressed, and their body temperature rises as high as 40-41°C, with loss of appetite, thirst and flushing of the eye conjunctiva. The suffering pigs lie down with lethargy, barely stand, and then lie down again. Clinical symptoms also include accelerated heartbeat, slight shortness of breath, cough, dry stool, and dark yellow urine. Some affected pigs have swollen hind limb joints, limp or paralyzed, and have unstable gait.

Pregnant sows often have a sudden miscarriage, with mild food loss or fever before the miscarriage. Miscarriage mostly occurs in the late pregnancy. Symptoms alleviate after miscarriage, and body temperature and appetite return to normal. A small number of sows show discharge of reddish-brown or even gray-brown mucus from the vagina after abortion. After abortion, the rebreeding ability of the sow is not affected. Most aborted fetuses are stillborn, mummified, or dying. Although some of the surviving piglets are normal in appearance, they are weak in physique and cannot stand or suck milk of their mothers. Some piglets also show neurological symptoms after birth, convulsions all over the body, fall to the ground, and die within 1 to 3 days. The survivors vary in size, with most of them weak. In addition to the above-mentioned general symptoms, the prominent manifestation in the boar is orchitis after fever. One or both testicles are obviously swollen, which is 0.5 to 1.0 times larger than normal testes. The scrotum folds disappear due to the swelling of the testis. The local temperature is high, with pain. The scrotal skin of the white pig becomes red, the swelling subsides and scrotum returns to normal after 2 to 3 days, or it may become smaller, harder, and loses its function.

Lesions

Lesions of the Porcine Japanese encephalitis include the endometrial hyperemia and edema of aborted sows, with small hemorrhages and sticky secretions on the mucosa. Scraping of secretions shows mucosal erosion, lower edema, and placenta showing inflammatory reaction. Cerebral edema, congestion of meninges and spinal cord, subcutaneous edema, pleural and abdominal effusion, discoloration of the muscles, with boiled-meat like appearance can be found in aborted fetuses. There are bleeding spots on the serosal membrane, congestion of lymph nodes, necrosis in the liver and spleen, and hypoplasia of the brain and cerebellum in some fetuses. The testicles of the boar are enlarged to varying degree, the testicular parenchyma is congested and shows bleeding spots. Cut surface of testicles shows yellow necrotic foci of varying sizes, surrounded by bleeding spots. The scrotal folds disappear and the scrotum appears shining with large amount of yellow-brown opaque liquid retained in the tunica cavity. In chronic cases, atrophy and hardening of the testicles, adhesion of the testicles to the scrotum and most of the connective tissue can be seen.

Diagnosis

Preliminary diagnosis of Porcine Japanese encephalitis can be made on the basis of its epidemic characteristics, clinical symptoms and lesion characteristics. For example, the epidemic of this disease has a strict seasonality, and it usually occurs from July to September. The main symptoms in sows are abortion, stillbirth, mummified fetuses, and the one-side enlargement of testicles in boars. However, the diagnosis requires laboratory investigations.

The method of isolation and identification of pathogens is the most classic diagnostic method for Japanese encephalitis. In the early stage of fever, blood and serum can be used to isolate the virus. After the animal dies, brain tissue and body fluids should be taken as soon as possible. Aborted fetuses and placenta can also be used to take samples. The blood sample can be used directly in the experiment. After the brain tissue is ground and homogenized, it is inoculated simultaneously into the brain and subcutaneously in the suckling mice. In recent years, BHK cells have been used for isolation of the virus. After the cells have shown regular lesions, the cell fluid is

taken to inoculate mice, and the disease is observed. If there is a suspicious disease, the brain tissue is taken and then the virus is identified.

The RT-PCR method mainly amplifies the more conservative M or E genes in the genome, which can be used for early pathogen diagnosis. Serological diagnosis can also be conducted. At present, complement fixation test, neutralization test, latex agglutination test, enzyme-linked immunosorbent assay, indirect immunofluorescence test, dot immune-filtration detection method and other serological methods are available to detect specific antibodies in the serum or cerebrospinal fluid of patients with Japanese encephalitis (Mansfield et al. 2017). Among these, the hemagglutination inhibition test (HI) is the most commonly used. Because the Japanese encephalitis hemagglutination antibody IgM appears earlier, HI can be used for early diagnosis. The diagnosis can also be made on the basis of a 4-fold increase in the titer of double serum IgG antibodies. In clinical diagnosis, it should be distinguished from porcine brucellosis, porcine parvovirus disease, porcine pseudorabies, porcine chlamydia, porcine reproductive and respiratory syndrome, and porcine infectious encephalomyelitis.

Prevention

Preventive strategies of this disease include the management of host animals, with the focus on the management of young animals that have not passed through the summer and autumn seasons and animals introduced from non-epidemic areas. Most of these animals have not been infected with Japanese encephalitis. Once infected, they will develop viremia and become a source of infection. Pigs are proliferation animals of Japanese encephalitis, and sick pigs with this disease should be quarantined immediately.

Another preventive strategy is the elimination of the transmission medium and the control of transmission source. Because mosquitoes are the main transmission vector of the disease, mosquito control measuresshould be strengthened. However, it is difficult to control the epidemic of the disease simply by killing mosquitoes. Therefore, immunization becomes an important preventive measure.

The third preventive strategy is immunization. Currently, a live vaccine of Japanese encephalitis for animals has been developed. In areas where this disease is endemic, immunization of antibody-negative pigs or breeding pigs over 4 months of age one month before mosquito activity, or vaccination one month before mating is advised. A booster vaccination should be given a week later, and then a vaccination is given every year before the start of mosquito season or before breeding.

Listeriosis

Listeriosis is a food-borne, sporadic zoonotic disease of animals and humans, caused by *Listeria mononucleosis*, which has a high fatality rate (Lepe 2020). Sick animals are mainly manifested by meningitis, sepsis and pregnancy miscarriage. Affected birds are mainly manifested by necrotizing hepatitis and myocarditis. The disease is widely distributed all over the world.

Listeria monocytogenes is Gram-positive micro-bacteria. Single or two bacteria in the smear are arranged in the "V" shape, without capsules or spores, and with flagella. There are currently 7 serotypes and 16 serovariants. Pigs are more commonly affected by type 1. The bacteria have strong resistance and can survive in soil and feces for several months. Pasteurization cannot kill these bacteria. However, they can be inactivated after 30-40 minutes exposure at 65°C. General disinfectants are effective. It is sensitive to streptomycin, tetracycline and sulfa drugs.

Epidemiology

The range of susceptible animals is very wide and at least 42 species of mammals and 22 species of birds have been proved to be susceptible to this disease. Sheep, cattle, pigs, and rabbits are more susceptible. In poultry, chickens, turkeys, and geese are more commonly affected. Wild animals, wild fowls, and rodents are all susceptible to infection, and they are often the storage hosts of the disease. Humans can also be infected naturally. The feces, urine, milk, mucus and secretions of eyes, nose, and reproductive tract of infected animals and bacteria-carrying animals contain the organism. The disease can be transmitted through the digestive tract, respiratory tract, conjunctiva and injured skin. Contaminated feed and water are the main transmission media, and blood-sucking insects can also transmit the disease. Lack of green feed in winter, sudden changes in weather, internal parasites or salmonella at the time of infection can be the inducement of the disease. The disease is more common in the fertile soil.

The disease is sporadic, with usually only a small number of cases, but the fatality rate is high (Chlebicz and Śliżewska 2018). Although animals of all ages can be infected, the young ones are more susceptible, and the disease is acute in nature. In some areas, the disease occurs mostly in winter and early spring (Belœil et al. 2003).

Clinical symptoms

The incubation period of the disease is generally 2 to 3 weeks; however, it can be as short as several days, and as long as 2 months. The clinical symptoms are mainly characterized by fever, neurological signs, abortion in pregnant animals, and sepsis in young pigs. The clinical manifestations in different animals are different.

The incubation period of natural infection is usually 2 to 3 weeks, and weaned piglets and suckling piglets show symptoms of meningitis. Body temperature at the beginning of the illness is generally normal, some animals may show low fever; the body temperature can drop below 36.5°C in the later stage. At the beginning of the illness, affected animals may show disturbance of consciousness, dyskinesias, circular motions or aimless

walk, or holding the head against the ground. Some have their heads and necks turned back, with their forelimbs or hind limbs open, showing a typical "star-gazing" posture. In some cases, muscles tremble and become tough, and it is obvious in the neck and cheek muscles. Some animals show paroxysmal spasms, foaming at the mouth, lying on the ground, and swimming-like movements of the limbs. The larger pigs are shaky and show ataxia. Some have paralyzed hind limbs, unable to stand up and drag the floor. Generally, 1-4 days of exhaustion and death are seen, the long course of disease can reach 7-9 days. Piglets often suffer from sepsis, showing increased body temperature, decreased or annulled appetite, cough, diarrhea, rashes, pulmonary edema, dyspnea, blue-purple skin, etc. The course of the disease is 1 to 3 days, and the fatality rate is high. Miscarriages often occur in pregnant sows.

Lesions

There are usually no obvious gross pathological changes. Diseased animals with neurological clinical symptoms may have changes in meninges with congestion, inflammation and edema in the brain. The amount of cerebrospinal fluid is increased, becomes slightly turbid and contains many cells, and the brainstem becomes soft. The diagnosis depends on histological examination. There are inflammatory changes in the pons and encephalon. Sick animals with sepsis show sepsis changes, small punctate necrosis or multiple abscesses in the liver, spleen, and myocardium, as well as yellow staining of the subcutaneous tissue.

Diagnosis

Sick pigs with special neurological symptoms show pregnancy miscarriage and increased blood mononuclear cells. Necropsy shows meningeal congestion, edema, and small necrotic foci in the liver. Microscopic examination of brain tissue shows vascular sleeves dominated by mononuclear cell infiltration. A preliminary diagnosis can be made for lesions such as tiny purulent foci, and laboratory tests are required to confirm the diagnosis.

Prevention

Sanitation, epidemic prevention and breeding management are preventive strategies. Attention should be focused to expelling rodents and other animals with ectoparasites, and not introducing livestock and poultry from epidemic areas. Sick livestock and poultry should be treated in isolation after the onset of the disease, and the livestock houses, cages, utensils, environment and feeding troughs should be disinfected with disinfectants such as bleaching powder, and comprehensive epidemic prevention measures should be taken.

The humans are susceptible to *Listeria bacilli*, and the symptoms of encephalitis are more common. Workers engaged with diseased livestock and poultry should pay attention to protection and food hygiene. Livestock with Listeriosis should not be used for food, but they can be allowed to be slaughtered if fully recover from the disease and their health is restored. *Listeria monocytogenes* is sensitive to some antibiotics, such as sulfa drugs, gentamicin, streptomycin, tetracycline, so they are often used in the prevention and treatment of this disease. But the organism is resistant to penicillin. Early high-dose use of sulfa drugs combined with gentamicin and tetracycline have shown good results. However, in cases with obvious neurological symptoms, treatment is difficult and may not be very effective.

Streptococcosis

Streptococcosis is a general term for different clinical types of infectious diseases caused by a variety of different groups of streptococci. It is characterized by acute cases with sepsis and meningitis, and chronic cases with arthritis, endocarditis and tissue suppurative inflammation (Alello et al. 2018). Streptococcus is divided into 20 serogroups (Good 2020). Acute cases of this disease are mainly caused by group C streptococci, which has a high incidence and fatality rate and is very harmful. The most common chronic disease is lymph abscess caused by group E streptococcus.

Bacteria of the genus Streptococcus are round or oval, and are often arranged in chains of varying lengths. They often appear as short chains on solid media and long chains on liquid media. They do not form spores and generally have no flagella. Some strains can form capsules in the body or in serum-containing media, and are Gram-positive. These bacteria grow better on the medium containing fresh blood or serum. The colony is small, transparent, shows beta hemolysis, and strong pathogenicity (Sriskandan and Slater 2006). The bacteria have strong resistance to the external environment and can survive for 6 days on a field at 29-33°C. They are sensitive to disinfectants and can be killed quickly by common disinfectants (Gottschalk and Segura 2019).

Epidemiology

Sick, recessively infected and convalescent pigs after recovery are the main sources of infection. Recessively infected pigs normally carry bacteria in their tonsils and upper respiratory tract, so that they are the most dangerous source of infection. Pathogens can be detected in the nose, saliva, urine, blood, muscles, internal organs and joints of sick pigs. The disease can spread through direct contact with wounds. The respiratory tract and digestive tract are also the main routes of transmission of the disease. There is no strict age difference for this disease; both adult and young pigs can be infected, with the young pigs show the highest incidence (Aragon et al. 2012). The morbidity and fatality rates are very high. The chronic type of the disease is sporadic. There is no obvious seasonality for the disease, but the highest incidence is seen in summer and autumn, when the weather is hot and humid. A pandemic may occur from July to October.

Clinical symptoms

Based on the length of the disease and clinical manifestations, swine streptococcal disease is divided into three types: acute sepsis, meningoencephalitis, and chronic lymph node abscess. Acute sepsis is common in the early stage of the epidemic. It has an acute onset and a short course. The symptoms include high fever (above 40°C), depression, idle standing, loss of appetite, flushed conjunctiva with bleeding spots and tears, shortness of breath, dry nose with serous and purulent nasal fluid discharge. The skin on the neck, ears and lower abdomen becomes purple with bleeding spots. Most patients die within 3 to 5 days.

Meningoencephalitis is more common in suckling and weaning piglets, with meningoencephalitis as the main symptom. Other symptoms include constipation, runny or mucous nasal juices. After that, neurological symptoms appear quickly, such as blindly walking, unstable gait, moving in circles, screaming or twitching when touched, foaming at the mouth, strokes of the limbs, and death within 1 to 2 days.

Chronic lymph node abscesses are mostly transformed from acute form the disease. The symptoms include arthritis, endocarditis and tissue suppuration. It is characterized by mild disease, slow epidemic and long course, with some up to more than one month, and developmental delay. Polyarthritis is manifested by inflammation of one or more limb joints, swelling of the muscles around the joints, high limp, pain, and difficulty in standing. In severe cases, the hind limbs can be paralyzed, and finally death occurs due to physical failure and paralysis.

Diagnosis

A preliminary diagnosis can be made based on clinical symptoms and pathological changes. This disease should be distinguished from swine pneumonia, swine erysipelas and swine fever. Confirmation of preliminary diagnosis requires further laboratory tests such as microbiological examination. Different samples from the patients are taken according to different disease types, such as pus, joint fluid, liver, spleen, kidney, blood, lymph nodes, cerebrospinal fluid and brain tissue of sick or dead pigs to make smears and staining. By microscopic examination, diagnosis can be made if positive Gram, spherical or oval, and single, paired or chain-shaped cocci of different lengths are found (Alello et al. 2018).

Prevention

If the bacteria have been isolated, the most effective antibacterial drugs can be used for treatment following drug sensitivity test. If the bacteria have not been isolated and the disease is suspected, the drugs of choice are penicillin, tetracycline, oxytetracycline, and gentamicin.

Immunization with vaccines is an important measure to prevent and control this disease. Swine streptococcal disease multivalent inactivated vaccine can be used (Alello et al. 2018). A disinfection and isolation system can be established. The pig house should be kept clean, dry and ventilated, and a strict disinfection system should be established. Diagnosis of the disease should be made as soon as possible after discovering the epidemic. Sick pigs should be isolated, and the contaminated pig houses and utensils should be thoroughly cleaned and strictly disinfected.

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THE EMERGENCE OF NOVEL INFLUENZA A VIRUSES OF PUBLIC HEALTH CONCERN IN PAST DECADES

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INTRODUCTION

Influenza A viruses (IAVs) belong to Genus *Alphainfluenzavirus* and family *Orthomyxoviridae,* and contain single-stranded, negative-sense, segmented genome, comprising of eight segments. The virus particles are spherical to pleomorphic in shape. Each viral RNA molecule is associated with viral polymerase complex (polymerase basic protein 2; PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA)) and nucleoprotein (NP) to form viral ribonucleoprotein particles (McGeoch et al. 1976). In turn, each viral particle encapsidates eight vRNP molecules named as PB2-, PB1-, PA-, hemagglutinin (HA)-, NP-, neuraminidase (NA)-, matrix protein (M)- and non-structural protein-(NS)- gene. Approximately 13 kb genome of IAVs encodes at least 18 viral structural and non-structural proteins. All viral genes segments, except HA, NA and NP, are so far known to encode only one viral protein (Manzoor et al. 2017). IAVs are divided into serotypes on the basis of combination of surface glycoproteins, named as hemagglutinin (HA) and Neuraminidase (NA). So far, 16 HA (H1-H16) and 9 NA (N1-N9) have been identified (Webster et al. 1992; Wille and Holmes 2020).

IAVs have the ability to infect a wide range of avian and mammalian host species. However, traditionally wild aquatic birds and shorebirds are considered as the main natural reservoirs for IAVs. At least, 105 species of wild birds have been identified as natural reservoirs of IAVs. The IAVs are being maintained in the "natural reservoir" via fecal-oral route and the prevalence level can reach up to 20%, or even more during the autumn migration season (Munster et al. 2007; Latorre-Margalef et al. 2014). The recent discovery of novel and highly divergent genome of H17N10 and H18N11 viruses from fruit bats suggest that wild aquatic birds are not the exclusive influenza A virus reservoirs (Tong et al. 2012; Tong et al. 2013). Occasionally, IAVs leave the natural reservoir, overcome the species barrier through genetic reassortment and/or genetic mutations and cause sporadic infections, epidemics or pandemics in other host species, such as poultry, various mammalian species and even humans (Webster et al. 1992; Wille and Holmes 2020).

Historically, IAVs have caused multiple pandemics. There have been at least 13 pandemics since 1500; and in the past 130 years, the mankind has undoubtedly faced pandemics in 1889, 1918, 1957, 1968, 1977 and 2009 (Morens and Fauci 2007; Taubenberger and Morens 2009). Interestingly, these pandemics, except those of 1968 and 1977, occurred approximately at 40-years intervals. The worst was the 1918 pandemic that claimed about 50 million human lives worldwide (Taubenberger and Morens 2006). In addition to the pandemics, the IAVs are also responsible for periodic seasonal flue and sporadic outbreaks. It is estimated that seasonal influenza viruses infect 15% of the human population each year, resulting in ~500,000 deaths worldwide (Clem and Galwankar 2009).

Although IAVs show species restriction, sometimes spillover event happens, allowing them to switch to a new host. These spillover events have allowed IAVs to establish new lineages in novel hosts, such as the emergence of swine or equine lineages. The most important of these spillover events is the emergence of novel IAVs causing pandemics in human populations. The adaptation of IAVs to a new host species involves adaptation at multiple levels i.e., entry into the host, attachment and entry into the host cell, successful replication inside the host cells and final release from the host cells and host. In addition to the mutations in the genome of IAVs, genetic reassortment between different IAVs plays a critical role in crossing the species barrier and adaptation to a new host. Following co-infection with different types of IAVs (different lineage or different HA and/or NA subtypes), viral progeny containing many gene segment combinations different from parental viruses is produced. Though many of these combinations will be harmful to the virus, some may facilitate adaptation to the new host. Thus, the emergence of novel IAVs might be a result of within-species reassortment or cross-species reassortment (Wille and Holmes 2020), as shown in Fig. 1. Sometimes, fine-tuning in viral genome facilitated by adaptive mutations is required, especially if an avian virus jumps to a mammalian host. Avian IAVs bind to α2, 3-linked sialic acid receptors with higher affinity, whereas human IAVs bind to α2,6-linked sialic acid receptors with higher affinity. However, recent studies showed that amino acid substitutions Q226L and G228S in receptor binding domain of H2 and H3 subtype IAVs changed the binding preference from avian to human sialic acid receptors. Similarly, A134V, N182S, S223N and R497K mutations in H5 HA were shown to increase viral affinity for human type sialic acid receptors. Human/mammalian adaptation mutations have also been reported in internal genes, such as E627K, D701N in PB2, N375S in PB1 etc. (Schrauwen and Fouchier 2014; Guo et al. 2019).

In recent decades, there have been multiple incidences of the emergence of novel IAVs, and in some instances, human transmissions were also recorded. In the following paragraphs, the avian influenza viruses (AIVs) that have posed a serious threat to public health in the past decades have been discussed.

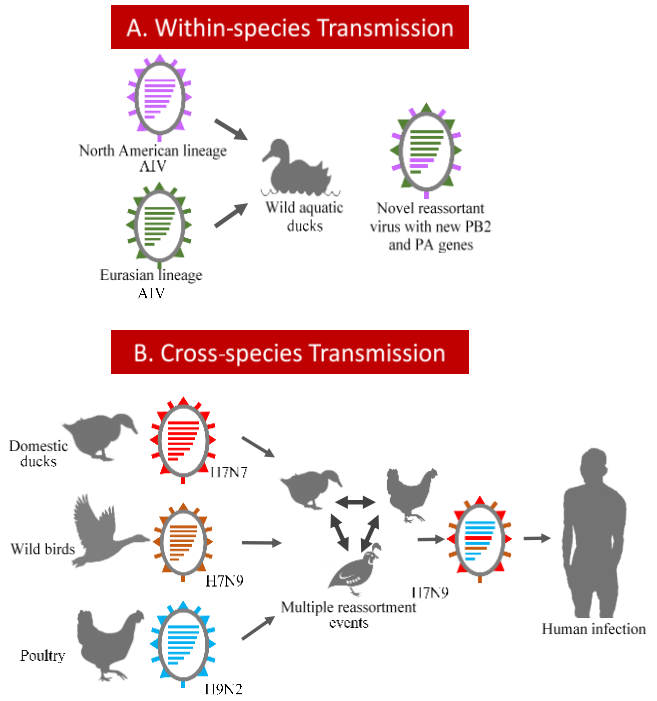

Fig. 1: Emergence of novel IAVs. A. Within-species reassortment of genes occurs when IAVs of different subtypes or lineages co-infect the same species. A/duck/Hokkaido/W95/2006 (H10N8) virus was isolated from migratory ducks during surveillance in northern Japan. The sequence analysis showed that NA and M genes were of American lineage, while the remaining six genes were of Eurasian lineage (Manzoor et al., 2008). B. Emergence of H7N9 influenza virus that infected humans. The virus emerged as a result of multiple reassortment events in domestic ducks, poultry and wild birds. The gene segments of this novel virus are closely related to the viruses found in domestic ducks, wild birds and poultry in Asia (Lam et al., 2013).

H5 subtype IAVs

Highly pathogenic avian influenza viruses (HPAIVs) have been limited to H5 and H7 HA subtypes, and not all viruses of these subtypes are highly pathogenic for chicken (Manzoor et al. 2008). These H5 and H7 subtypes of avian influenza viruses (AIVs) are maintained in their natural reservoirs as apathogenic avian influenza viruses (APAIVs). Many studies suggest that these APAIV strains are first transmitted to terrestrial poultry, such as domestic waterfowls, quails or turkeys. Then, during multiple transmission events in chicken population, they may acquire pathogenicity for chicken to become HPAIVs (Swayne and Suarez 2000; Ito et al. 2001; Nao et al. 2017). This shift in pathogenicity results from the accumulation of multiple basic amino acids at the HA cleavage site, allowing the virus to gain maturity outside the gastrointestinal tract and establish systemic infection. In 1996, several outbreaks occurred in geese farms at Sanshui, Foshan, a rural area in Guangdong province of China (Wan 2012; Nunez and Ross 2019) and a virus, A/goose/Guangdong/1/1996 (H5N1) (Gs/Gd/96) was isolated from a sick goose. Later, extensive surveillance for influenza was conducted in Guangdong province between January 1996 and December 1997. Surprisingly, no H5N1 HPAI isolate could be detected, suggesting that Gs/Gd/96-like virus probably was not efficiently transmitted among poultry (Zhao et al. 2008; Wan 2012). Later, Gs/Gd/96-like viruses mutated by genetic reassortment were found to be associated with many outbreaks in poultry. These mutations allowed the viruses to be easily transmitted by fecal-oral route from waterfowl to terrestrial poultry (Webster 2002). In 1997, three chicken farms in Hong Kong were affected by an H5N1 HPAIV, with mortality rates ranging between 70 and 100%. In May 1997, the first human case of a 3-year-old boy infected by a novel H5N1 virus was reported in Hong Kong. Later, 17 more human cases were reported between November 1997 and January 1998, raising the concern about potential pandemic (Claas et al. 1998; Subbarao et al. 1998; Bender et al. 1999). Interestingly, there were outbreaks in chicken farms in Hong Kong just before the first and second waves of human infections. Comparison of all gene segments of human and avian isolates, and temporal relationship between avian and human outbreaks, strongly suggested direct chicken-to-human transmission (Claas et al. 1998; Suarez et al. 1998; Subbarao et al. 1998: Shortridge 1999). Later, several studies were conducted to identify the origin of these lethal H5N1 viruses isolated in Hong Kong. These studies showed that only HA gene was derived from A/goose/Guangdong/1/1996 (H5N1), whereas other genes might have been derived from locally circulating IAVs, particularly H9N2, since both H5N1 and H9N2 viruses co-circulated in Hong Kong in 1997 (Subbarao et al. 1998; Guan et al. 1999; Xu et al. 1999). Another study suggested that A/teal/Hong Kong/W312/97 (H6N1) might have acted as gene donor, since it showed >98% nucleotide homology with six internal genes of A/Hong Kong/156/1997 (H5N1) (Hoffmann et al. 2000).

Although the 1997 outbreak was controlled by large scale stamping out of 1.5 million poultry in Hong Kong poultry markets, the putative precursor viruses continued to circulate in Hong Kong poultry i.e. H5N1 Gs/Gd/96-like viruses in geese (Cauthen et al. 2000; Webster et al. 2002) and the H9N2 (G1)- and H6N1 (W312)-like viruses in quails (Guan et al. 2000; Chin et al. 2002). Continued surveillance activities in Hong Kong poultry markets during 2000-2001 revealed the existence of multiple genotypes of H5N1 viruses that differed from H5N1/97 viruses. The genetic analysis of the isolates suggested that these H5N1/2001 viruses originated as a result of reassortment among viruses from aquatic birds. Until then, these H5N1 did not kill their natural hosts and were thought to be in evolutionary state Unexpectedly, in late 2002, these H5N1 viruses caused deaths of many resident avian species (such as waterfowls and greater flamingoes), as well as migratory birds (such as egrets, grey herons) in two Hong Kong parks. It was the first report of a fatal case in the natural reservoir since 1996 (Becker 1966). At the same time, H5N1 viruses were also isolated from dead chicken in live bird markets and poultry farms in Hong Kong. In 2003, concurrently, H5N1 HPAIVs were isolated in China and these viruses displayed higher pathogenicity for ducks in a species dependent manner (Sturm-Ramirez et al. 2005; Pantin-Jackwood and Swayne 2007). In February 2003, H5N1 viruses were isolated from two

humans in Hong Kong (Wuethrich 2003). Since 2003, there had been multiple outbreaks by various genotypes of H5N1 viruses across Asia, Europe and Africa. In April – June 2005, an outbreak caused by a novel H5N1 genetic variant killed more than 6000 wild migratory birds at Lake Qinghai in Southern China and this variant further spread to Middle East, Europe and Africa (Chen et al. 2005; Wang et al. 2008). These outbreaks confirmed the establishment of a new lineage with virulence potential for the natural reservoir. Since January 2003, there have been 862 cases of human infections with H5N1 AIVs, reported from 17 countries, with case fatality rate of 53% (WHO 2021).

The extensive circulation of H5 subtype IAVs has resulted in the emergence of multiple lineages. Therefore, the Southern part of China is considered as a potential influenza epicenter due to its unique ecological system (Shortridge 1997). It is noteworthy to mention that since 1970, at least ten HA subtypes and nine NA subtypes in various combinations have been isolated from ducks and geese in southern China (Wan 2012). These enzootic viruses posed a continuous threat for the emergence of novel AIVs due to potential reassortment with viruses associated with HPAIV outbreaks in poultry and aquatic birds. During 2010-11, a new H5 HA clade (2.3.4.4) emerged. This HA gene displayed a unique tendency to reassort with NA subtypes other than N1, resulting in the emergence of novel AIVs. Since 2010, there were multiple outbreaks of H5N2, H5N3, H5N5 and H5N8 HPAIVs in poultry across Asia, Europe and North America. These viruses emerged as a result of reassortment events with H5N1 and other circulating viruses, such as H9N2, H6N6 etc. (Jhung et al. 2015; Shin et al. 2015; Verhagen et al. 2015; Claes et al. 2016). The H5N6 IAVs spread to countries in Asia and H5N8 IAVs spread to Europe and North America (Claes et al. 2016). The first laboratory-confirmed case of human infection by H5N6 was reported in April 2014 in Sichuan Province, China. Since then, a total of 32 laboratory-confirmed cases of human infection by H5N6 IAV with 19 deaths have been reported in the Western Pacific region. In February 2021, seven laboratory confirmed cases of human infection by H5N8 IAV were reported from Russian Federation (WHO 2021). The human infections by H5N6 and H5N8 viruses were the result of direct contact with infected poultry and no human-to-human spread has been reported.

H6 subtype IAVs

The first H6 subtype IAV was isolated from turkeys in Massachusetts in 1965. Since then, H6 subtype IAVs have been frequently isolated from aquatic birds and terrestrial poultry from various parts of the world in combination with all NA (N1-N9) subtypes (Downie et al. 1973). Classically, H6 subtype IAVs are responsible for low pathogenic avian influenza virus (LPAIV) infections in poultry. Globally, LPAIV infections by H6 virus are becoming increasingly an economical burden on the poultry industry. In China, H6 subtype AIVs rank second after H9 subtype AIVs in prevalence in aquatic birds and terrestrial poultry (Pepin et al. 2013). According to a surveillance study conducted from 1998–2006 (8 years) on more than 36,000 wild birds from Europe and the Americas, the H6 was the most abundantly detected influenza virus subtype (Munster et al. 2007). Several studies suggest that H6 subtype IAVs display the broadest host range among all IAVs. More than 30% of the H6 subtype IAVs isolated from Southern China showed enhanced affinity to human-like α2,6-linked sialic acid receptors (Wang et al. 2014). A study evaluated the replicative potential of 14, non-adapted, H6 subtype AIVs (H6N1, H6N2, H6N5, H6N8 and H6N9) in mice. The tested viruses infected the mice and produced neutralizing antibodies (Gillim-Ross et al. 2008). Another study demonstrated the ability of the tested H6 subtype viruses to be transmitted by direct contact between guinea pigs (Wang et al. 2014). Lin et al. (2015) isolated an H6N1 IAV from a dog in Taiwan, and genetic analysis showed that the virus was closely related to the circulating H6 AIVs in Taiwan. So far, there is only one case of human infection reported in 2013 in Taiwan by H6N1 IAV, and the sequence analysis showed that the virus was closely related to the circulating avian H6 viruses. However, there have been some studies that reported 0.4-2.5% seroprevalence for H6 HA IAVs among poultry workers (Myers et al. 2007; Quan et al. 2019). These findings clearly warrant the continuous surveillance for H6 subtype influenza A viruses to avoid any future zoonotic infections.

H7 subtype IAVs

H7 AIVs have been shown to infect a wide range of host species, encompassing wild and domestic aquatic birds and terrestrial poultry, and mammals including seals, pigs, horses and humans (Abdelwhab et al. 2014; Mostafa et al. 2018; Naguib et al. 2019). Like H5 AIVs, H7 AIVs also can acquire HPAIV potential while circulating in poultry asymptomatically. From infected poultry, human infections with LPAIV or HPAIV have been reported. H7 subtype IAVs of low and high pathogenic potential have caused several outbreaks in poultry (Table 1), with occasional transmissions to humans (Table 2). Before 2003, there were very few cases of human infection by H7 subtype IAVs. Mostly, these cases were reported in laboratory workers. A brief history of various instances of human infections by different H7Nx IAVs is discussed below.

H7N2

The first evidence of human infection by LPAIV H7N2 was reported in a poultry worker in USA during 2002 (Terebuh et al. 2018) and the first LPAIV H7N2 was isolated from an immunocompromised patient in USA in 2003 (Ostrowsky et al. 2012). Other instances of human infections by LPAIV H7N2 include four humans in 2007 in UK and infection of a veterinarian in a cat shelter in USA in 2016 (Belser et al. 2017; Marinova-Petkova et al. 2017; Naguib et al. 2019).

H7N3

Serological analysis using microneutralization assay showed that 7 of 185 poultry workers, who had direct

Table 1: Outbreaks of LPAI/HPAI caused by H7 subtype viruses in poultry

Country	Year	Subtype	Virulence	Species	Reference
UK	1963	H7N3	HPAI	Turkeys	(Wells 1963)
Australia	1976	H7N7	HPAI	Chicken	(Turner 1976)
UK	1979	H7N7	HPAI	Turkeys	(Alexander et al. 1979)
Germany	1979	H7N7	HPAI	Chicken	(Rohm et al. 1996)
Australia	1985	H7N7	HPAI	Chicken	(Barr et al. 1986)
Australia	1992	H7N3	HPAI	Ducks	(Forsyth et al. 1993)
Australia	1994	H7N3	HPAI	Chicken	(Dhingra et al. 2018)
Pakistan	1994	H7N3	HPAI	Chicken	(Naeem and Hussain 1995)
USA	1996-98	H7N2	LPAI	Chicken	(Henzler et al. 2003)
Australia	1997	H7N4	HPAI	Chicken	(Selleck et al. 2003)
Ireland	1998	H7N7	LPAI	Chicken	(Campbell and De Geus 1999)
Northern Ireland	1998	H7N7	LPAI	Chicken	(Graham et al. 1999)
Italy	1999-01	H7N1	HPAI, LPAI	Turkeys	(Capua and Alexander 2004)
Canada	2000	H7N1	LPAI	Turkeys	(Pasick et al. 2003)
Germany	2001	H7N7	LPAI	Chicken	(Werner et al. 2003)
Pakistan	2001	H7N3	HPAI, LPAI	Chicken	(Swayne and Suarez 2001)
Chile	2002	H7N3	LPAI, HPAI	Chicken	(Rojas et al. 2002)
USA	2002-04	H7N2	LPAI	Turkeys & chicken	(Spackman and Suarez 2003)
Italy	2002-03	H7N3	LPAI	Turkeys	(Capua and Alexander 2004)
Pakistan	2003-04	H7N3	HPAI	Chicken	(Abbas et al. 2010)
The Netherlands	2003	H7N7	HPAI	Chicken	(Fouchier et al. 2004)
Belgium	2003	H7N7	HPAI	Chicken	(Capua and Alexander 2004)
Germany	2003	H7N7	HPAI	Chicken	(Schrauwen and Fouchier 2014)
Canada	2004	H7N3	LPAI, HPAI	Chicken	(Hirst et al. 2004)
USA (Delaware, Maryland)	2004	H7N2	LPAI	Chicken	(Capua and Alexander 2004)
Korea	2005	H7N7	HPAI	Chicken	(Dhingra et al. 2018)
Canada	2007	H7N3	HPAI	Chicken	(Berhane et al. 2009)
UK	2008	H7N7	HPAI	Chicken	(Dhingra et al. 2018)
Spain	2009	H7N7	HPAI	Chicken	(Iglesias et al. 2010)
Mexico	2012	H7N3	HPAI	Chicken	(Maurer-Stroh et al. 2013)
Australia	2012	H7N7	HPAI	Chicken	(FAO)
Italy	2013	H7N7	HPAI	Chicken	(Bonfanti et al. 2014)
Australia	2013	H7N2	HPAI	Chicken	(Dhingra et al. 2018)
Germany	2015	H7N7	HPAI	Chicken	(APHA 2015)
UK	2015	H7N7	HPAI	Chicken	(APHA 2015)
USA	2015	H7N8	HPAI	Turkeys	(Killian et al. 2016)
USA	2017	H7N9	HPAI	Chicken	(Naguib et al. 2019)

Table 2: Human infections by H7 subtype influenza A viruses

Country	Year	Subtype	Pathotype	No. of human cases	Reference
USA	1959	H7N7	HP	1	(Campbell et al. 1970; DeLay et al. 1967)
Australia	1977	H7N7	HP	1	(Taylor and Turner 1977)
USA	1979-80	H7N7	LP	4	(Webster et al. 1981)
UK	1996	H7N7	LP	1	(Banks et al. 1998; Kurtz et al. 1996)
USA (Virginia)	2002	H7N2	LP	1#	(Terebuh et al. 2018)
Italy	2002-03	H7N3	LP	7#	(Puzelli et al. 2005)
USA (New York)	2003	H7N2	LP	1	(Ostrowsky et al. 2012)
The Netherlands	2003	H7N7	HP	89	(Du Ry van Beest Holle et al. 2005; Koopmans et al. 2004)
Canada (British Columbia)	2004	H7N3	HP	2	(Tweed et al. 2004)
UK (Norfolk)	2006	H7N3	LP	1	(Nguyen-Van-Tam et al. 2006)
UK (Wales)	2007	H7N2	LP	4	(Belser et al. 2017; Naguib et al. 2019)
Mexico	2012	H7N3	HP	2	(Lopez-Martinez et al. 2013)
China	2013~	H7N9	LP/HP	1568	(WHO 2021)
Italy	2013	H7N7	HP	3	(Puzelli et al. 2014)
USA	2016	H7N2	LP	1	(Marinova-Petkova et al. 2017)
China	2018	H7N4	LP	1	(Gao et al. 2018; Tong et al. 2018)

Serologic evidence only

contact with poultry during 2002-03 outbreaks by LPAIV H7N3, became seropositive to H7 HA (Puzelli et al. 2005). In 2004, two laboratory-confirmed human infections with HPAIV H7N3 were reported from British Columbia, Canada (Tweed et al. 2004). In 2006 in UK, an LPAIV H7N3 was isolated from a poultry worker, who attended an outbreak in poultry by LPAIV H7N3, and infection was only limited to conjunctivitis (Nguyen-Van-Tam et al. 2006). Later, in 2012, two laboratory-confirmed cases of human infection with HPAIV H7N3 were reported from Jalisco, Mexico (Lopez-Martinez et al. 2013).

H7N4

So far, only one case of human infection with LPAIV H7N4 has been reported from Jiangsu, China. The virus was isolated from a woman (68-year-old) in February 2018. The entire genome of the virus was of avian origin, and the evidences suggested that the infection was probably acquired directly from poultry during a visit to the live bird market (Gao et al. 2018; Tong et al. 2018).

H7N7

In 1959 in USA, H7N7 virus was isolated from the blood of a man diagnosed with infectious hepatitis (DeLay et al. 1967; Campbell et al. 1970). In 1977, a laboratory technician accidentally got infected with H7 virus while handling infectious allantoic fluid (Taylor and Turner 1977). During 1979-80, four technicians got infected after conducting necropsies of the seals died of H7N7 infection (Webster et al. 1981). The first evidence of direct avian-to-human transmission of H7N7 AIV was reported in 1996 in UK, when one woman developed conjunctivitis after cleaning her duck house (Kurtz et al. 1996; Banks et al. 1998). In 2003 in Netherlands, an outbreak with H7N7 HPAIV was reported in poultry farms and human transmission was confirmed by PCR in 86 poultry workers, mostly developed conjunctivitis and/or mild respiratory symptoms. Three households also got the infection from poultry workers, suggesting limited human-to-human transmission. This outbreak also claimed one human life, a veterinarian who visited multiple farms and was hit by the virus (Koopmans et al. 2004; Du Ry van Beest Holle et al. 2005). The genetic analysis showed that the virus had avian origin and was related to previously circulating LPAIVs in ducks (Fouchier et al. 2004). In 2014 in Italy, three cases of human infection with HPAIV H7N7 were reported from poultry workers and the clinical signs were only limited to conjunctivitis. The sequence analysis confirmed that all gene segments were closely related to the virus isolated from the same poultry farm (Puzelli et al. 2014).

H7N9

H7N9 has caused the highest number of human infections among all reported human infections caused by other than H7N9 subtype IAVs. Since March 2013, a total of 1568 laboratory-confirmed human infections with LPAIV H7N9 have been reported, including 616 deaths, the case fatality rate was 39% (WHO 2021). The majority of the cases were reported from China, though a few cases were reported from Taiwan, Malaysia and Canada. So far, there is no evidence of sustained human-to-human transmission of H7N9 infection. Since the first report in 2013, there have been 7 waves of infection, mainly occurring in winter. Extensive genetic analysis suggests that the LPAIV H7N9 virus originated as a result of several reassortment events between three different AIV subtypes. The zoonotic H7N9 virus acquired HA gene from an H7N7 virus, NA gene from an H7N9 virus and six remaining genes from an enzootic H9N2 virus, as shown in Figure 1B (Lam et al. 2013).

H9 subtype IAVs

The first H9 subtype (H9N2) AIV was isolated from an LPAIV outbreak in turkeys in February 1966 in northern Wisconsin, USA (Homme and Easterday 1970). Later, H9N2 viruses were found to be associated with multiple outbreaks, particularly, in turkey production states of Minnesota and Wisconsin (Halvorson et al. 1983; Carnaccini and Perez 2020). Then, in the following years, these viruses were isolated from various parts of Asia and Africa. Three H9N2 viruses, for the first time, were isolated from terrestrial poultry in 1988 in Hong Kong. Now, these viruses are endemic in many parts of Asia, the Middle East and Africa (Peacock et al. 2019). Like other IAVs, H9 subtype IAVs are also being maintained in wild aquatic birds. Although H9 subtype IAVs have been isolated in combination with all NA (N1-N9) subtypes, the huge number of isolated viruses has been reported to be in combination with NA N2 subtype (H9N2), suggesting the preferred association between H9 and N2 molecules (Yan et al. 2016; Carnaccini and Perez 2020). Due to low pathogenic potential, H9N2 viruses are often found co-circulating with other pathogens, like other AIVs subtypes or bacteria, thereby causing significant morbidity and production losses.

Phylogenetically, HA gene of H9 viruses can be divided into two broader lineages i.e., American and Eurasian lineages. H9 viruses belonging to American lineage have mostly been isolated from wild aquatic birds, especially sea birds with sporadic outbreaks in poultry. On the contrary, H9 viruses in the Eurasian lineage are stably circulating in poultry, as well as aquatic birds, with occasional transmission events from wild aquatic to terrestrial poultry. The endemicity of H9 viruses has led to the emergence of phylogenetic diversity, resulting in the emergence of many clades/subclades. Broadly, viruses in a Eurasian lineage can be categorized into the G1-h9.4, BJ94-h9.3 (also known as Y280 or G9) and Y439-h9.2 (Korean) sub-lineages (Guo et al. 2000; Chen et al. 2009; Liu et al. 2009).

The enzootic nature of H9 viruses in Asia poses a major threat to public health. The first human infection with H9N2 AIVs was reported from Hong Kong in 1998 (Peiris et al. 1999). Later, many cases of human infections have been reported from China, Pakistan, Bangladesh, Egypt and Oman (Butt et al. 2005; Chakraborty 2011; Ali et al. 2019; Almayahi et al. 2020). As of 25th June 2021, a total of 59 laboratory-confirmed cases of human infections with H9N2 have been reported (WHO 2021). The majority of the infections were reported in young children usually with mild respiratory symptoms. In most human infections, direct or indirect contact with poultry was confirmed. The phylogenetic analysis of human isolates showed that H9 HA gene belonged to G1 or BJ94 sub-lineages. Additionally, many surveillance studies, conducted among poultry workers in countries where H9 subtype AIVs are endemic, suggested significant exposure

to H9 subtype IAVs. Fortunately, there has been no report of human-to-human transmission of H9N2 viruses (Carnaccini and Perez 2020). However, seroconversion in poultry workers indicates poor management practices in the poultry industry in these countries. It is very important to point out that H9 subtype AIVs have the propensity to donate genes to other AIVs that can cause zoonotic infections, for example, recent H10N8, H7N9 and H5N6 viruses acquired internal gene cassette from circulating H9N2 viruses (Lam et al. 2013; Chen et al. 2014).

H10 subtype IAVs

The first H10N7 AIVs was isolated from a chicken in Germany in 1948 (Feldmann et al. 1988). Since then, H10 AIVs with different NA combinations have been isolated from various species of wild and domestic aquatic and terrestrial birds across the globe. Genetic analysis showed that these H10 AIVs could be grouped into two lineages i.e., Eurasian and North American lineage (Liu et al. 2009; Zhuang et al. 2019). H10 AIVs were thought to infect only avian species. However, in 1984 in Sweden, the first report of infection of farmed minks with H10N4 AIV was reported. In 2004, two laboratory-conformed cases of human infection (two infants) with H10N7 were reported from Egypt. Later, the same virus was isolated from samples collected from wild ducks (Organization. 2004). In 2008, an H10N5 AIVs was isolated from pig samples in the slaughterhouse of Hubei province of central China. The genetic analysis showed that the virus was of avian origin and belonged to Eurasian lineage (Wang et al. 2012). In 2010, an outbreak by low pathogenic avian influenza H10N7 virus (LPAIV) was reported from a poultry farm in New South Wales, Australia. Later, the virus was isolated from abattoir workers, who reported conjunctivitis and mild rhinorrhea. The genetic analysis showed that H10 gene belonged to American lineage (Arzey et al. 2012). In 2014, H10N7 AIV was found to be associated with deaths in harbor seals in Sweden. The phylogenetic analysis showed that H10 gene belonged to Eurasian lineage and clustered with avian viruses isolated from wild aquatic birds (Zohari et al. 2014). Interestingly, all of these events were isolated, short-term and lacked sustained animal-to-animal or human-to-human transmission. Each of the human case had a history of either direct interaction with, or proximity to the live poultry or aquatic birds, and the infections was non-fatal.

A novel H10N8 reassortant IAV was isolated in December 2013 from the tracheal aspirate specimen of a patient suffering from pneumonia in Nanchang, Jiangxi province China; the patient passed away 9 days later. Later, three more human infections with H10N8 novel IAV were confirmed, with two of them proved fatal (Chen et al. 2014; Zhang et al. 2015). The high fatality rate caused by the novel H10N8 virus raised a significant public health concern. Extensive surveillance was conducted and H10N8 AIVs were isolated from wild aquatic birds and terrestrial poultry in live poultry markets (Qi et al. 2014; Zhang et al. 2014). In May 2014, this H10N8 AIV was reported from feral dogs living in close proximity of live bird markets in Guangdong Province, China (Su et al. 2014). Based on extensive genetic analysis, it has been proposed that the H10N8 virus that infected a human emerged as a result of interaction between wild birds and farmed ducks in China, leading to the transfer of H10 and N8 genes to the live poultry markets, where reassortment with enzootic H9N2 viruses occurred. The human infections with H10N8 viruses were coincident with H10 virus outbreaks in live poultry markets in China (Ma et al. 2015), and the patients infected with the H10N8 virus had a history of visiting live-poultry markets or exposure to live poultry before disease onset, thus further strengthening the postulate.

Conclusion

Investigations on most of the recent human infections clearly show that infected people were in direct contact with the poultry and most of these contacts took place in live bird markets. Live bird markets are thought to act as sanctuary for the mixing and reassortment events, since different species of wild and domestic aquatic birds and terrestrial poultry are kept together. A retrospective epidemiological study showed that most patients infected with H5N1 visited the live bird market in China (Yu et al. 2007). Therefore, continuous monitoring of IAVs and proper control measures to bring various avian species to these markets is necessary. Moreover, proper training of people involved in handling poultry and poultry products should be made mandatory. Additionally, continuous surveillance activities in wild and domestic aquatic birds should be ensured to monitor the emergence of any novel IAVs and to prevent their transmission to terrestrial poultry.

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RECENT TRENDS IN IMMUNOLOGY AND VACCINATION AGAINST NEWCASTLE DISEASE VIRUS

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INTRODUCTION

Poultry is one of the largest groups of livestock all over the world. Newcastle disease (ND) is an infectious, contagious, septicemic, fatal and destructive poultry disease that primarily affects chicken and turkey. This disease is responsible for huge economic losses to the global poultry industry. It has also devastating effect on the domestic poultry production. This viral disease was first time appeared in 1926, in England (Newcastle). This disease is now found all over the world, causing symptoms such as nervous manifestation, diarrhea and acute respiratory disease. Newcastle Disease Virus (NDV), the causative agent of ND, belongs to the *Paramyxoviridae,* which is a large family of RNA viruses having envelope and negative strand of RNA. The virus envelope is lipid in nature and derived from plasma membrane of host cell (Lamb and Jardetzky 2007).

Traditional vaccines and variety of diagnostic tests are available for prevention and diagnosis of NDV, respectively. Traditional vaccines are still used, but they have been linked to disease symptoms and failure to respond under field conditions. To address the issues with conventional vaccines, recombinant vaccines have been developed by expressing various immunogenic genes.

Newcastle Disease

ND is an infectious disease of birds that can be transmitted to humans. It is a disease that affects both domestic and wild avian species. It is highly contagious viral infection that affects poultry and birds of all ages and both sexes. The most susceptible avian species of ND are: chickens, pigeons, Japanese quails, turkeys, guinea fowls, ducks and other wild birds irrespective of their ages. Some mammals, including dogs, cats and humans, are also susceptible to ND (Ashraf and Shah 2014). Since 66% of the population in developing countries lacks access to a proper protein diet, ND is considered as vital in the poultry industry in these countries, as it poses a significant economic threat to the industry and causes huge losses in poultry all over the world (Abdisa and Tagesu 2017).

History

Although the ND panzootics occurred in all over the world but specifically epizootics have been reported in Africa, Asia, South and Central America and sporadic epizootic have occurred mostly in Europe. The very first panzootic was reported from 1926 to late 1950s in Newcastle-upon-Tyne (England) from Europe, as well as Java (Indonesia) from Southeast Asia (Seal et al. 1995; Qiu et al. 2011). In 2012, APMV type I serotype caused the serious disease in birds of Jallo Wildlife Park in Lahore, Pakistan, resulting in the deaths of 190 peacocks. Overall, it resulted in a 100% mortality rate, as well as 50% damage to the susceptible birds. After isolating the causative agent and conducting various serological and molecular diagnostic investigations, such as PCR, ELISA, and the HI test, the velogenic strain NDV was verified. Different strains of NDV have different rates of morbidity and mortality in birds (Shahzad et al. 2015; Abdisa and Tagesu 2017).

Importance of Poultry

Poultry is the most common domestic animal stock in the world in terms of animal numbers. In the backyard animal production of developing countries, poultry occupies very important position. Poultry is a source of income for villagers in developing countries. Backyard poultry production poses a high risk of infection, such as ND in poultry, due to fewer biosecurity activities. Poultry serve as inexpensive and a high-quality source of protein in the developing countries. Availability of eggs is approximately increasing up to 4% each year (Thomazelli et al. 2012). According to a Pakistani survey, there are approximately 1105.91 million poultry birds in the country, with approximately 152.44 million rural poultry. Poultry provides a significant contribution to the village's economy, accounting for 3611 million eggs and 100.42 metric tons of total poultry meat (Khan et al. 2010).

Newcastle Disease Virus

Epidemiology

The most destructive NDV panzootics have occurred over the last ten decades. The first one began in Asia and Europe and spread across the world over the course of 20 years, beginning in the mid-1920s. The second pandemic spread to the entire world in just four years due to the commercialization of the poultry industry and increased foreign exchange in cage birds, which served as a source of NDV. The third pandemic, during 1980s, in the racing pigeons was believed to be caused by genotype VI isolates. This pandemic also showed wreaking havoc on a variety of

birds uncontrollably due to excessive racing pigeon's husbandry (Meng et al. 2016). Similarly, fourth pandemic, which spread in late 1980s, also caused huge economic losses to a vast number of countries, including Middle East, Africa, Europe, and South East Asia. The fourth pandemic is thought to be caused by NDV genotype VII, which is the fastest-evolving strain of the virus. The emergence of virulent NDV strain has always posed to the poultry industry. Major source of reservoir for the emergence of virulent strain is the lentogenic strain. In term of ecological interference, this strain can be easily transmitted form wild birds to domesticated poultry. The emergence of virulent NDV strains has always posed a risk to the poultry industry. Lentogenic strains are a major reservoir for the emergence of virulent strains. In terms of environmental impact, this strain can easily be transmitted from wild birds to domesticated poultry (Ayala et al. 2016).

Structure

Newcastle diseases virus (NDV) is the causative agent of Newcastle Disease. This is a non-segmented, single stranded, RNA virus. It belongs to *Avian Paramyxovirus* (APM) type I, genus *Avula Virus*, family *Paramyxoviridae* (Fauquet and Fargette 2005). A wide range of both domestic and wild birds can be affected by NDV. The genome of NDV consists of six genes, named as nucleocapsid (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), large protein (L) and hemagglutinin-neuraminidase protein (HN). All the viral genes are monocistronic; forming a single structural protein, except the P gene. P gene is transcribed to give three mRNA encoding, two nonstructural (W and V) and one structural (P) protein (Yusoff and Tan 2001). Virus

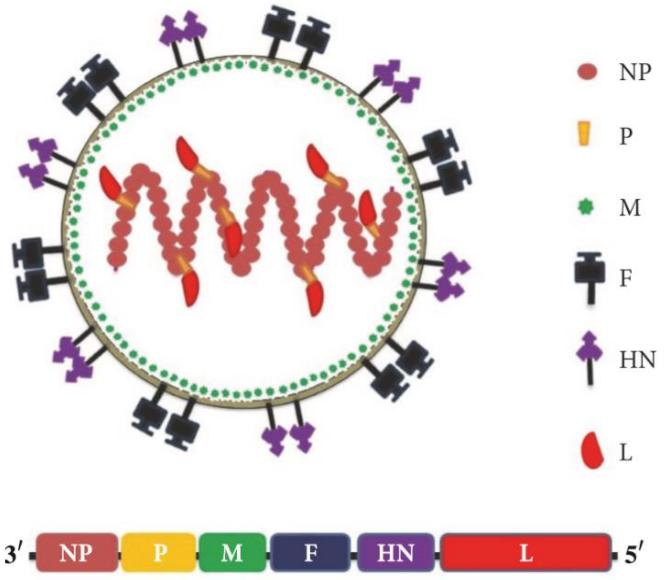

Fig. 1: Structural feature of New Castle Disease Virus. Figure displaying morphology of virus showing all the associated proteins. NP, P and L linked with RNA Genome. M. F and HN attached with envelop (Ganar et al. 2014).

(Yusoff and Tan 2001). Virus transcription and replication are regulated by the 3' and 5' ends of the viral genome, which accommodate the regulatory signal (Conzelmann 2004). NDV appears to be polymorphic with spike projection if glycoproteins from the surface of enveloped particle; that initiate the virus infection cycle. M protein is just beneath the viral envelope, maintains shape of virus and assists in packaging and release of virus (Fig. 1). Therefore, viral genome, associated with these three proteins, is responsible for replication related function. L protein serves as transcriptase and replicase during infectious cycle, with the P protein acting as a cofactor (Steward et al. 1993).

The severity of illness in birds ranges from subclinical symptoms to 100% mortality. On the outside of the envelope of virus, both of these proteins form the protrusions and act as the neutralizing, as well as protective, proteins of NDV. The F protein is produced in the form of inactive form or precursor called F_0. Proteases from host cells cleave this inactive form into two subunits called F_1 and F_2; both are biologically active subunits. This cleavage of inactive form is precondition and essential requirement for the viral entry and fusion with cells. The sequence of the chief element of pathogenicity, that is F protein cleavage site, is a well characterized. To initiate the process of fusion, homotypic interaction between the F and HN proteins is required (Kim et al. 2013).

Categories

Due to great variation in the severity of disease, NDV was not identified as only one clinico-pathologic identity; it is categorized into 4 pathotypes. These pathotypes are; Doyle, Beach, Beaudette, and Hitchner. But recently, the NDV has been categorized on the basis of pathogenicity of the disease from most to least pathogenic (Kammon et al. 2015). These categories are: (i) viscerotropic velogenic (ii) velogenic (iii) mesogenic and (iv) lentogenic.

Among these strains, velogenic strains are considered extremely lethal, with a 100% mortality rate. These strains cause lesions that have adverse effects on digestive system, respiratory system and even the nervous system. The velogenic strain has further 2 sub-categories, depending on the organ affected. These sub-categories are: (a) viscerotropic and (b) neurotropic. Viscerotropic was formerly called as Doyle and specifically affects the visceral organs of birds. The neurotropic was formerly called as Beach and symptoms caused by this strain are associated with nervous system. After the velogenic strains, the mesogenic strains possess intermediate virulence that causes proven disease in chickens, characterized by intermediate effects on nervous and respiratory systems. The mortality rate of mesogenic strains is low. The lentogenic strains include the members like Ulster strains, LaSota and V4; these strains generally are not associated with remarkable clinical illness in mature and grown-up chickens or birds. These strains are used for the production of live vaccines (Abdolmaleki et al. 2018).

All strains of NDV belong to a single serotype but all these strains are identified or recognized even by the slight antigenic and genetic variation. The study of successive years on the recognition of various increasing changes in the NDV strains proved that the virus is constantly evolving. Depending upon the sequence of F gene and genome size, all strains of NDV are classified into two classes, class I and class II. Class I has 9 genotypes, while class II has eleven genotypes. Strains in class I are generally avirulent. But in one recent study it is reported that there is only a single genotype and 15 groups on the genetic basis in class I and class II, respectively. Class II has both types of strains that is virulent and avirulent and includes the previously identified 10 genetic groups and five new genetic groups. Avirulent strains, like LaSota and B1 of class II, are consider as avirulent vaccine strains. It is reported that V, VI and VII genetic groups of class II are associated with the outbreaks of the disease in the whole world (Khan et al. 2010; Kammon et al. 2015).

Clinical manifestations of Newcastle disease

The symptoms of ND vary, depending on the strains of causative agent. Some strains are asymptomatic, while other cause disease with 100% mortality rate. Clinical manifestation of ND has involvement of gastrointestinal tract, respiratory tract, as well as nervous system, causing ruffled feathers, conjunctivitis, diarrhea, paralysis, dyspnea, prostration, nasal discharge and tremors. Ulcers and haemorrhages in the various regions of digestive tract, as well as patches showing necrosis, have been observed in GALT, liver and spleen through internal examination (Siddiqui et al. 2014). All four types of NDV strains show different but closely related clinical signs (Abdisa and Tagesu 2017).

In viscerotropic velogenic ND, remarkable lesions, depression, profused greenish yellow diarrhea, swollen heads, decreased egg production, and cyanotic combs can be seen in infected animals. Mortality rate is 90%. Neuroptopic velogenic strain shows acute symptoms of nervous system and respiratory tract. All signs appear suddenly and simultaneously. Mortality rate is around 10-20% in adult fowls, while higher in young birds. In case of mesogenic pathotype, clinical symptoms from respiratory tract with coughing dominate, while depression, decreased egg production and weight loss can also be observed. Mortality rate is approximately 10%. Lentogenic pathotype shows mild signs of respiratory tract and a very small drop in egg production. Mortality is very low, and may be negligible.

Onset of ND is rapid, and its signs appear as the aerosol exposure take places within 2 to 12 days, throughout the flock. When the virus is transmitted through fecal-oral route in caged birds, the spread rate is slower. Eggs from infected birds are usually abnormal in shape, color, surface and having watery albumen. Ataxia, body or head tremors and torticollis develop in poorly vaccinated fowls within 10 to 14 days. A well vaccinated bird may not show any symptoms of infection, except decreased egg production and shedding of virus in feces and saliva. Severity of the disease depends on the tropism and virulence of virus, susceptibility of the host species, age of bird, immune status, environmental conditions, and presence of other diseases.

Besides avian species, humans can also be affected by the NDV (MacLachlan and Dubovi 2017). On the exposure of virus, conjunctivitis is caused in humans. The conjunctivitis recovers quickly after 4-7 days of infection, but the causative agent tends to be shed in the ocular fluid. In case of mild infection, the disease appears as influenza with symptoms of headache and fever. Laboratory persons and vaccinators are at the great risk of disease. Risk can be reduced in the laboratory persons by using personal protective equipment (PPEs) and safety cabinet. The disease rarely occurs in farm workers and consumers of poultry products. It has been reported in previous studies that NDV can easily transmit from birds to human and vice versa but not from human to human (Abdisa and Tagesu 2017).

Diagnostic tests of NDV

Proper diagnosis of a causative agent is crucial for successful cure of any disease. Clinical signs and symptoms always give clue to the medical personnel to diagnose that disease, which then can further be confirmed through other diagnostic tools to better combat the disease. It is critical to quickly identify the NDV strain and distinguish it from other closely related pathogenic strains, so that the virus can be controlled. The diagnostic tests of NDV are based on clinical symptoms, serological testing, conventional and molecular based assays, microarray techniques and next generation sequencing (Table 1).

Conventional assays

NDV is initially diagnosed on the basis of clinical features that distinguish the NDV from other viruses and one strain of NDV from another strain. All pathotypes show different but closely related symptoms i.e, depression, diarrhea, tremors, paralysis, decreased egg production and have different severity and mortality rates (Marks et al. 2014). Then the virus samples are isolated from the sites of replication, viral shedding, and routes of transmission. The samples may be oropharyngeal and cloacal swabs in live birds. In case of recently died animals, the samples may be taken from liver, kidneys, spleen, caecal tonsils, lungs or intestine (OIE 2017). After that, hemagglutinin (HA) and hemagglutinin (HI) tests are performed to confirm the presence of virus in infected cavity fluid. It is advisable to perform virus isolation both in allantoic cavity and cell cultures (Bello et al. 2018). Enzyme linked immunosorbent assay technique (ELISA) is another test used to detect antibodies against all pathotypes of NDV. It is possible to develop ELISA that can distinguish between infection antibodies and vaccine antibodies (Ge et al. 2016). It can also eliminate the cross reactivity of NDV with other paramyxoviruses, using C terminal extension NP recombinant protein as a detecting antigen (Zhao et

al. 2018). Virus neutralization test is another serological test to measure NDV specific neutralizing antibody.

Molecular based assays

The conventional techniques are found to be relatively slow and expensive. In order to overcome these shortcomings, molecular based accurate NDV detection methods have been developed. Among these molecular techniques, RT PCR is commonly used assay in developing countries. A wide range of PCR protocols (from conventional RT-PCR to real time RT-PCR) have been used. These are sensitive and rapid diagnostic techniques. Singleplex and multiplex real time RT PCR have also been used for rapid detection of viruses. Triplex real time PCR has been found to have 10 times more detection limits for NDV, avian influenza and duck tembusu virus (Zhang et al. 2020). qPCR is also a molecular assay which gives greater detection sensitivity of specific viral disease. Another non-PCR amplification technique is used for rapid diagnosis of pathogens. It works on the principle of strand displacement reaction in which a stem looped structure is formed allowing the rapid and sensitive amplification of target gene.

Microarray hybridization technique is used to diagnose avian influenza and NDV simultaneously in a mixed infection sample (Lung et al. 2012). In recent years, multiplex Luminex suspension microarray system has been used for rapid detection of NDV (Sultankulova et al. 2017). Most recently, different researches on the use of microarray have been carried out which showed it as the most reliable and promising technique for diagnosis of NDV (Xiao et al. 2019). Biosensors are another emerging diagnostic tool of 21st century, which gives incredibly inexpensive, effective, sensitive and rapid results of pathotype. These are made up of transducers (physicochemical detectors) and biorecognition molecules, which can convert biomolecular interactions in the measurable signals. In recent years, a label free immunosensing scheme, having tilted fiber grating equipped with gold nanospheres, has been developed. This system is more sensitive than qPCR (Luo et al. 2018; Mauriz 2020).

Table 1: Diagnostic tools for the detection of NDV

Diagnostic techniques	Basis	Advantages	Limitations	References
Conventional Assays				
Differential diagnosis	- Clinical symptoms	-Easy to perform	- Similar symptoms with other paramyxoviruses - Cause confusion in treatment	(Bello et al. 2018)
Virus isolation	- Used swabs for sampling - HA and HI tests for confirmation - Cynctia formation	- Definitive method	- Not reliable	(Alexander 2000)
Serological Assays	-Antigen antibody serum detection - ELISA - VNTs	- Important for humoral immune responses	-Expensive - Cannot identify distinguished strains	(Choi et al. 2013)
Molecular based assays				
RT-PCR	-Target F gene	- Sensitive and rapid	- Cause mutation in primer binding region - Dire need to improve primers according to evolution	(Wang et al. 2001)
Real Time RT-PCR	-TaqMan probes are used to target F gene - Singleplex and multiplex RT PCRs	-More sensitive and rapid - Non cross reactive	-Need proper expertise	(Zhang et al. 2020)
qPCR	- Target Fusion (F) and matrix gene - Improved method known as matrix polymerase multiplex-qPCR	- Less tiring - Reliable - Faster - Greater sensitivity	- Detect only class II NDV	(Miller et al. 2010)
No- PCR amplification	- LAMP test - Detect 6 independent regions - SYBR green is used for visualization	- Inexpensive - Sensitive and rapid	- Different primers for different independent sites	(Pham et al. 2005)
Microarray hybridization technique	-Various DNA probes are used	- Efficient for genotyping and phenotyping - Reliable	- Need expertise	(Lung et al. 2012)
Biosensors	- Used detectors and biorecognition molecules	- Rapid and effective	-Sensitive to pH	(Shi et al. 2015)
NGS	- Differentiate virulent from avirulent strains	- Promising tool Accurate and reliable	-Expensive	(Deurenberg et al. 2017; Chen et al. 2020)
Random priming technology	-Random amplification of gRNA and sequence analysis	- Detect virus at very low concentration	- Expensive	(Byarugaba et al. 2014)

Next generation sequencing is the most recent technology used for rapid and most reliable diagnosis of NDV. It can detect mixed infection within the same host and low frequency variants. Recently, genotype VI of NDV has shown continuous evolution in NDV genome (He et al. 2018). Various researches have been done on studying NGS technology for the diagnosis of NDV genes (P-gene), which showed that NGS is an excellent, revolutionizing technology used for this purpose (Chen et al. 2020).

Vaccines against NDV

Development of vaccines against NDV is a major concern. Vaccines that are presently in use are a few decades old. In USA, live vaccines were started in 1945. Live vaccines and inactive vaccines were available from 1950 to 1990. During that time, researchers investigated the development of symptoms after the vaccination using live vaccines. The problems related to live vaccines became clear in 2016 (Dimitrov et al. 2017). The major problems related to live and inactivated vaccines are: (i) high dose of live vaccines elevate the cost, (ii) live vaccines are able to reduce the symptoms but unable to stop the replication of NDV and, (iii) the inactivated vaccines are not very effective (Dimitrov et al. 2017). To overcome these problems, the recent trend is set to develop the recombinant vaccines. Recently, plant based Edible vaccine, a type of recombinant vaccine, which can be fed to animals orally to induce immune response, has also gained attention of scientists. In different studies, transgenic potato plant, tobacco root hair, maize and yeast have been reported for the expression of HN and F proteins of NDV.
The two major categories of vaccines are: i) conventional vaccine which is an old vaccine technology and, ii) recombinant vaccines used as recent technology to prevent NDV in poultry animals (Table 2).

Status and consideration of vaccine development

A number of studies on NDV control strategies in poultry industry have been conducted. Different vaccines have been developed for preventing the occurrence of disease and saving the poultry birds for food security. Previously, live inactivated vaccines (Inactivated Chick-ND Hester) and attenuated vaccines (LaSota) were available in the market and licensed in many countries to control the spread of disease. These vaccination techniques do not offer sterilizing immunity and fail to make the flocks highly resistant to natural viruses. However, the main strategy is to vaccinate the fowls with live lentogenic strains at two to four weeks or day-old birds, which should be revaccinated at 2-4 weeks and again at tenth week with an inactivated mesogenic or lentogenic strain. Vaccine delivery through aerosol or water drinking method has found to be convenient and effective but still due to varying uptake rate and stocking densities, the success rates have been reduced to 53 and 60% through these methods, respectively. Live vaccines may mask the clinical signs when the NDV infection occurs in vaccinated animals, but these are inexpensive and can be directed on large scale to produce mucosal immunity. Vaccination of the flocks depend on the national and international policies under the regulations of OIE. In many European countries, emergency and prophylactic vaccines are in use. Vaccination strategies are routinely used in developing countries where NDV is endemic. Booster vaccines of the live mesogenic pathotype are used to improve immunogenic responses, but their use is still illegal in many countries (Mayers et al. 2017). Currently, with the emergence of molecular biology and recombinant DNA technology, new vaccine strategies named as recombinant vaccines have been developed to prevent the disease and protect the birds from virus.
While designing a vaccine for poultry flocks, various factors should be considered to avoid adverse reactions and to protect birds from infection; these factors include maternal antibodies level, age, breed of the fowl and other infections which can weak the immune system of animals. Vaccine should be cost-effective, easy to store and appropriate for mass administration. There is a dire need to improve control strategies through enhanced biosecurity practices and good vaccination protocols to cut down the burden of NDV on poultry industry in developing countries.

Old vaccination strategies

Old vaccination strategies include conventional vaccines, which are either live attenuated vaccines or inactivated vaccines.

Live Attenuated Vaccines

NDV has been decreased in many countries due to the isolation of naturally occurring immunogenic strains of NDV. A number of strains, like B1, F, LaSota, V4 and I2, among the lentogenic category of NDV have been used as live vaccine for disease control (Commission and Committee 2008). LaSota is the most widely used vaccine strain due to its high immunogenicity. B1 strain is less immunogenic than the LaSota strain but it is also widely used for vaccine purpose due to its quality of being highly attenuated and lack of respiratory reactions in birds after vaccination. The advantage of V4 and I2 is their thermostability, which makes them suitable for villages with limited refrigeration capacity (Bensink and Spradbrow 1999). Other NDV strains, like Komoroy and Mukteswar, were used as booster vaccines following the administration of the lentogenic strain. All these live attenuated vaccines are highly effective because they stimulate both systematic and mucosal response like that of natural response, because of their ability to replicate in chicken. Furthermore, a single dose of live NDV vaccine stimulates enough immune response to protect against clinical disease but does not stop the shedding of virulent virus by oropharyngeal and cloacal routes. Studies have shown that this shedding can be reduced when increased doses of live vaccines are administered. Due to the high cost of vaccine per bird, this method is not proved to be economically acceptable, therefore, improved and cost-

effective approach is requited to overcome the problem of virus shedding. Tissue tropism is the most important factor in determining the effectiveness of a vaccine. Among the conventional vaccines, the most famous strain is the LaSota strain, which provides strongest immunity along the path where initial exposure to virus may occur. Other than this, VGGA strain was proved to be enterotropic, stimulating mucosal immunity. The greatest advantage of live NDV vaccines is their ease of mass application in water or even in spray, which makes them highly cost effective and manageable. In addition, the concept of herd immunity is also studied under the administration of live vaccines (Dimitrov et al. 2017).
Nevertheless, despite their variety and advantages, these vaccines have certain shortcomings. The first and foremost disadvantage is that live vaccines have the potential to revert to virulent form, inducing the disease again. Secondly, different strains may cause different post vaccination respiratory infections. Another major problem is that vaccines are mostly based on genotype I and II, which are different from present day genotypes in many countries. Although a vaccine protects against clinical disease, but these factors could be very dangerous. Therefore, the live attenuated vaccines must be used with extreme caution and care, and a well improved vaccine is needed to overcome the weaknesses of conventional live attenuated vaccines.

Inactivated vaccines

Chicken immunization with inactivated vaccine is the earliest strategy of controlling NDV. The vaccine is produced by growing desired strain of NDV, followed by inactivation using chemical and physical methods. The method of inactivation should be able to separate the immunogenic epitopes of viral surface glycoprotein (HN and F), which are the most crucial determinants of neutralizing antibody. Widely used chemicals for inactivation are binary ethylenimine (BIE) and formaldehyde. The vaccine is administered subcutaneously or intramuscular, after being prepared in emulsion of mineral oil. These vaccines cannot be administered in masses but have to be given individually via parenteral route because these viruses do replicate and spread horizontally. As a result, the entire procedure is costly and time-consuming (Kapczynsk et al. 2013). Moreover, a great deal of care is needed during inactivation process of the virus, as too much inactivation may destroy immunogenic epitopes, while less exposure to chemicals may not completely inactivate the virus. To improve the efficacy of vaccines, they are primed with live vaccines before administration and also require use of adjuvants to trigger immune response. Besides, these adjuvants may cause some unwanted reaction in birds. In addition, inactivated vaccines are known to be bad inducers of mucosal immunity. Thus, to ensure better protection and care, a rationally designed vaccine with improved characteristics is required in the poultry industry (Zhai et al. 2011).

New vaccine technologies

Recombinant vaccines

Currently, recombinant vaccines are used as an alternative to conventional vaccines to overcome their limitations. Some of the recombinant vaccines are as follows:

DNA vaccines

The advancements in recombinant DNA technology, paved the way of developing recombinant DNA vaccines through cloning process. In this process, the gene encoding of different neutralizing epitopes or an immunogen is cloned into an expression vector. Then the cloned vector is injected into the fowl host, where the transgene is transcribed and translated into protein, acting as epitope which induces immune response (Bello et al. 2018). F and HN gene cloned vectors are used to vaccinate chickens, which then are boosted through inactivated vaccines, as a result, a very strong response is observed. It has been noticed that recombinant DNA vaccines can be used to boost the immune response induced through inactivated vaccines. These can be improved further by using nanoparticles as a vehicle to deliver a vaccine (Firouzamandi et al. 2016).
In recent years, F gene-based DNA prime protein vaccine strategy has been experienced and its results showed that DNA prime protein boost technique can easily boost up the immune response in poultry birds. This approach gave 91.6% protection from NDV and showed higher immunity level than DNA vaccines (Khulape et al. 2019). These are safer alternatives against ND vaccination challenges and more capable of inducing both humoral and cell mediated (CD4+ and CD8+) immune responses. Still, they have some limitations, including high cost, poor immunogenicity, and difficult mass delivery system. These limitations can be overcome by using suitable adjuvants (Zhao et al. 2017).

Nanoparticle vaccines

Recent advances in nanotechnology have made vaccine delivery much easier. Nanoparticles are used as delivery vehicles, which protect the delivered antigen from external disruption. This process is beneficial in controlled release of immunogen; it also enhances the duration of immune response. Various types of nanomaterials are used as carriers such as chitosan, polylactic acid, magnesium sulphate, carboxymethyl-cellulose and calcium phosphate. It has been seen in a study that two chitosan used as delivery vehicles can enhance humoral, mucosal and cell mediated responses (Renu and Renukaradhya 2020).

Viral Vector Vaccines

Recombinant viral vector vaccine strategy is the most promising approach to control infectious diseases. In poultry, most used vectors are vaccinia virus, herpes virus

and fowl pox virus because of their large dsDNA genome, which enhance their capacity of transgene expression. These are most effective vaccines but still have challenges to their performance due to the presence of maternally derived antibodies in neonatal chicks (Baron et al. 2018). In fowl pox virus vaccine, thymidine kinase is replaced with HN or F or both genes, and showed high level of immunity against NDV. Although there are no post-vaccinal reactions in fowl pox vectors, anti-fowl pox antibodies may interfere with its reactivity, due to which it is not suitable for young birds. This limitation can be overcome by using herpes virus vector, which shows long lasting and strong humoral, and cell mediated immune responses. Herpes vector is an ideal vaccine carrier, which has ability to induce 95-100% immune response in chicken against NDV. Avian paramyxovirus-3 can also be used as an efficient avirulent vector to induce immune response (Bello et al. 2018). They show high level of immune responses and are highly immunogenic. They can activate TLR and induce strong innate inflammatory immune response. But they still have some challenges, including their sensitivity to preexisted immunity against vectors.

Virus like particles as vaccines

Virus like particles are composed of outer core only without any genome, have natural configuration of infectious virion and do not have ability of self-replication. Absence of genome makes them effective candidates of vaccines, inducing cell mediated and humoral responses, without the use of any adjuvant. The particulate nature, size and their repetitive structure are important for innate immunogenicity. These are more protective and safer mode of vaccination than conventional vaccines (Liu et al. 2012). NDVLPs have many unique features, including the protein ratio which is similar to wild type NDV and these are released with the efficiency of 84% (Bello et al. 2018). These are safe to vaccinate and produce robust immune responses. The only limitation is that these cannot be produced in a large amount for massive vaccination.

Reverse genetic based vaccines

Reverse genetic based vaccine is the latest vaccine used for NDV vaccination; it is the recovery of recombinant virus from cloned cDNA. In reverse genetic based vaccines, the cleavage site of virus is modified from polybasic to monobasic (Kim and Samal 2018). It has been observed in different studies that this approach is superb in making the virus avirulent and inducing significant protective immunity, and reduces the viral shedding when the immunization is achieved. Recently, reverse genetic based vaccine was isolated from naturally recombinant Malaysian strain (NDV IBS025/13) against NDV genotype VII. In this experiment, pOLTV5 vector was used to recover virus after modifying their F cleavage site. The results showed that virus was highly attenuated and induced higher level of HI titre (Bello et al. 2020). This approach is pertinent in rapid production of stable attenuated NDV genotype matched vaccines against infectious NDV. These demonstrate the ability to distinguish between virulent and avirulent strains. The disadvantage of this method is the high cost of sequencing and other molecular processes.

Oral Edible Vaccines

Although already available live attenuated, inactivated, and recombinant vaccines have played an important role in combating NDV but still they show some limitations and challenges, including their high cost, low reactivity, difficulty in massive vaccine delivery and multiple dosage system. To overcome these challenges, plant-based oral edible vaccines have been developed, which provide safer, affordable, and attractive platform for vaccine delivery. In this approach, *Agrobacterium* mediated gene transfer system or genetically modified plant virus transformation methods are used, through which the desired gene encoding epitopes of specific disease are integrated into plant genome (Laere et al. 2016). Tobacco, potato and maize are most commonly used transgenic plants for the preparation of oral edible vaccines.

In the construction of transgenic potatoes, NDV genes under the control of 35S CaMV promoter are incorporated into a binary vector (pGJ357) and transferred to potato through *agro*-mediated transformation (Berinstein et al. 2005). In another study, gene encoding HN and F proteins of NDV, controlled by promoter and terminator as CaMV35S and NOS respectively, were integrated into a plant-based expression vector (pBI121) and transferred to tobacco plant through *Agrobacterium tumefaciens* mediated transformation. Results showed high immune response in chicken (Ghaffar et al. 2016). Recently, corn has been observed as a successful candidate of inducing immune response in chicken against NDV. In this research, transgenic corn seeds having F and HN genes under the regulation of 35S and Zein promoters were observed. Results showed approximately 7 and 28 folds higher expressions of F and HN genes, respectively. Their analysis showed that chicken fed with these transgenic leaves produced specific antibodies against NDV antigen proteins (Shahid et al. 2020).

Challenges to plant based oral edible vaccines

Edible vaccines have been used for decades, as they are cost effective, safer, and efficient, but they still have some limitations. Their commercialization and implementation are biggest challenges faced by scientific personnel. Plant based vaccines can also produce hypersensitive responses. Plants need regular monitoring, so that they may not be mixed with non-transgenic plants during pollination or cross-contamination. Quality and safety of GM plants are difficult task to be achieved. These can also contaminate the human food through accidental mixing of plants (Kurup and Thomas 2020).

Table 2: Vaccines to control NDV

Vaccine strategy	Process	Pros	Cons	References
Conventional vaccine				
Live attenuated vaccine	-V4 and I2 thermostable vaccine; it can bear high temperature	-Mass application in water -Herd immunity -Systematic and mucosal response	-Ability to revert back to virulent form -Post vaccine infection	(Mielcarek et al. 2006)
Inactivated vaccines	-Physical and chemical damage of immunogenic viral glycoprotein	-Can administered subcutaneously and intramuscularly	-No mass administration -Expensive and hectic	(Tlaxca et al. 2015)
Recombinant vaccine				
DNA vaccines	-F and HN gene cloning into vector	- Produce both humoral and cell mediated immune responses	- Expensive - Poor immunogenicity	(Dimitrov et al. 2017)
Nanoparticle vaccines	- Nanoparticles used as delivery vehicles of genes	-Controlled release of immunogen Enhanced immune responses	-Expensive	(Bello et al. 2018)
Viral vector vaccines	- F and HN genes in Vaccinia, herpes virus or fowl pox viruses	- Produce strong innate inflammatory immune response	-Sensitivity to preexisted immunity against vector	(Dunn et al. 2019)
Virus like particles	-Genomeless VLPs are used	- Safer - Produce robust immune responses	- Difficult massive vaccine production	(Bello et al. 2018)
Reverse genetic based vaccines	-Recovery from cDNA - Modified cleavage site (polybasic to monobasic)	-Produce stable vaccines - Differentiate virulent from avirulent strain	-High cost	(Bello et al. 2020)
Oral edible Vaccines	-*Agrobacterium* mediated transformation of gene F and HN into plants	- Higher expression - Safer - Efficient and effective	- Hypersensitive responses - Need regular monitoring	(Shahid et al. 2020)

Immune response against infection and vaccination

The key goal of any vaccine against NDV is to provide sterilizing immunity, protecting all bird species from a specific disease. The target areas of NDV are the epithelial cells of respiratory system and may spread to other essential tissues based on the pathogenicity of virus, increasing the level of mortality. The incubation period of vaccine for the onset of clinical disease is from 3 to 6 days, followed by the induction of active immunity (humoral, mucosal and cell mediated). Rather, innate immunity (interferon system) provides the first line defense against virus by inhibiting its replication to limit the spread of virus within the host. Various *in vitro* studies have shown that NDV induces the expression of innate immunity genes, like IFN-a, IFN-b, IFN-c and pro-inflammatory cytokines 6 and 1. This was also verified by *in vivo* studies with an addition of regulation of chemokine genes. NDV antagonizes IFN response and host tropism qualities, as demonstrated by *in vitro* studies. There is a lot of progress and development of avian immunology but precise role of cellular immunity against NDV has not been studied yet. Antibodies (humoral response) have been detected approximately six days after the infection or vaccination with live virus. This method of producing antibodies by the use of vaccination is considered as an essential method for the protection against NDV by preventing viral spread. According to previous studies, use of inactivated vaccines or mesogenic strains via ocular and nasal routes results in the highest antibody titres 6-10 days after vaccination. However, preliminary measures should be considered in day-old chicks rather than vaccination because it proves to be ineffective due to MDA. The efficacy of live vaccination in young birds was also affected by high level of MDA, which caused delay in immune response, resultantly about 55% of birds remained unprotected. Application of live vaccine added the advantage of inducing humoral and cellular immunity, along with mucosal immunity, which plays an important role in the protection against ND. The production of antibodies can be detected by hemagglutination test, ELISA and various other methods but does not necessarily relate to the level of virus transmission. Therefore, further research is needed for improved understanding of immune response against NDV in avian species.

Future Prospects

Much research work has so far been done on NDV vaccines and the trends are likely to be followed in future. The government should prioritize the protection of livestock and agriculture sectors in the country by following rules and regulations of biosecurity. Vaccine technology should be upgraded and more new vaccination techniques must be introduced in near future. New strategies, expansion of laboratories, regular training and proper expertise would help in meeting the basic vaccine demands. Private sectors must also invest for research and development of vaccines at large scale for mass administration.

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NEWCASTLE DISEASE IN MAMMALIAN SPECIES: THE PROPENSITY OF CROSS-SPECIES TRANSMISSION TOWARDS PUBLIC HEALTH PERSPECTIVE

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INTRODUCTION

With the significant advancement in the diagnostics and medical countermeasures in the last decade, the jeopardy of cross-species transmission of known and unknown pathogens has emerged as a health threat to susceptible hosts, including human and animal populations (Parrish et al. 2008; Pavia 2011). Such episodes of inter- and intra-species transmission of viruses are greatly influenced by various factors, including climate change, rapid transportation, urbanization, and intensive farming (Turner et al. 2004; Parrish et al. 2008; Pedersen and Davies 2009). The incidence of human infections has increased due to spillover from natural reservoirs and improvements in diagnostic methods (Letko 2020). Different outbreaks of the paramyxoviruses provide conclusive evidence of spillover from naïve hosts to other mammalian species, including humans (Table 1). Novel paramyxoviruses continue to emerge from naïve/reservoir hosts and represent an on-going threat to public health worldwide (Thibault et al. 2017). During the last three decades, as a result of spillover, a considerable number of diverse paramyxoviruses have been identified from the wildlife species and terrestrial mammalian species, including humans (Virtue et al. 2009; Coffee et al. 2010; Kurth et al. 2012; Sieg et al. 2020; Table 1). Thus, paramyxoviruses have traditionally been associated with global epidemics with human and animal health burdens (Chua et al. 2000; Wang et al. 2008; Spires et al. 2017).

The paramyxoviruses are a group of negative-sense, single-stranded, RNA viruses that are classified under the family *Paramyxoviridae*. The paramyxoviruses mainly concerned with public health include Hendra virus (HeV), Nipah virus (NiV) and Menangle paramyxovirus (MenPV) (Bowden et al. 2001; Aljofan 2013). Globally, these viruses are recognized as zoonotic paramyxoviruses, and are associated with neurological and respiratory infections in humans and animals. The HeV has also been reported from equine and canine species (Playford et al. 2010; Kirkland et al. 2015). Similarly, outbreaks of NiV were observed among human populations in Malaysia, India, Singapore, and Bangladesh (Chew et al. 2000; Clayton et al. 2012; Arunkumar et al. 2019). The MenPV virus is also known as a zoonotic paramyxovirus, as it caused severe influenza-like infection during numerous epidemics (Bowden et al. 2001). Moreover, Mojiang paramyxovirus (MojPV) has been associated with human casualties in China during 2012 due to spillover from naïve hosts (Wu et al. 2014). Consequently, paramyxoviruses exhibit the highest cross-species transmission rates among RNA viruses with variable clinical presentation in humans.

One of the most significant paramyxoviruses, known as the Newcastle disease virus (NDV), caused Newcastle disease (ND) in a wide range of avian species, significantly affecting poultry production (Alexander et al. 2012; Aziz-ul-Rahman et al. 2018a,b; 2019a,b; Absalón et al. 2019; Du et al. 2020). Newcastle disease virus, formally known as avian paramyxovirus 1 (APMV-1) and avian avulavirus 1, is recently renamed as *Avian orthoavulavirus* 1 by the International Committee on Taxonomy of Viruses (ICTV) (Kuhn et al. 2020). In 1926, the first outbreaks of ND occurred in chickens in Indonesia and England. Newcastle disease virus is a major poultry pathogen and mainly causes respiratory, neurological, and gastrointestinal symptoms (Alexander et al. 2012; Absalón et al. 2019; Du et al. 2020). In terms of pathogenicity, owing to their virulence in chickens or the presence of conserved amino acids in the F protein, NDVs can be categorized into velogenic (virulent), mesogenic (intermediate), and lentogenic (non-virulent) (Alexander et al. 2012; Absalón et al. 2019; Du et al. 2020).

Based on viral fusion protein, NDV can be categorized into nonlytic and lytic strains, with antitumor potential. Lytic NDV strains can produce infectious progeny in host cells, whereas nonlytic NDV strains have a tendency to elicit immune response that results in antitumor effects (Zhao and Liu 2012). Owing to the potential of antitumor activities, NDV is now ascertained as an effective oncolytic agent in various *in vitro* and *in vivo* investigations against a considerable number of carcinomas, such as renal carcinoma, breast, and pancreatic adenocarcinoma, hepatocellular carcinoma, colorectal carcinoma, pleural mesothelioma and glioblastoma (Tayeb et al. 2015). Similar to other paramyxoviruses, NDV also tends to infect non-avian hosts, including humans (Goebel et al. 2007; Kuiken et al. 2018; Shabbir et al. 2021), monkeys (Kuiken et al. 2017), rabbits (Charan et al. 1984), minks (Zhao et al. 2017), hamsters (Samuel et al. 2011), mice (Khattar et al. 2011), cattle (Ozawa and Chow 1958; Subbiah et al. 2008) and pigs (Chen et al. 2013). A shred of evidence about natural and experimental infection in different mammalian species highlighted the zoonotic spillover and the potential of NDV (Kuiken et al. 2018; Ul-Rahman and Shabbir, 2019; Shabbir et al. 2021). Newcastle disease virus

is a major poultry pathogen but can cause respiratory infections in humans and pigs, neurological infection in the monkey and mink, and gastrointestinal infection in pigs, along with generalized symptoms (Charan et al. 1984; Samuel et al. 2011; Chen et al. 2013; Kuiken et al. 2017; Zhao et al. 2017; Kuiken et al. 2018; Shabbir et al. 2021).

The recent molecular identification and serological detection of NDV in non-avian hosts, including humans, raised concerns about its zoonotic potential and underscored the near-global threat. Recently, NDV infections have been associated with casualties in human beings, which highlighted the potential zoonotic risk and sparking interest in public health worldwide (Kuiken et al. 2018; Shabbir et al. 2021). Despite shreds of evidence, information on the emergence and zoonotic potential of NDV focusing on the propensity of cross-species transmission is scattered. An insight into the zoonotic potential and cross-species transmission of NDV undoubtedly plays a vital role in linking within and between all susceptible hosts and larger evolutionary dynamics of the virus. As a matter of fact, no one looks for NDV infection, specifically in mammalian species, assuming that it would not be present, even though NDV can naturally and/or experimentally infect a large number of mammalian species. Therefore, this chapter aims to compile together all scattered information about the evidence of NDV infections and genetically diverse strains prevailing in non-avian hosts, including humans, and draw the global scientific community's attention towards its public health concerns. A brief description of Newcastle disease in different mammalian species is presented below.

Family *Huminidae*

The Newcastle Disease virus was first recognized as a zoonotic pathogen many decades ago, and the virus has recently been placed in hazard group 2 by the advisory committee on dangerous pathogens in the United Kingdom. According to this committee, the NDV is a biological agent that can cause infection in humans and may be a hazard to personnel associated with veterinary practices or laboratory employees; however, it is unlikely to spread to the community. The first report on human infection with NDV was documented in 1942 in Australia (Burnet 1943). From 1942 until now, 485 human cases were collectively reported in approximately 20 investigations from Israel, USA, UK, Netherland, Canada, Australia, and Pakistan (Figure 1). Communally, the highest number of cases of NDV infection in humans were observed in UK (n = 288), followed by Pakistan (n = 82), Israel (n = 34), USA (n = 17), Australia (n = 3), Netherland (n = 2) and Canada (n = 1). The first case of human infection with NDV was described as a case of conjunctivitis in a laboratory worker, who had accidentally squirted NDV-infective or positive allantoic fluid into the eye (Burnet 1943).

Most of the reported instances of NDV infection in humans have been the result of accidental inoculation of high-titer NDV-contaminated egg fluid and/or touching of NDV-contaminated fingers into the eyes, the imprudent handling of infectious tissue samples by laboratory employees or workers in poultry processing plants, veterinary laboratory diagnosticians and practitioners, who performed post-mortem examinations on infected birds or handled vaccines (Burnet 1943; Anderson 1946; Yatom 1946; Shimkin 1946; Ingalls and Mahoney 1949; Freymann and Bang 1949; Mitchell and Walker 1951; Keeney and Hunter 1951; Hunter et al. 1951; Gustafson and Moses 1951; Nelson et al. 1952; Quinn et al. 1952; Lippmann 1952; Reagan et al. 1956; Pilsworth and Wall 1964; Alby, 1965; Trott and Pilsworth 1965; Shabbir et al. 2021). Overall, the transmission of ND infection from birds to humans is rare. However, it is believed to have resulted from close contact with infected birds or materials (Goebel et al. 2007; Kuiken et al. 2018). For instance, conjunctivitis cases in poultry workers were observed during an ND outbreak in backyard poultry (Nolen 2003). These workers were involved in the identification, euthanasia, and disposal of NDV-morbid birds. No evidence exists to support human-to-human transmission, but the potential for birds-to-human transmission exists (Rasmussen 1964). A history of aerosol exposure was associated with most individuals, who developed a generalized infection (Hanson and Brandly 1958). Because NDV infections of humans are usually mild and self-limiting, the concern is much greater about the possibilitythat infected individuals may transmit the virus through their contact with susceptible birds and poultry, and thus extend the outbreak concern that such infections will become a human health problem. Seemingly, six outbreaks of NDV infection in humans have been observed, exhibiting conjunctivitis and influenza-like symptoms with 17-288 cases (Yatom 1946; Shimkin 1946; Nelson et al. 1952; Pilsworth and Wall 1964; Trott and Pilsworth 1965; Shabbir et al. 2021). Most of the reported human infections with NDV have been self-limiting, non-life-threatening, without permanent health consequences, and usually not debilitating for more than 4-5 days. Notably, three casualties of immuno-compromised patients, exhibiting pneumonia have been observed due to respiratory failure (Goebel et al. 2007; Kuiken et al. 2018). Of these, two deaths were observed during 2003 in Netherland, while one death was observed during 2007 in the USA (Goebel et al. 2007). The NDVs isolated from human casualties cases showed the highest similarity to the strains originating from pigeons (Kuiken et al. 2018; Ul-Rahman and Shabbir 2019). It is believed that feral pigeons are also playing a vital role in the dissemination of various zoonotic pathogens, including *Escherichia coli*, *Mycoplasma*, *Cryptococcus*, *Aspergillus*, *Chlamydia* species, and pathogenic avian influenza virus into the environment (Haag-Wackernagel and Moch 2004; Magnino et al. 2009; Phan et al. 2013; Michiels et al. 2016; Borges et al. 2017; Pakshir et al. 2019). These pathogens have been transmitted by inhalation of airborne excreta and dried feces, ocular discharges, and crop milk (Phan et al. 2013). However, the exact route of transmission of NDV to immunocompromised individuals

is yet to be explored. The transmission of NDV from pigeons to humans may be instructive for humans, who keep pigeons as pet or racing birds. Close contact between feral pigeons and humans commonly occurs in public gardens, squares, railway stations and historical places in urban areas (Harris et al. 2016). Therefore, immunocompromised individuals living in urban areas are more likely to have an ND infection than in rural areas.

Table 1: Evidence of spillover events of emergent paramyxoviruses from naïve host to other mammalian species

Virus	Naïve host	Spillover host	Geographical distribution	References
Achimota virus 1 (AchV-1)	African fruit bat (*Eidolon helvum*)	Humans	Accra, Ghana, Tanzania, Guinea	Baker et al. 2013
Ghanaian bat henipavirus (GhV)	African fruit bat (*Eidolon helvum*)	Humans	Ghana, Cameroon	Drexler et al. 2012; Pernet et al. 2014
Hendra virus (HeV)	Flying bats (*Pteropus alecto*, *P. conspicillatus*)	Horses, humans, dogs	Australia, Papua, New Guinea	Field et al. 2013; Field 2016
J paramyxovirus (JPV)	Mice (*Mus musculus*)	Humans, pigs, cows	Australia	Mesina et al. 1974; Jun et al. 1977; Drexler et al. 2012
Menangle paramyxo-virus (MenPV)	Fruit bats (*Dobsonia magna*, *Pteropus* spp.)	Pigs, humans	Australia, Papua, New Guinea	Chant et al. 1998; Breed et al. 2010; Barr et al. 2012
Mojiang paramyxovirus (MojPV)	Yellow hair rat (*Rattus flavipectus*)	Humans	China	Wu et al. 2014
Nipah virus (NiV)	Fruit bats (*Pteropus spp.*, *Eidolon helvum*, *Dobsonia magna*)	Pigs, humans, horses, dogs, goats, cows, cats	Bangladesh, Ghana, India, Malaysia Papua, New Guinea, Singapore, Thailand, Philippines	Wacharapluesadee et al. 2005; Hayman et al. 2008; Breed et al. 2010; Chowdhury et al. 2014; Luby and Gurley 2015; Ching et al. 2015
Porcine rubulavirus (PorV)	Pig (*Sus scrofa*), Yellow bat (*Rhogeessa parvula* majo)	Pigs, humans	Mexico	Salas-Rojas et al. 2004; Cuevas-Romero et al. 2015
Sosuga virus (SosV)	Egyptian fruit bat (*Rousettus aegypticus*)	Humans	South Sudan, Uganda	Amman et al. 2015
Tioman virus (TioV)	Fruit bats (*Pteropus* spp.)	Humans, pigs	Tioman Island, Malaysia Madagascar, Papua, New Guinea, India	Yaiw et al. 2008; Breed et al. 2010; Yadav et al. 2016

Note: Data only with full information was compiled from a previous report (Thibault et al. 2017).

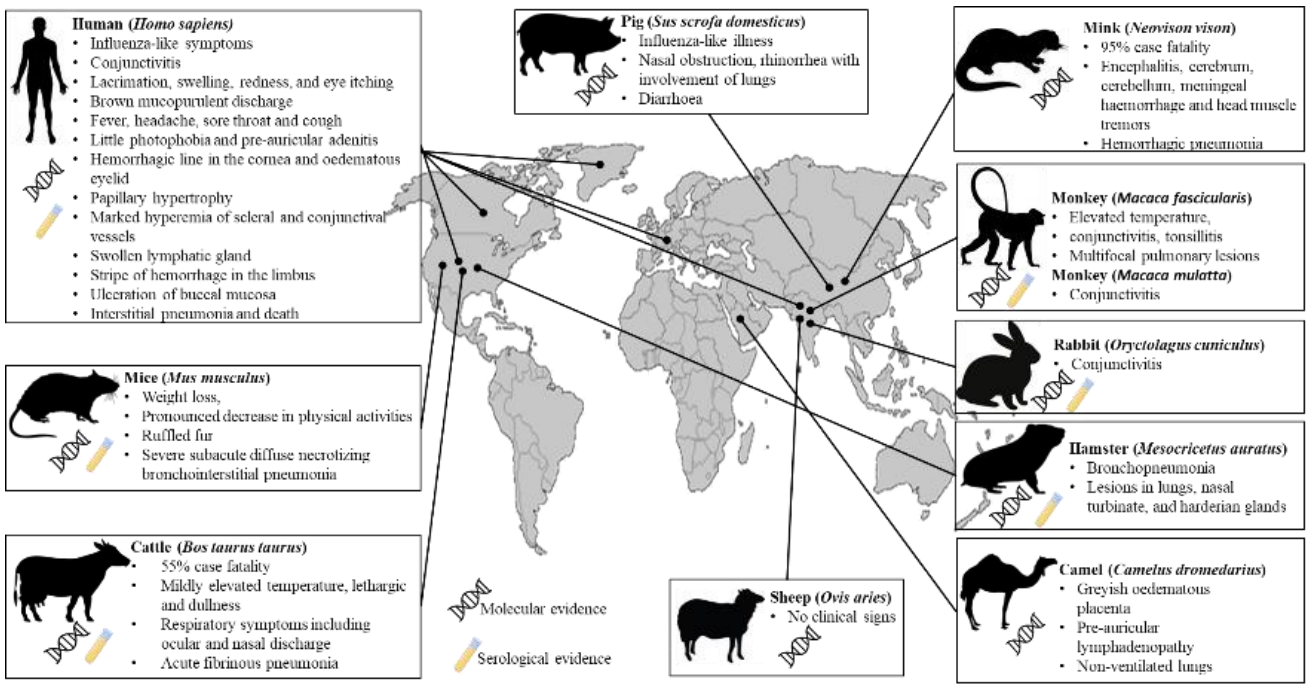

Figure 1: Map illustrating reports of Newcastle disease infection in non-avian species. Data compiled from published reports on the evidence of NDV infection and detection of viral genome in humans (Burnet 1943; Shimkin 1946; Yatom 1946; Anderson 1946; Freymann and Bang 1949; Ingalls and Mahoney 1949; Gustafson and Moses 1951; Hunter et al. 1951; Lippmann 1952; Keeney and Hunter 1951; Mitchell and Walker 1951; Quinn et al. 1952; Nelson et al. 1952; Reagan et al. 1956; Pilsworth and Wall 1964; Trott and Pilsworth 1965; Alby 1965; Goebel et al. 2007; Kuiken et al. 2018; Shabbir et al. 2021), mice (Khattar et al. 2011), cattle (Yates et al. 1952; Ozawa and Chow 1958; Subbiah et al. 2008), sheep (Sharma et al. 2012), camel (Teng et al. 2019), hamster (Samuel et al. 2011), rabbit (Charan et al. 1984), monkey (Charan et al. 1984; Kuiken et al. 2017), mink (Zhao et al. 2017) and pigs (Lu et al. 2009; Ding et al. 2010; Yuan et al. 2012; Chen et al. 2013).

From 1950-2014, the proportion of the global human population living in urban areas increased from 30% to 54% and is projected to be 68% by 2050 (United Nations Department 2015). This suggests that the number of vulnerable individuals at risk of contracting NDV from feral pigeons will increase in the coming decades. Considering factors mentioned above, along with increased urbanization and a high proportion of immunocompromised individuals, there is a realistic coincidental increase in the risk of severe human cases of NDV infection. Recent evidence suggested that NDV infections in immunocompromised individuals may not be commonplace and that when occur, they may be life-threatening, with public health concerns (Goebel et al. 2007; Kuiken et al. 2018). Furthermore, the virulence of NDV isolates does not appear to differ from humans to the vast differences in virulence in poultry. Even infection with low virulence NDV strains, which are commonly used in vaccine production, may cause similar clinical signs in humans (Dardiri et al. 1962). The pathogenesis of NDV infection in humans is not extensively reviewed so far. In brief, the duration and severity of NDV infection in humans lasts for 6-8 days. Following the incubation period (1-2 days), humans develop unilateral or bilateral conjunctivitis and/or influenza-like symptoms (Nelson et al. 1952; Alby 1965; Shabbir et al. 2021). During NDV infection in humans, various clinical manifestations may be present, such as fever ≥100°F, headache, eye itching, redness, lacrimation, mucopurulent nasal discharge, chilliness, sore throat, depressed appetite, pain, malaise, little photophobia, pharyngitis, slight unproductive cough, and marked insomnia, with general apathy (Figure 1). However, a lack of involvement of neurological and digestive systems was observed. Histopathological manifestations include hemorrhagic strip/line in limbus and cornea, oedematous eyelid, marked hyperemia of scleral and conjunctival vessels, pre-auricular lymphadenopathy, papillary hypertrophy, ulceration of buccal mucosa, palpebral edema, and interstitial pneumonia (Figure 2). The NDV has been isolated from the conjunctival sac washing, lacrimal fluid, nasal discharge, and saliva (Anderson 1946; Hunter et al. 1951; Quinn et al. 1952; Reagan et al. 1956; Figure 3). Successful isolation of NDV from blood cells and blood serum is suggesting that NDV can produce a viremia. Rarely, inclusion bodies have been noted in the cytoplasm of epithelial scraping (Hunter et al. 1951). There is marked serological evidence of NDV exposition to humans in Pakistan (Ahad et al. 2013) and India (Charan et al. 1981). These retrospective serological investigations revealed that humans associated with poultry farming and/or veterinary services are more susceptible to NDV infection with or without clinical disease. In Pakistan, poultry vaccinators showed the highest seroprevalence (85.7%), followed by poultry attendants (46.6%), laboratory technicians (38.9%), veterinary practitioners (30.4%), and poultry butchers (20.6%) (Ahad et al. 2013). Likewise, a higher seroprevalence of NDV (79.8%) was observed in individuals working in poultry farms, veterinary and vaccine institutes in India (Charan et al. 1981).

Family *Suidae*

The first report of NDV infection in pigs (*Sus scrofa domesticus*) was recorded in 2009 from China (Lu et al. 2009). Later, the occurrence of NDV outbreaks suggested a high susceptibility of pigs. Recently, a high proportion of NDV infected cases (>600) of pigs was observed during an outbreak in 2009 (Chen et al. 2013). As a consequence of NDV infection, pigs developed high fever, together with respiratory and intestinal signs (Lu et al. 2009; Ding et al. 2010; Yuan et al. 2012; Chen et al. 2013). Classical symptoms mainly include nasal obstruction, eye redness, and rhinorrhea. Lethal lesions include interstitial pneumonia, bronchopneumonia, and diarrhea (Figure 1). Besides, profound generalized influenza-like symptoms were observed in affected pigs. Thus, the emergence and spread of NDV among the pig population in China indicated the potential of NDV to replicate in the broad host range, even in non-avian hosts. Remarkably, the isolation and detection of NDV strains in nasal swabs and intestine tissues collected from affected pigs highlighted the potential of this virus to replicate in the respiratory and digestive tracts, from where they may subsequently shed in feces and respiratory secretions, which may act as a source of infection for other mammalian species (Lu et al. 2009; Yuan et al. 2012; Chen et al. 2013). Respiratory tract infection may range from asymptomatic to acute life-threatening diseases with the secretion of infectious viral progeny, thereby posing a significant threat to public health.

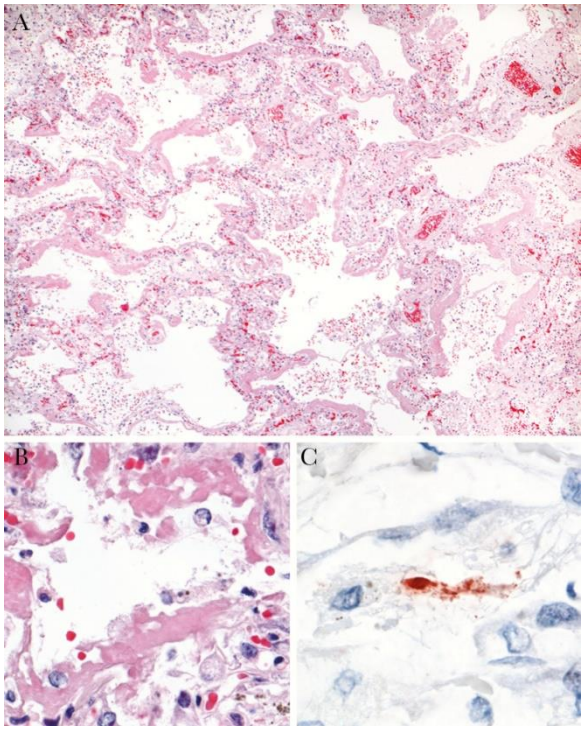

Figure 2: Histopathology and immunohistochemistry of lung tissue collected from NDV-infected human patient. The thickness of alveolar septa with diffuse damaged boundaries and alveolar lumina (A), the presence of hyaline membranes in alveolar septa (B), and granular staining in the cytoplasm of degenerative alveolar epithelial cells (C) (Kuiken et al. 2018)

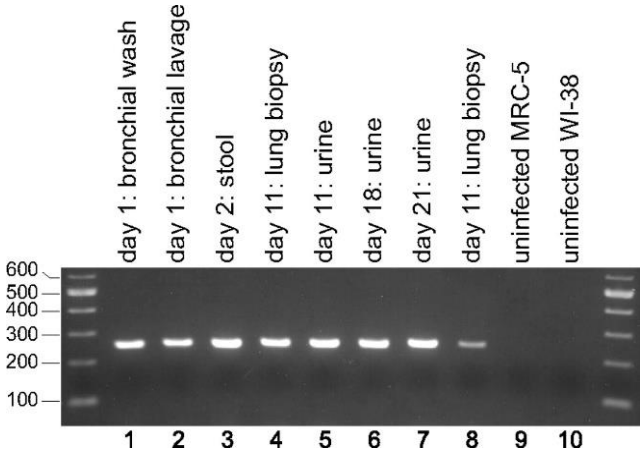

Figure 3: Detection of NDV genome in different samples collected from NDV-infected humans (Goebel et al. 2007). The analysed samples in lanes 1 through 7 had been cultured in MRC-5 cells; the sample in lane 8 had been cultured in WI-38 cells. Samples in lanes 9 and 10 were uninfected cell controls.

Family *Cercophitecidae*

So far, no investigation of natural infection with NDV in monkeys is noted. However, three experimental studies claimed monkeys' susceptibility against all three NDV pathotypes, including velogenic, mesogenic and lentogenic strains (Collier et al. 1950; Charan et al. 1984; Kuiken et al. 2017). Besides natural/experimental infection with NDVs in poultry birds, the experimental infection in monkeys caused similar clinical presentation in respiratory and neurological systems, as observed in the case of birds (Aziz-ul-Rahman et al. 2018, 2019a,b; Ul-Rahman et al. 2020). Even though the Indian Rhesus monkey (*Macaca mulatta*) in captivity may also succumb to low virulent and/or vaccine strains (Charan et al. 1984). Clinical manifestations mainly include conjunctivitis, tonsillitis, and encephalitis, with generalized symptoms such as elevated temperature (Collier et al. 1950; Charan et al. 1984; Kuiken et al. 2017). The NDV infection with genotype VI class II may cause multifocal pulmonary lesions. Subsequent to intra-tracheal inoculation, the mesogenic NDV strain showed a potential to replicate in nasal turbinate, trachea and bronchus of cynomolgus monkey (*Macaca fasicularis*). The viral genome of NDV has been detected in throat swabs, eye, nose, and lung samples (Charan et al. 1984; Kuiken et al. 2017).

Families *Bovidae* and *Camelidae*

Among ruminants, the evidence of NDV infection was first noted in Holstein cattle (*Bos taurus taurus*) during 1952 in the USA (Yates et al. 1952).Later, an outbreak of NDV infection was observed among the Holstein cattle population in the USA during 1953 (Ozawa and Chow 1958). Inclusively, influenza-like signs, including conjunctivitis, ocular and nasal discharge with 55% case fatality, were observed (Figure 1). Pathologically, acute fibrinous pneumonia was also observed in affected cattle. The viral genome of NDV may be detected in lung tissues from dead calves (Ozawa and Chow 1958). The evidence of viral antigens in different tissues and their characterization under electron microscopy indicated the potential of NDV to replicate in different tissues (Ozawa and Chow 1958). Following the experimental inoculation of lentogenic NDV strain via intra-nasal and intra-tracheal routes, few clinical signs, including mildly elevated temperature, lethargy, and dullness, were observed in Holstein cattle (Subbiah et al. 2008). In 2012, the NDV genomes were detected in two healthy sheep (*Ovis aries*) in India, which were isolated using the cell culture technique (Sharma et al. 2012). In 2015, an abortion case in a dromedary camel (*Camelus dromedarius*), with slight respiratory signs, was noted in Dubai (Teng et al. 2019). Pathology was characterized by the greyish oedematous placenta, pre-auricular lymphadenopathy, and non-ventilated lungs (Figure 1). Utilizing Next Generation Sequencing (NGS), the NDV genome was detected from nasal swabs collected from the aborted fetus (Teng et al. 2019). During that period, an NDV outbreak occurred in a pigeon flock living in close vicinity where the dam of the aborted camel fetus was housed. Therefore, there is likely a chance that the pregnant camel had acquired the NDV from infected pigeons and subsequently transmitted it to the fetus.

So far, indirect cross-species transmission of NDV to mammals has been reported. Inter- and intra-species transmissions between non-avian hosts, including humans and domestic animals, are an increasingly challenging threat to public and veterinary health.

Families *Musteliade*, *Leporidae*, *Cercetidae* and *Muridae*

So far, the information on natural infection in rodents and rabbits is not available, except for an NDV outbreak in mink (*Neovison vison*) in China during 2014 (Zhao et al. 2017). A total of 456 cases with 95% case fatality were noted. As a result of NDV infection, mink showed both respiratory and neurological signs, including respiratory distress, encephalitis, and head muscle tremors. Pathological lesions were characterized by hemorrhagic pneumonia, and hemorrhages in cerebrum, cerebellum and meninges e(Figure 1). Utilizing cell culture and embryonated eggs, NDVs were isolated from brain and lung tissues, and the virus could also be detected from these tissues using RT-PCR (Zhao et al. 2017). Experimental infection with three NDV pathotypes *via* subconjunctival route caused mild respiratory infection limited to conjunctivitis in rabbits (*Oryctolagus cuniculus*) (Charan et al. 1984). The experimental infection with lentogenic NDV strain *via* the intranasal route caused mild to severe pneumonia in mice (*Mus musculus*) (Khattar et al. 2011). Clinically, weight loss, with profound decrease in physical activities, ruffled fur, and slight respiratory distress were observed in infected mice (Figure 1). Histopathologically, severe sub-acute diffuse necrotizing bronchointerstitial pneumonia was observed in mice. Likewise, the experimental infection with lentogenic NDV strain *via* the intranasal route caused bronchopneumonia in hamsters (*Mesocricetus*

auratus) (Samuel et al. 2011). Moreover, pathological lesions were observed in the hamster's nasal turbinate, lungs, and harderian glands. The NDV genome has been detected and isolated from brain and lung tissues, and the virus can be detected from these tissues using RT-PCR (Samuel et al. 2011; Khattar et al. 2011).

NDV binding on cellular surface

The binding of NDV on the cell surface is an important phenomenon and crucial for the viral replication and multiplication within the host cells (Zaitsev et al. 2004). This phenomenon is also crucial for species and/or tissue tropism, to cross/jump to other species and cause infection in the host. In NDV, the hemagglutinin-neuraminidase (HN) appears to be a promiscuous binding type-II glycoprotein and contains C-terminus and T-terminus domains that may serve as receptor-binding domains (Huang et al. 2004). This protein is a multifunctional protein and binds with sialic acid-containing receptors, performs sialidase and neuraminidase activities for virus budding, and enhances the fusion activity of the fusion protein (Ferreira et al. 2004). The sialic acid receptors are present in red blood cells (RBCs) of mammalian species. The ability of NDV to induce agglutination of mammalian RBCs was studied as part of the problem of characterizing strains of the virus, but not because of cross-species potential (Winslow et al. 1950; Abu Elzein et al. 1993; Ibu et al. 2009). The ability of NDV to bind at sialic acid receptors leads to virus entry inside the cellular host. The actual rate of NDV spillover into mammalian species is greatly overlooked, as it did not cause any severe or lethal infection, except in immunocompromised individuals. However, it is suggested that if NDV can bind to sialic acid receptors of mammalian RBCs, it may cause clinical to subclinical infection in the host. Previous studies have shown that NDV strains can agglutinate the RBCs of goats, sheep, horses, cattle, camels, dogs, pigs, mice, rabbits, and humans, achieving hemagglutination titer comparable to the level obtained with chicken RBCs (Winslow et al. 1950; Abu Elzein et al. 1993; Ibu et al. 2009). Such evidence highlighted the presence of required binding receptors on the mammalian RBCs membrane, which suggests that mammalian species may be susceptible to NDV infection. However, there is limited information on the agglutination of a few mammalian RBCs. Therefore, extensive investigations, including all possible mammalian species are a prerequisite for further understandings about the issue.

Molecular evolution

The emergence and cross-species transmission of viruses in a natural system are influenced by different ecological, evolutionary, and genetic factors (Geoghegan and Holmes 2017). Amongst these factors, evolution is a crucial phenomenon, which is generally driven by the possibility of mutations in viral genomics (Moelling and Broecker 2019). Subsequent to natural selection, the generation and spread of positive or beneficial mutations increase the survival fitness of a virus in a specific environment (Dolan et al. 2018). The evolution manifests itself in a variety of ways, such as the repeated fixation of the positive selection site or mutation or more extensive genetic changes in significant biological and structural motifs of the NDV genome (Aziz-ul-Rahman et al. 2018a,b; Ul-Rahman and Shabbir 2019; Rahman et al. 2019). Such positive selection sites usually favor the replication and cross-species transmission of viruses. Several substitutions have been noted in NDV strains originating from different mammalian species (Ul-Rahman and Shabbir 2019). A previous study revealed copious substitutions at different significant motifs, including N-N assembly motif in nucleoprotein (NP), ATP-binding motif and domain-III in large (L) protein, M domain in matrix (M) protein, the sialic acid-binding motif in HN protein, and cleavage motif in the fusion (F) protein (Ul-Rahman and Shabbir 2019). It is hypothesized that such types of substitutions

Figure 4: Fusion protein-based phylogenetic analysis of NDV strains originating from mammalian species concerning representative strains of different genotypes. The phylogenetic tree was constructed using the maximum-likelihood statistical method in MEGA® X software. The NDV strains originating from mammalian species are highlighted with different shapes, as human-originated NDV strain with a circular shape, mink-originated NDV strain with a square shape, camel-originated NDV strain with a triangle shape, and pig-originated NDV strain with diamond/rhombus shape (Ul-Rahman and Shabbir 2019).

are needed to adapt a novel host species or evade host immune responses and ultimately derive the recurrent evolution of certain NDV strains. Based on the fusion protein sequence, NDV strains are categorized into two classes (class I and II), and NDV strains belonging to class II are further classified into 18 genotypes (I-XVIII) (Dimitrov et al. 2019). Almost five distinct NDV genotypes

(G-I, G-III, G-VI, G-VII and G-XIII) have been identified in mammalian species and recognized to date-based on their unique genomics, as determined by bioinformatics analysis of fusion protein (Figure 4). A total of 14 genomic sequences of NDV originating from four distinct mammalian species, including humans, pigs, camels, and mink, are available in the NCBI database. Phylogenetic analysis clustered the NDV strains originating from mammalian species together with those strains isolated from different birds. Comparative residue and phylogenomic analysis of fusion protein of NDV originating from diverse mammalian species substantiates the theory of cross-species transmission, with probable transmission directly from birds to mammalian species. Comparative genomic analysis revealed that structurally and functionally essential features of velogenic NDV strains were also present in those strains originating from mammalian species (Ul-Rahman and Shabbir, 2019). Comparative residue and phylogenetic investigation also suggested the evolutionary dynamics of these NDV strains. Such genomic analysis may help to forecast virus emergence and cross-species transmission events in the near future (Geoghegan et al. 2017). Utilizing a comparative genomic analysis, the emergence and cross-species transmission can be predicted; however, *in vitro* investigations and experimental evolutionary studies in the same phylogenetic context are prerequisites. Moreover, it is necessary to investigate viral and host factors associated with cross-species transmission in order to strengthen effective epidemiological, disease-control, and therapeutic intervention strategies.

Importance of advanced molecular techniques

Over time, the advancement in novel molecular techniques has helped to identify previously unsuspected or unknown pathogens, including emerging viruses (Mokili et al. 2012; Artika et al. 2020). Many advanced molecular techniques are being used to explore the unknown origin and different genomic characteristics of newly emerged viruses (Artika et al. 2020). Among diverse molecular techniques, the next-generation sequencing (NGS) technique has proven as a significant break-through in the discovery of many novel viruses in animals, plants, and humans (Kumar et al. 2019; Maljkovic et al. 2020; Minicka et al. 2020). Likewise, the use of NGS has led to identifying the SARS-CoV-2 virus, which is responsible for a recent pandemic of COVID-19 infection in the human population (Bhoyar et al. 2021; Tillett et al. 2021). As a result of the COVID-19 pandemic, the discoveries of different mammalian species as reservoirs and/or carrier hosts of emerging viruses have boosted interest in searching for more novel or known viruses in those suspicious hosts that were not previously associated with any infection against a specific virus. Similarly, NGS has made it possible to identify NDV strains in dead immunocompromised patients and other apparently healthy or clinically ill animals (Goebel et al. 2007; Sharma et al. 2012; Kuiken et al. 2018; Teng et al. 2019). These metagenomic studies have remarkably highlighted the spectrum of NDV to cause infection in a wide range of mammalian species. At present, it is hard to predict the risk of spillover potential of NDV strains that have been detected in mammalian species. Therefore, it is crucial to conduct insightful investigations to explore the cross-species potential of NDV strains.

Future Perspectives

Identification of distinct NDV strains in mammalian species not only emphasized the expansion of their host range and genetic diversity but immediately raised the question about their zoonotic potential and, ultimately, public health concerns. Since NDV is not screened in mammalian species, it is presumed that NDV is also prevalent in non-avian hosts, and thus screening for this virus in these species may give surprising results. Based on these facts, it may be recommend that screening for NDV should be included in the differential diagnosis of respiratory infection and disease surveillance cases, especially in poultry workers and immunocompromised individuals in which common respiratory pathogens cannot be routinely tested. Being evolving paramyxovirus, the emergence of novel NDV variant or strain within the same genotype is not uncommon, and thus increasing evidence proposed the adaptation of NDV to novel host species for its survival fitness (Aziz-ul-Rahman et al. 2018a; Ul-Rahman and Shabbir 2019; Rahman et al. 2019; Afonso 2021). It is, therefore, crucial to conduct *in vitro* investigations to understand the likelihood of cellular receptors mediating entry, host factors involving tissue tropism, and possible zoonotic transmission of NDV among non-avian species. Moreover, it is essential to assess the clinical and public health relevance of NDV by determining its origin and cross-species transmission aspects or spillover event in all susceptible mammalian hosts. Poultry workers or any individual, especially immunocompromised patients, linked to poultry processing or vaccine manufacturing units, are advised to follow recommended biosecurity measures. The use of appropriate personal protective equipment, biological safety cabinet, and careful attention to hand hygiene may undoubtedly reduce vulnerable individuals' exposure to NDV infection. Besides, appropriate biosecurity measures in the poultry industry, careful handling of infected birds or infectious material, and continuous surveillance among high-risk professionals should be practiced as a critical element to the possible reduction in the opportunities of cross-species transmission and ultimately to combat against diseases. Bird and/or poultry owners, who keep birds, whether as pets or as commercial production, should be aware of the zoonotic potential of NDV and possible routes of its transmission, including contact with infected birds, contaminated surfaces, and feces from domestic, commercial, or wild birds. Most importantly, bird owners should undoubtedly seek medical assistance if they suspect to have contracted an NDV-infected bird or develop any clinical signs of the disease.

Conclusion

In the last seven decades, numerous studies have provided evidence on the zoonotic potential of emerging NDV strains worldwide and suggested that the number of mammalian species at risk of contracting a zoonotic infection of NDV will increase in the coming decades. Since most of the investigations suggested the spillover of NDV from naïve bird hosts, the cross-species transmission among non-avian hosts should not be underestimated, as shown in the recent SARS-CoV-2 pandemic. The data presented here suggest that, under particular circumstances, it is indeed possible for NDV strains to cause severe respiratory infections in humans. Concerning the evolutionary dynamics of NDV strains from distinct genotypes, it is supposed that NDV can cause more disease outbreaks among different non-avian hosts. Cumulatively, the data presented in this chapter indicate a probability of different mammalian species getting NDV infection. However, we are still at an infancy stage in understanding the NDV capability in the context of comparative immunology, pathogenesis, and cross-species transmission studies at human- or animal- and birds-interface. Up till now, the knowledge about the impact of NDV on public health is minimal. Therefore, it should be cautioned that these are preliminary studies based on the genomic and bioinformatics analysis only. More in-depth pathobiological and functional studies are prerequisites to fully understand the NDV infection in a wide range of non-avian hosts, particularly humans.

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HEPATITIS E: ZOONOTIC PERSPECTIVES

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INTRODUCTION

Hepatitis E virus (HEV) is a single stranded hepatotropic virus which primarily affects the liver, resulting in acute hepatitis in humans. Every year, approximately 20.0 million HEV infections are reported globally, with 3.3 million symptomatic cases. The infection results in 3.0 million acute cases and 57,000 deaths per year (WHO, 2020).

Infection caused by HEV may be severe, ranging from imperceptible infection to fulminant liver and, if untreated, can lead to death. It was previously considered as an acute infection; however, chronic infections have also been observed in people suffering from chronic liver disease (CLD) and kidney or liver transplant. Despite the growing awareness about the HEV, the origin of the disease remains complicated to understand (Guerra et al. 2017). The HEV was first reported in 1983 by a Russian virologist (Balayan et al. 1983), who examined the virus under electron microscope while investigating his own feces after intake of a pooled fecal extract of soldiers, who were previously infected by the virus.

The virus enters the human body through the intestine, while it is eliminated in the stools of infected persons. The infection is generally self-limiting and resolves within 2–6 weeks. In the past, HEV was not considered to be prevalent in developed countries. However, now-a-days, it is believed to be endemic, as it is observed to be a zoonotic contagious agent in many developed countries. In these countries, acute hepatitis is commonly caused by autochthonous infections due to HEV3 and HEV4. The available literature shows that in previous years the most frequent genotype in China was HEV1, but due to improved hygiene and sanitation conditions, HEV4 surpassed HEV1 (Dai et al. 2013).

With regard to developing countries, waterborne acute hepatitis occurs due to HEV. Furthermore, the infection is more prevalent in areas with poor sanitation and hygiene conditions. Since HEV is a single stranded RNA, it exhibits high rate of mutations in genome, which has been estimated from clinical isolates in previous studies. The mutation rate of HEV has been observed to be more than 1.5 base substitutions per site in a year, which is comparable to that reported for hepatitis C virus (HCV).

Novel HEV variants in different animals have been identified, which is constantly evolving the diversity of the virus. This chapter will discuss about the basic virology, genotypes of HEV, epidemiology, zoonotic transmission, diagnosis, clinical complications, and treatment of the infection, based on the currently available data.

Basic Virology of Hepatitis E virus

Hepatitis E virus is currently the only species classified within the genus Hepevirus, family Hepeviridae. The hepatitis E virion is non-enveloped and 27–35 nm in size. The viral genome consists of three partially overlapping open-reading frames (ORFs) (Nagashima et al. 2017), as shown in Fig. 1a.

ORF1

ORF1 encodes non-structural polypeptides i.e. methyltransferase, RNA helicase, cysteine protease and RNA-dependent RNA polymerase activity (Fig. 1b). The pORF3 and pORF2 encode for sub-genomic RNA fragment of 2.2-Kb into two different reading frames.

ORF2

The viral capsid ORF2 is translated into a multifunctional 88 kDa protein with 660 amino acids, which is helpful in encapsculating viron and maintaining host viral interaction.

ORF3

ORF3 encodes for a phosphoprotein carrying 114-amino acids with molecular weight of 13-kDa. This protein bears proline rich residues, which interact with SH3 domain and MAPK (mitogen activating phosphokinase factor) of the host to mediate cellular response. Viral genomic fragment pORF3 transcribes by regulating MAPK/ERK and JAK/STAT pathways via epidermal growth factor receptor (EGFR), which thereby hinders immune activation. In addition, pORF3 is supposed to play a vital role in budding of virion by Golgi synthesis (Ikram et al. 2018).

Identified mutations of ORF1 and ORF2 regions of HEV are shown in Fig. 1. The box numbers represent the amino acid position; the letters above the box refer to the wild type amino acid and the letter below the box are relevant mutations cited before (Ikram et al. 2018). During genotyping of HEV, it was revealed that genotype 1 possessed 8 sites of mutation compared with genotypes 3 and 4, which possess only 6 and 3 mutations, respectively.

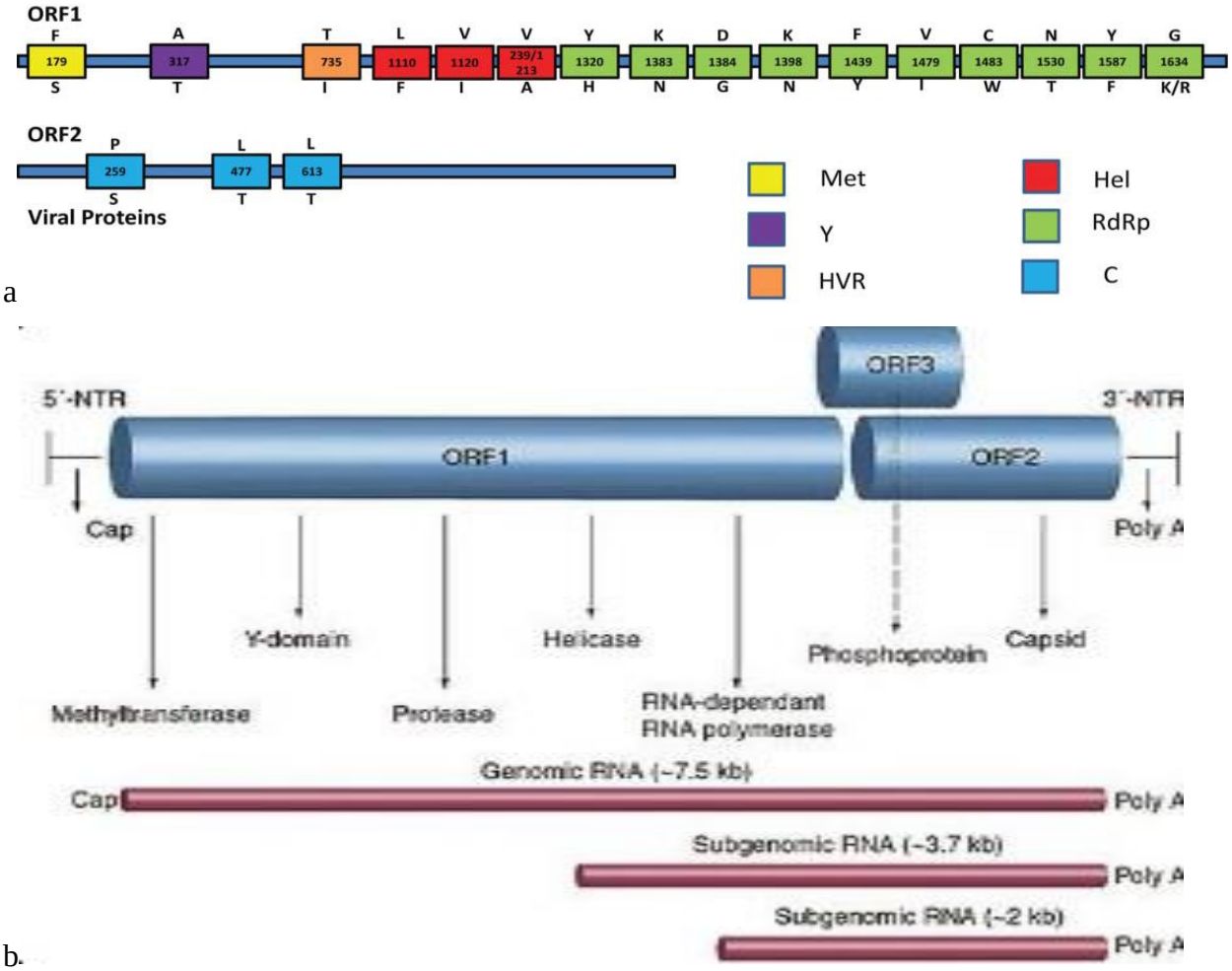

Fig. 1a: Mutagenic regions of open reading frame (ORF)1 and 2 of Hepatitis E virus (Hel: RNA helicase; RdRP: RNA-dependent RNA polymerase; C: capsid protein, Met: methyltransferase; Y: Y-domain; HVR: hypervariable regions). 1b: Three main ORF with their genomic regions for Helicase, capsid methyltransferase etc.

Epidemiological aspects of Hepatitis E

HEV infection in humans has two different epidemiological designs. The virus is responsible for the worldwide transmission of hepatitis and has caused higher than 50% of acute hepatitis cases in endemic countries. Contaminated water in unhygienic areas is the foremost cause of transmission of HEV1 and HEV2 in people through the fecal-oral route. Previous studies demonstrate that poor quality of water has resulted in a large number of sporadic cases and large outbreaks. Although HEV is self-limiting, it can lead to the development of liver failure, particularly in pregnant females. Moreover, patients having solid organ transplant or taking immunosuppressant and HIV patients suffering from AIDs can experience cirrhosis due to HEV infection (Labrique et al. 2010). Screening strategies have been significantly improved and periodic cases were observed in previous years due to zoonotic transmission of HEV3 and HEV4 through animal reservoirs.

One of the most common reasons for acute liver infection world-wide is HEV (Hepatitis E virus). In India, HEV is responsible for more than 50% of acute viral infection in liver, while 25% in Africa and 15-20% in Eastern-Oriental countries.

HEV was originally recognized like non-A non-B hepatitis, which creates infection through water borne illness like hepatitis A (Donnelly et al. 2017), and epidemics in developing countries are mainly connected with genotypes 1 and 2. However, currently it is the prominent reason of icteric hepatitis and acute failure of liver in underdeveloped areas around the world, the predicted occurrence of HEV illnesses is 20 million per anum, which can result in about 56,600 mortalities (Lozano et al. 2012). A major instance during severe infection is in pregnant women, who are more vulnerable and may exhibit the clinical development linked with poor results (3000 stillbirths are annually recorded due to HEV3). Genotypes 3, 4 and 5 are involved in locally acquired HEV infections since the last decade in western countries. However, since 2008, chronic HEV infections are considered (Kamar et al. 2008) and this may cause cirrhosis and organ failure in transplant recipients (Kamar et al. 2011). It has been suggested that in developed countries, HEV pathogen is under-consideration, while frequency of confirmed cases has also been reported (Koot et al. 2015; Adlhoch et al. 2016). According to various cohort studies, seroprevalence rate HEV infection was reduced in some countries (Wenzel et al. 2014; Holm et al. 2015), whereas few have reported

increased ratio of (HEV-RNA-positive) blood donors (Hogema et al. 2014; Tedder et al. 2016). World Health Organization (WHO) have reported through systemic review the range ofo.03% to 52% HEV seroprevalence (having IgG antibody) in general population and blood donors. However, in 2014, WHO reported the maximum incidence of HEV seroprevalence in France and Netherlands. In European countries, there are 77% patients having symptomatic acute hepatitis through HEV infection and show a significant relation of the virus with liver morbidity (WHO 2014). However, seroprevalence estimation was remarkably affected through the use of different serological test systems.

Hartl et al. (2016) conducted a research through which they observed HEV illness during 1983-2015 around UAE, North and East Africa and Pakistan in Dromedaries. For this purpose, serum and fecal samples were collected from dromedary camels in the UAE, Somalia, Sudan, Egypt, Kenya, and Pakistan during 1983–2015 (Aggarwal 2016; Lee et al. 2016). Through RT-PCR (reverse transcription PCR), analysis of 2,171 serum samples and 267 fecal samples for HEV RNA was done (Drexler et al. 2012). The positive serum samples (about 46% from 6 countries) were compared with the seroprevalences characteristically seen in pigs responsible for zoonotic reservoirs of HEV-3 in developed countries (Krumbholz et al. 2012). Another study reported the occurrence of non-A, non-B liver illness, which was present in an armed academic community in Abbottabad, Pakistan. This was due to an isolate of HEV genetically different from the HEV type responsible for an epidemic in Sargodha, (Pakistan). Genetically, the HEV strains isolated from Sargodha resembled the isolates from China around Hindu Kush hills from Pakistan. However, the type isolated from Abbottabad genetically resembled with south Asian isolated strain from Nepal, India and Burma (Cuyck-Gandre et al. 2000). Therefore, two genetically different HEV strains were responsible for hepatitis E infection in the Pakistani military.

HEV Genotypes

HEV is a non-enveloped, positive-sense, RNA virus (Emerson et al. 2010), which is approximately 7.2 kb long and 27 to 34 nm in diameter, and is capped with 7-methylguanylate at 5′ and polyadenylated at 3’ end (Kabrane-Lazizi et al. 1999; Debing et al. 2016). It is comprised of a short 5′ untranslated region (UTR), three open reading frames (ORFs), namely ORF1, ORF2 and ORF3, and a 3′ UTR. Non-structural proteins, as well as methyltransferase, cysteine protease, RNA-dependent RNA polymerase and helicase, are encoded by ORF1, (Koonin et al. 1992). ORF2 encodes the viral capsid protein, whereas a phosphorylated protein (113 or 114 amino acids long) is encoded by ORF3, which plays an important role in morphogenesis and release of the virus (Emerson et al. 2010). Frameshifted segments from a sub genomic mRNA species are encoded by overlapping reading frames ORF2 and ORF3 (Graff et al. 2006). Inter-species variation was observed at the junction of both frames, with the variant cutthroat trout virus comprising of an ORF3, which is displaced towards the core of ORF2 (Siddharth et al. 2017).

HEVs found in humans are sub-catagorized in the genus Orthohepevirus and species Orthohepevirus A (Smith et al. 2014). Nucleotide sequencing of HEV genome from samples isolated from humans and animals made it clear that Orthohepevirus A viruses can be grouped into 8 genotypes (HEV-1 to HEV-8), which are further subdivided in subtypes (Enouf et al. 2006).

While 8 HEV genotypes have been identified, there are 4 distinct genotypes, including genotype 1, 2, 3 and 4, that infect humans. Genotypes 1 and 2 are present in humans (usually found in young and adults, aged 15-40), causing self-limited acute infection, which can hardly become chronic. In developing countries, these genotypes contribute the most to cause the infection. Previous findings suggest that animals, like wild deer, boars and pigs, carry genotypes 3 and 4 which are thought to be the primary causative agents of periodic infection in the developed countries and are transferred to humans by consuming raw meat of animals, such as pig and dear, and are mainly found in United States, China, Australia and Japan (Woo et al. 2016). These genotypes are mainly seen in older adults, who are above 40 years of age (Lu et al. 2006), and they usually cause acute infections which can lead to chronic diseases in some cases.

Furthermore, Genotype 3 is divided into 10 sub genotypes, ranging from 3a-3j, while genotype 4 is divided into 7 (4a-4g) sub genotypes, and both are found in swine and humans. Although viral factors play an important role in the pathogenesis of the disease, condition of immune system of the host determines the severity of acute hepatitis (Rutjes et al. 2009). Hence, genotype of HEV contributes to the pathogenesis of HEV-associated hepatitis. It has also been observed that people infected with genotype HEV4 show more severe form of viral hepatitis than genotype HEV3 infected patients. Thus, genetic changes in HEV genotypes have a profound impact on the efficacy of viral transmission and, eventually, the severity of HEV-associated hepatitis. A single case of infection by genotype 7 has also been reported, but it is still unidentified that either this genotype is easily transmitted to humans or its transmission occurs only in exceptional cases (Lee et al. 2016). Genotype 8 was found in Bactrian camels from Xinjiang, China (Woo et al. 2013).

Genotypes 1 and 2 Infection

In underdeveloped countries, HEV isolates with genotypes 1 and 2 are more endemic; Genotype 1 is mostly responsible for acute liver infection in Asia (especially in India), while genotype 2 is predominant in Africa, Central America and Mexico. However, these genotypes are limited to humans. Data received from 9 out of 21 Global Disease Burden areas world-wide showed that out of 20.0 million HEV infections, around 3.3 million had HEV symptoms, with 56,600 HEV associated expiries according to the World Health Organization (WHO) findings, while

the exact burden of disease is drastic. However, genotypes 1 and 2 are more responsible for infection in underdeveloped countries, with fatality rate ranges from 0.2 to 4.0%. This mortality rate is about 20% in infected pregnant patients in endemic countries. A novel study designed on human liver cells on chimerical mice for HEV illness proved that intravenous injection of HEV virions was consequently excreted through stool. There was HEV genotype 1 infection, while there was no active HEV infection by injecting HEV-positive serum intraperitonealy or via intravenous route (Allweiss et al. 2016). These results show that stool positive for HEV virion is due to HEV genotype 1.

Genotype 3 and 4 Infection

Both, human beings and animals are targeted for HEV isolates of genotypes 3 and 4. The highest prevailing infection in advanced countries is mainly due to HEV genotype 3. Pigs, deer and wild boar are responsible for this in comparison with genotype 1 and 2. In South-East Asia, genotype 4 infection is more prevalent (Hoofnagle et al. 2012).

HEV Zoonotic Transmission

Initially, it was believed that HEV infection was limited to humans, responsible for deaths from 0.5 to 3.0% in general. However, this mortality approached 30% in pregnant women. But presence of HEV in pigs in 1997 reflects that there is variety of HEV hosts and it is truly zoonotic. There are 70,000 mortalities and 3,000 stillbirths (Holm et al. 2015) from nine out of 21 parts of the world recommended as GDB (global burden of disease) study.

Primarily, it was suggested that Hepatitis E was only confined to undeveloped countries (Khuroo and Khuroo, 2016). But after evidences of infrequent hepatitis E infections in advanced countries, these findings became conflicted and showed an extended host range world-wide (Anheyer-Behmenburg et al. 2017).

Cross specie transmission

Hepatitis E has become one of the worst active and powerful animal-born viral illness (Dalton et al. 2015). In these countries, major way of transmission of HEV is from animal sources to humans via cross-species transmission (Pavio et al. 2015; Salines et al. 2017). However, HEV is not best known commonly in contrast to the other liver viruses, like hepatitis B and C viruses. But, mostly in developed countries, the sporadic hepatitis E cases may be transmitted from endemic areas or associated with the persons traveling from an endemic part, while they are not transmitted through conventional fecal-oral way. HEV may have less endemicity in advanced countries having unknown infectious reasons. The advancement of diagnostic tools for HEV and sero surveillance analysis on anti-HEV antibodies reflected high proportion (about 28% in few parts) in persons living in the US and other advanced states, which showed that hepatitis E is not pervasive. Analytical data showed that there are unidentified HEV infection sources and unknown non-infectious or less infectious HEV isolates also present in advanced countries.

In advanced countries, occasional cases of HEV infection were basically associated with the visitors to HEV-pervasive undeveloped countries (Abid et al. 1997). These persons were expected to infect through impure water drinking via travelling. But there is high autochthonous HEV infections in developed countries (Preiss et al. 2006). However, the exact causes for infection in autochthonous cases are still unknown and there are several risks which may be associated with this infection (Chaussade et al. 2013).

Evaluation of pervasiveness for anti-HEV antibodies to measure connection amongst HEV contagion risk and profession shows that there is significantly higher HEV seroprevalence in farmers than that in other professions in China (34.4%) (Jia et al. 2005). It is about 1.5 times higher in persons (veterinarians) handling pigs than in standard blood donors in the USA (Moal et al. 2013). Additionally, there is 3.5 times greater danger for HEV infection in swine farmers than general people in rural Taiwan (Lee et al. 2016). Furthermore, the occurrence of serum HEV-reactive antibodies is also greater in persons working on swine-associated activities, like veterinarians, meat inspectors, pig farmers and slaughter houses than in registered blood donors in Spain and Germany (Galiana et al. 2008; Krumbholz et al. 2012). About 41.7% persons working in slaughterhouses are reported as carriers of serum HEV-reactive antibodies. All these findings reflect that persons who are in contact with pigs or other farm animals have great danger towards infection than those who are not associated with animals. However, currently, some authors claim that the HEV seroprevalence rates in people who are in contact with pigs are not remarkably higher in comparison with those people who are not in contact with pigs in Northern Thailand and Austria (Hinjoy et al. 2013; Lagler et al. 2014).

Swine zoonotic transmission

Women employed on swine farms are observed to have less HEV infection rates in comparison with common people in Guangdong province in China (Liang et al. 2014). These findings reveal the occupational effect in spreading the infection. Therefore, there is need for more studies to solve this conflict concerning the professional based hazard factors in HEV infection. The following clues give significance about the reasons of autochthonous HEV illness HEV sequences separated from hepatitis E positive are nearly similar to the sequences of swine HEV isolates (Widén et al. 2011). Along with, swine HEV sequences in sera acquired from native Netherland's patients who had not moved to HEV-endemic countries (Herremans et al. 2007).

Zoonotic transmission by farm animals and meat

It is generally believed that acute hepatitis E native infectious cases are caused by pork eating and same is true

in Japan (Miyashita et al. 2012). These autochthonous illnesses are usually seen in middle-aged to elderly persons, who generally eat pork meat (Péron et al. 2006). HEV infection cases due to eating uncooked or undercooked pork are also found in other countries. Consequently, now-a-days, pigs are considered as the basic risk factor for HEV positive population in advanced countries. Furthermore, in European countries, HEV infection is recognized by pork liver sausage, and wild boar are considered as risk factors (La Rosa et al. 2011). In France, this sausage seems to contribute remarkably in HEV infection (Colson et al. 2010). Illness of human hepatocarcinoma is also due to HEV infection present in pork liver sausages and it is thought that the occurrence of this danger lies in usage of these sausages (Berto et al. 2013).

A study reported acute hepatitis E infection in a Korean citizen, who used raw bile juice of wild boar (Kim et al. 2011). In wild boar, the HEV-4 is responsible for this danger. However, meat from other animal as carrier of HEV-3 strains has been identified in both human beings and animals like, wild boars, deer, and swine in Hungary (Reuter et al. 2009).

Remarkably similar HEV infection sequences were recognized in both roe deer and human beings. In Japan, similarity in HEV infection was identified among wild boars, deer and hepatitis E infected persons fed on raw deer meat (Takahashi et al. 2004). These data specify that transmission of HEV among species is possible, and that wild animals like boars and deer take part in the transfer of HEV to human beings. However, both HEV genomes and infectious HEV particles have been testified in sewerage water tasters (Masclaux et al. 2013) and this wastewater may also pollute seawater and shellfish. Certainly, in Japan, an HEV-3 isolate was found in saline water and it showed similarity with the human strain (Ishida et al. 2012). Polluted saline water contain shellfish and its consumption can cause infection of about 4 million people annually (Shuval et al. 2003). Among the shellfish samples collected from coastal area of China, around 18% were infected with HEV-4 strains and again there was close association with swine and human strains (Gao et al. 2015). Additionally, oysters of about 8.7% gathered from costal area having HEV in Korea also resembled with swine HEV in nucleotide sequence (Song et al. 2010). Various HEV infection cases in developed countries confirmed that another causative agent for HEV infections in humans is due to eating habits of shellfish (La Rosa et al. 2011), as shown in Fig. 2.

Together with all these findings, it appears that most of the HEV infections in humans are through eating of animal meat, meat products, and seafood, especially in advanced countries. Initially, it was considered that HEV spread among animals and humans is through fecal-oral route.

Transmission *via* blood donors

Furthermore, it is proposed that in HEV endemic countries, blood transfusions also take part in spreading the infection (Arankalle and Chobe 2000). This is due to the presence of HEV-specified antibodies and high levels of alanine aminotransferase (ALT) in HEV-negative persons after blood transfusion. This was also confirmed in one study on rhesus monkeys, where the monkeys were infected with acute hepatitis after blood transfusion from hepatitis E-viremic donors. Blood transfusion-mediated transmission is also seen in autochthonous HEV infections currently observed in advanced countries where HEV is not a zoonotic pathogen. Cases are reported after blood transfusion from HEV positive blood donors (Matsubayashi et al. 2004). Mostly, HEV infections are present basically in persons aged 15 to 45 years in HEV-prevalent areas, but studies also suggest that kids can also get infected with HEV via blood transfer (Colson et al. 2007). So, blood transfusion is now considered as a new risk factor for HEV transmission.

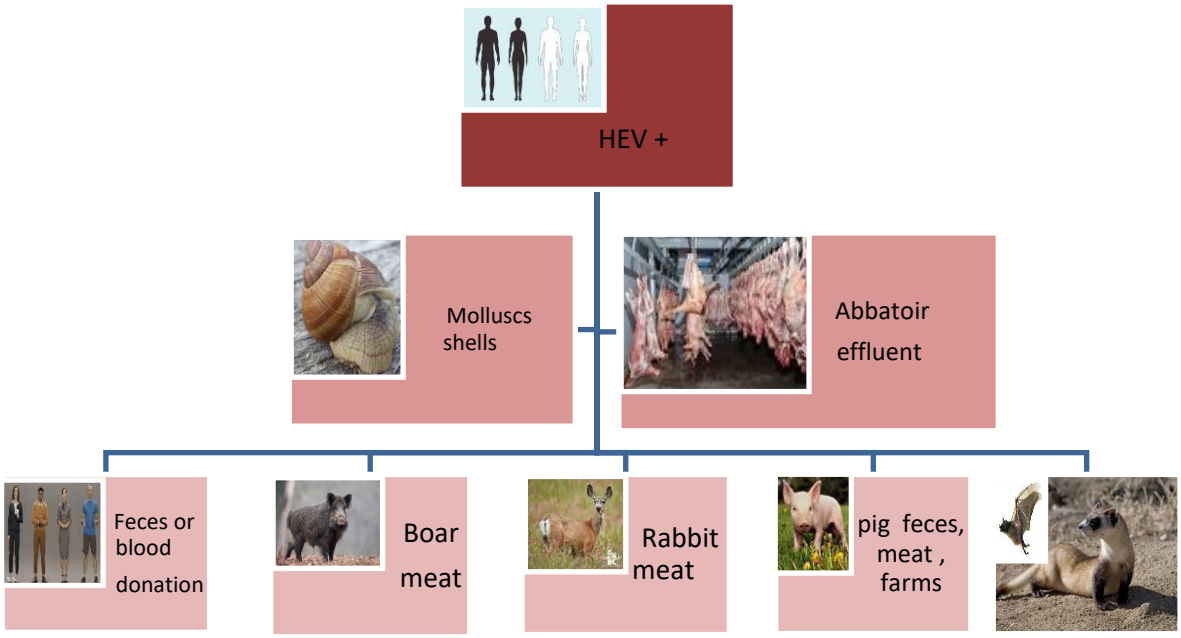

Fig. 2: Possible, probable, and definite routes of transmission of hepatitis E virus.

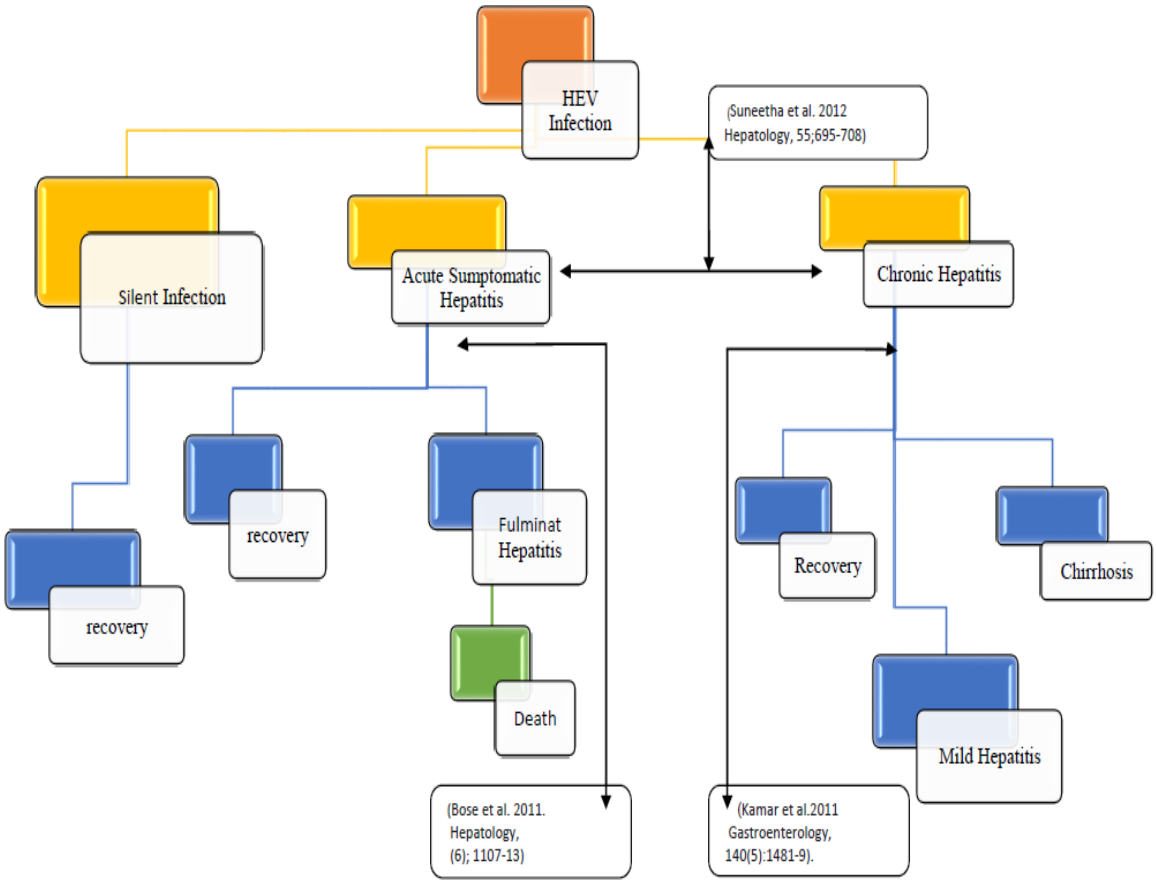

Fig. 3: Course of infection of Hepatitis E and its possible routes.

Table 1: Symptoms of acute autochthonous hepatitis E

Common symptoms	Less common symptoms
Jaundice	Myalgia
Anorexia	Headache
Lethargy	Pruritis
Vomiting	Arthralgia
Abdominal pain	Neurological disorders

Symptoms of Hepatitis E

Acute hepatitis E infection

Mostly, viral hepatitis has common symptoms. As already mentioned, acute hepatitis E infection is a self-limiting disease, which requires conventional treatment (Dalton et al. 2014). Majority of patients are asymptomatic and their causes of illness are not completely identified, or they develop clinical signs and symptoms of acute hepatitis (viral), like malaise, anorexia, nausea, vomiting, jaundice, and abdominal pain (Table 1). Patient may present with the symptoms of acute jaundice with liver enlargement and tender abdomen. In case of fulminant hepatitis, adverse results of 0.5% fatality rate may be seen (Zhao et al. 2016).

Chronic Hepatitis E

Cases with severe hepatitis and underlying chronic liver disease have a poor prognosis (Dalton et al. 2008). Several of them have extrahepatic manifestations, such as thrombocytopenia, hemolysis, aplastic anemia, acute thyroiditis, membranous glomerulonephritis, and neurological diseases such as acute transverse myelitis and septic meningitis (Geurtsvankessel et al. 2013), as depicted in Fig. 3.

Other extra-hepatic signs and symptoms are also seen, which show the line of spectrum for other clinical complications. In UK and France, basically one of the main reasons for neurological disorder may be locally developed acute and chronic HEV infected persons. In hospitals, about 5.5% patients out of 126, who were infected with acute hepatitis E virus, also showed neurological disorders during 2004 to 2009 (Kamar et al. 2011). Besides this, Sridhar et al. (2018) revealed that Guillain-Barre syndrome in China was also associated with this acute HEV, while transplant patients also showed chronic infections. However, the occurrence of acute Hepatitis infection aspects in immuno-suppressed recipients is mostly due to the followings:

- Intensity of immune-suppression
- Time interval between last episode of acute rejection and HEV infection since the time of transplantation
- Reduced leukocyte and total-lymphocyte counts (Kamar et al. 2011).

Serological Evaluation of Disease

In clinical diagnosis of HEV infection, laboratory findings are fundamental. Elevated serum concentrations of aspartate aminotransferase (AST), bilirubin and alanine aminotransferase (ALT) are index for acute Hepatitis.

Table 2: Vaccines derived from various candidate biological systems.

Pre-clinical evaluation of HEV vaccine candidates

	ORF-2 protein	Amino acids	Source	Remarks
Expressed in E. coli	TrpE-C2	221 – 660	Burma	
	pE2	394 – 607	China	
	HEV 239	368 – 606	China	Human CT
Expressed in insect				
Baculovirus-mediated	56-kDa protein	112 – 607	Pakistan	Human CT
	53-kDa protein	112 – 577	Burma	Oral route
Spodoptera litura larvae	62-kDa	112 – 660	Burma	
	50 kDa (VLPs)	112 – 534	Burma	
	62-kDa	112 – 660	India	
Expressed in other system				
Yeast Pichia pastoris	HBV/HEV	551 – 607	China	
Transgenic tomato plants	pE2	394 – 607	China	
DNA vaccines				Stability
Naked	pJHEV	1 – 660	Burma	Easy preparation
	pcHEVORF2	1 – 660	Burma	
DNA plus protein	+26 kDa		India	

VLPs: Virus Like Particles

Serological analysis through ELlZA kits for anti-HEV IgM and IgG are available commercially (Cao et al. 2018). Diagnosis of acute HEV infection is performed with a positive serum HEV IgM in the proper clinical setting. Detection of HEV infection is confirmed by PCR of serum or stool samples. Testing through PCR should be the keystone of diagnosis in population; however, in immune-compromised individuals the antibody level is not high enough to produce PCR response (Khuroo and Khuroo 2016). In 2018, EASL (European Association for the Study of the Liver) recommended guideline for both nucleic acid amplification technique (NAT) and serum findings for the diagnosis of acute and chronic HEV infection (Dalton et al. 2014).

HEV Molecular testing

WHO International standards are available since 2011 for nucleic acid testing of HEV genotypes (Baylis et al. 2013). Conventional RT-PCR technique is used for investigation of HEV genotype. The latest marketed commercial HEV viral load assays provide better sensitivity (20–100 IU/ml) and specificity (Mokhtari et al. 2013). Previously, partialORF2 sequences were commonly employed for this purpose. Zhai et al. (2006) determined 306-base pair region of RdRp (ORF1) while completing statistically complete HEV genome bordered by conserved primer sequence (Dalton et al. 2014).

Treatment/Management

Acute Hepatitis E (HEV) infection mainly requires supportive care, as it is self-limiting. Within 6 weeks after onset of infection, the fate of course of Hepatitis E infection is resolved. Victims developing fulminant liver failure require transplantation of liver. Among Hepatitis E chronic patients which are immuno-compromised, amendment in immuno-suppressive medication and use of anti-viral drugs such as peginterferon along with ribavirin is recommended (Khuroo and Khuroo 2016; Kamar et al. 2020).

Ribavirin

Therapy with Ribavirin for the treatment of 9 chronic HEV transplant recipients showed no viral relapse after 5 months (Pischke et al. 2013). In cases of pregnancy, ribavirin is not recommended due to the risk of teratogenicity. The optimal daily dose of Ribavirin is suggested to be between 200 and 1,200 mg for less than 3 months according to virological response, depending on case situation (Koning et al. 2013). Important parameters like RNA clearance of HEV in plasma and ALT stabilizing to normal levels are monitored during the course of therapy (Versluis et al. 2013; Koning et al. 2013).

Peg interferon

HEV infection in immune-compromised patients is treated with Pegylated interferon-alpha-2a (Peg-IFN-α-2a) and oral ribavirin (Dalton et al. 2014).

Hepatitis E vaccines

At present for the prevention of Hepatitis E, commercial vaccines are under trial, which are of two types (depicted in Table 2).

1- Recombinant vaccines
2- Subunit HEV vaccines

HEV vaccine candidates, including recombinant neutralizing epitope protein in models of mouse and insect larvae-derived recombinant hepatitis E virus ORF-2 proteins, are being explored for their efficacy (Zhao et al. 2016). A vaccine of 56 KD and HEV239 vaccine are under 2nd and 3rd phase of clinical trial (Zhu et al. 2010). HEV239 vaccine (Hecolin) is commercially presented in China (Nelson et al. 2014; Li et al. 2015), as mentioned in Table 2.

HEV antigen in insect cell

The HEV capsid antigen was expressed in Baculovirus-infected insect host system. The 56 kDa candidate vaccine showed highly immunogenic effects in rhesus monkeys in 6 to 12 months (Cao et al. 2018).

Proteins expressed in yeast

HEV ORF2 protein is expressed in yeast after truncation. Research expressed truncated genotype IVHEV ORF2 (aa112–607) in a system of yeast Hansenulapolymorpha. The protein which was truncated exhibited high immunoreactivity, which provided the base for the

Table 3 : To date vaccines used for hepatitis E (Zhao 2016).

Study of HEV	Region	Patient status	Feotal out comes	Protocol
HEV vaccine	China 2007 -2009	Pregnant women (No pre term delivery) Elective abortion 151% , natural abortion.	No congenital anomalies	HEV 239 (30µg /per dose) at interval 0, 1, 6 month

Proteins expressed in yeast

HEV ORF2 protein is expressed in yeast after truncation. Research expressed truncated genotype IVHEV ORF2 (aa112–607) in a system of yeast Hansenulapolymorpha. The protein which was truncated exhibited high immunoreactivity, which provided the base for the further advances in hepatitis E recombinant vaccine development. Among plants, tomato is used as a carrier HEV ORF2 fragment. The plant binary expression vector p1301E2 was introduced into agrobacterium tumefaciens EHA105.87 for developing vaccine (Cao et al. 2018).
Up till now China has given shots of vaccine among pregnant patients of Hep E. Outcomes are shown in Table 3.

Complications

Several patients may develop complications like hepatic failure, cholestasis, jaundice or chronic hepatic failure. Some extrahepatic manifestations for HEV genotypes, such as encephalitis, peripheral neuropathy, Guillain-Barre syndrome and ataxia, are being studied (Kamar et al. 2011). Yet vaccines have shown good results in their last trials.

Conclusions

In modern era, world has turned into a global village, hence HEV infection prevalence is not confined only to developing countries. Highly developed countries also suffer from endemic and zoonotic diseases. Following few aspects must be focused to develop guidelines for the intervention and management of Hepatitis E:

- Immune and hormonal factors of patients
- Genotypic heterogeneity of HEV
- HEV variants virulence
- Screening of voluntary blood donors (VBDs)
- Hygiene and healthy intervention

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SOME IMPORTANT VIRAL DISEASES OF FARMED FISH

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INTRODUCTION

Aquaculture is the rearing and harvesting of aquatic organisms like fish and shellfish, mollusks, crustaceans and aquatic plants. With the increase in human population, the demand for animal-based proteins is increasing. Fish is an important source of animal proteins and around 354 species of fish are cultured around the globe for human consumption (FAO 2012). Carps are mainly cultured in Asia, especially China, members of salmonids are cultured in Europe and South America, while tilapias are mainly cultured in Africa and Asia (Hall 2011). As the demand of fish increases, the aquaculture practices shift from extensive to intensive culture. High stocking density in intensive culture practices may lead to chronic stress in fish (Yada and Nakanishi 2002). If not managed properly, stressed fish are more prone to infectious diseases. Viral diseases are increasing rapidly in aquaculture, causing huge economic losses.

Viruses are infectious organisms that have a genome (a property of living organisms). Viruses are inactive outside the host body, once they enter the host, they become functionally active and exploit the host machinery to produce their protein, genome, and RNA that assemble and makes new viruses. According to "International Committee on Taxonomy of Viruses" (ICTV), genome of about 400,000 viral strains has been sequenced (Sharma et al., 2015). Viruses are classified into various hierarchical levels, *i.e.* order, family, genus and species. Some viruses are diverse, so they are further classified in to subfamilies. First letter of viral order, family, sub-family and genus is capital and italicized. Viruses are classified based on (i) structure- presence and absence of envelope, shape, size and capsomers, (ii) viral genome- DNA or RNA viruses, single stranded or double stranded, positive sense stranded or negative sense stranded, (iii) mode of replication- viruses replicating in host nucleus using cytoplasmic enzymes or viruses replicating in host cytoplasm using their own enzymes, and (iv) presence of enzymes.

Standard virological procedures are used to isolate and identify viruses from aquatic organisms. Viral isolation is the most sensitive and crucial phase; after isolation viruses are identified using "gold standard" procedures like PCR and RT-PCR (Goodwin et al. 2010). Viruses are then cultured in fish cell lines.

Fishes are susceptible to many viral diseases, most of which are yet to be discovered. Viruses that affect aquacultural important fish species are listed in Table 1. In this chapter, some important viral diseases, with special emphasis on the disease management and prevention, have been discussed.

Infectious Pancreatic Necrosis (IPN)

Infectious pancreatic necrosis (IPN) is caused by the infectious pancreatic necrosis virus (IPNV), which is a double-stranded RNA (dsRNA) virus, belonging to the family *Birnaviridae* (Leong et al. 2000; ICTV 2014).

Infectious pancreatic necrosis (IPN) is a well-known disease that was first named as acute catarrhal enteritis by M'Gonigle (1951), but was renamed as Infectious Pancreatic Necrosis by Wood et al. (1955) on the basis of histopathological analysis of brook trout (*Salvelinus fontinalis*) suffering from an infectious disease that resembled catarrhal enteritis. Infectious pancreatic necrosis virus (IPNV) belongs to the family *B*irnaviridae and the genus *Aquabirnavirus.* It is the first double-stranded RNA (dsRNA) virus of fish which was isolated *in vitro* (Sano and Okamoto 2017). The IPNV mainly infects salmonids, especially Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and other species of salmon (*Oncorhynchus spp.*) (Smail 2012).

Clinical signs and transmission

Affected fish are usually sluggish, show a darker coloration, and swim abnormally. Fish infected with the virus also have a distended belly, milky substances and mucus in the anterior intestine, with no food in the stomach. Long, thin, whitish fecal casts are common in fish (Reno 1999). The liver, spleen, heart, kidneys and gills are normally paler than the rest of the body. Blood spots may appear on visceral fat along the intestine and also on the ventral fins. The fluid in the body cavity may become bloody as well (Dopazo 2020). Hemorrhages of the visceral organs are usually visible in fish infected with IPNV (Munro and Midtlyng 2011). Sudden increase in mortality of fish fry and fingerlings is characteristic of IPN infection (Jensen and

Kristoffersen 2015), however, mortality depends on age, physiology and developmental stage of host and also on the environmental conditions (Munro and Midtlyng 2011). Viral infection in stressed fish is more virulent (Gadan et al. 2013). Even if the fish survives the IPN infection, it may contain virus without showing any clinical signs and serve as carrier that transmits infection either horizontally through urine and feces or vertically through eggs and sperms (Roberts and Pearson 2005). Figure 1 showed rainbow trout suffering with IPNV.

Prevention and control

Spread of the IPN virus can be prevented by the use of virus-free water, disinfection of fertilized eggs, purchase of disease-free stock, and strict biosecurity steps. Treatment of incoming water with UV is a suitable preventive measure. Despite the ability of the virus to survive, a variety of chlorine (5-minute treatment at concentration of 30 ppm) and iodine-based disinfectants, as well as per oxygen compounds (10-minute treatment with NaOH pH 12.5 or 5-minute treatment with 3% formalin), have been shown to be useful against viral infection (OIE 2003). Younger fish can be protected with an oral vaccine.

Infectious Hematopoietic Necrosis (IHN)

Infectious hematopoietic necrosis (IHN) is caused by a single stranded RNA virus (infectious hematopoietic necrosis virus-IHNV) of genus *Novirhabdovirus* and family *Rhabdoviridae*. This virus causes disease in salmonids including Pacific and Atlantic salmon and trout (rainbow and steel head trout). This virus causes necrosis in the hematopoietic tissues of the fish, therefore named as infectious hematopoietic necrosis virus (Amend et al. 1969).

Clinical signs and transmission

All stages of fish are susceptible to viral infection. Fish become lethargic and infected fry has pale gills, dark coloration and distant abdomen. Hemorrhages are usually visible on the vent, fins, base of gills and mouth. Juvenile and adult fish have less clinical signs, the most common sign being hemorrhage near the vent, gills and eyes. Many adult fish succumbed to virus without showing any clinical signs. Both horizontal and vertical transmission of the virus is seen (Bootland and Leong 1999). Virus enters the fish through the base of the vent, gills and skin to stomach or intestine and then goes to internal organs, where it causes hemorrhages. Spleen and liver are the first organs to become severely infected with the virus. Ultimately, the virus reaches the circulatory system and causes hemorrhages, resulting in anemia. A farmed rainbow trout infected with IHNV is shown in Figure 2.

Prevention and control

Prevention strategies to avoid IHNV infection involve use of virus free water, disinfection and quarantine of new fish (Meyers et al. 2003; Breyta et al. 2016). Iodine based disinfections *e.g.* iodophor are effective against IHNV. Intramuscular injection of DNA based vaccine (APEX-IHN by Novartis Animal Health Canada Inc.) is used to control IHVN infections.

Table 1: Some important viral diseases of farmed fish and their aetiological agents

Disease	Causative agent/ aetiological agent	Family	Genra	Viral genome
Pancreatic necrosis (IPN)	Infectious pancreatic necrosis virus (IPNV)	*Birnaviridae*	*Aquabirnavirus*	Double stranded RNA virus
Infectious hematopoietic necrosis (IHN)	Infectious hematopoietic necrosis virus (IHNV)	*Rhabdoviridae*	*Novirhabdovirus*	single-stranded RNA
Viral hemorrhagic septicemia (VHS)	Viral hemorrhagic septicemia virus (VHSV)	*Rhabdoviridae*	*Novirhabdovirus*	single-stranded, negative-sense RNA
Epizootic hematopoietic necrosis (EHN)	Epizootic hematopoietic necrosis virus (EHNV)	*Iridoviridae*	*Ranavirus*	Double stranded DNA
Infectious salmon anemia (ISA)	infectious salmon anemia virus (ISAV)	*Orthomyxoviridae*	*Isavirus*	Single stranded RNA virus
Spring viraemia of carp (SVC)	spring viraemia of carp virus (SVCV)	*Rhabdoviridae*	*Sprivivirus*	Single-stranded RNA virus
Channel catfish viral disease (CCVD)	Ictalurid herpesvirus 1 (IcHV1, commonly known as channel catfish virus, CCV),	*Alloherpesviridae*	*Ictalurivirus*	Double stranded DNA virus
Largemouth bass Viral Disease	largemouth bass virus (LMBV)	*Iridoviridae*	*Ranavirus*	double-stranded DNA-viruses
Koi herpesvirus disease (KHVD)	Cyprinid herpesvirus 3 (CyHV-3)	*Alloherpesviridae*	*Herpesvirus*	Double stranded DNA virus
Viral nervous necrosis (VNN) or Viral encephalopathy and retinopathy (VER)	Viral nervous necrosis	*Nodaviridae*	*Betanodavirus*	Two single stranded RNA-virus
Red sea bream iridoviral disease (RSIVD)	red sea bream iridovirus (RSIV)	*Iridoviridae*	*Megalocytivirus*	Double stranded DNA

Viral Hemorrhagic Septicemia

Viral hemorrhagic septicemia virus (VHSV), a single stranded RNA virus of *Rhabdoviridae* family and genus *Novirhabdovirus,* is the causative agent of viral hemorrhagic septicemia (VHS) (Baillon et al. 2020). Two closely related viruses, viral hemorrhagic septicemia virus and infectious hematopoietic necrosis virus are most economically important rhabdovirus, belonging to genus *Novirhabdovirus*.

First reports of viral hemorrhagic septicemia were from Europe and Japan, where it infected rainbow trout. Later, wild fish collected from Baltic Sea, Pacific and Atlantic oceans were also infected with VHS. Korea has also been hit by this virus (Hedrick et al. 2003). Infection of VHS from Great Lakes of North America was first reported in 2005. This virus infected almost 50 freshwater and marine species of Northern hemisphere (Faisal et al. 2012). The VHSV infects a wide range of fish which belong to Salmoniformes (trout and salmon), Scorpaeniformes (Sticklebacks and rockfishes), Gadiformes (cod), Clupeiformes (anchovy and herring), Pleuronectiformes (soles and flatfishes) and Osmeriformes (smelt).

Clinical signs and transmission

Hemorrhages on the body, gills, eyes or at the base of the fins are common in VHS-infected fish, along with the swollen (fluid-filled) abdomens, bulging eyes ("pop-eye"), lethargy and darkened coloration (OIE 2105). Infected fish can exhibit unusual swimming patterns. Hemorrhages in the muscle and organ tissues are also possible. Some of the VHS-infected fish display no symptoms (asymptomatic), but they can infect other fish and spread the disease (horizontal transmission) (Groocock et al. 2007). Disease outbreak mostly occurs in spring season when water temperature is less than 5°C (59°F). Fish mortalities due to VHS are uncommon at temperatures above 18°C (64°F). Once the infection spreads, mortality rate becomes very high. Mass mortality up to 80-100% has been reported after VHS infection. Healthy fish become infected by uptake of virus through gills. Horizontal transmission is common mode of spread of VHS infection (Al-Hussinee et al. 2016). Common histopathological lesions in fish infected with VHS are degeneration and necrosis of spleen, liver and kidneys.

Prevention and control

Viral Hemorrhagic Septicemia is a highly contagious, and the best practice to limit the outbreak is use of quarantine. VHSV has been shown to be transmitted from wild to farmed fish and vice versa. Fish health monitoring systems, as well as eradication and fallowing are currently used as control methods. In certain parts of Europe, the VHS has been eradicated thanks to these procedures. If farm ponds are not dried and disinfected, VHSV can survive for a long time at the bottom of the pond (OIE 2015).

Many popular disinfectants, such as iodophor, formalin disinfectants, sodium hypochlorite and sodium hydroxide, are used for VHSV. The efficacy of lime disinfection is still debatable. When disinfectants are dissolved in seawater, their virucidal activity is decreased. Irradiation of inflow water for hatcheries and recirculation system with UVC (280-200 nm wavelength) is very effective against VHSV. As this virus is quite thermostable, drying and a pH of 2.5 or 12.2 can inactivate this virus (Bovo et al. 2005).

Epizootic Hematopoietic Necrosis (EHN)

The causative agent of epizootic hematopoietic necrosis is epizootic hematopoietic necrosis virus (EHNV). This virus belongs to the family *Iridoviridae*, which includes four genera; *Iridovirus*, *Lymphocystivirus, Megalocytivirus,* and *Chloriridovirus* and six species; Epizootic hematopoietic necrosis virus, Santee-Cooper ranavirus, Ambystoma tigrinum virus, Frog virus 3, Bohle irido virus and European catfish virus. This virus cannot be identified through conventional antibody-antigen interaction assays. The identification of this virus is through host specificity, protein profiling of virus, DNA sequence analysis and RFLP (Wolf 1988).

The genome of EHNV is 127kb. With the diameter of 175nm, this is a massive icosahedral virus with a double-stranded DNA. Replication of this virus is in the nucleus and cytoplasm and the virus is assembled intracytoplasmic. The virus gets its outer limiting membrane by budding from the plasma membrane of the host cell. An internal bilayer of lipids, which is identical to FV3, surrounds the nucleoprotein center of the inner capsid (Yan et al. 2009) and comprises a circularly permuted and terminally redundant genome.

The virus was first reported in juvenile redfin perch (*Perca fluviatilis*) in 1985 from Victoria, Australia. EHNV was the first virus to be isolated from freshwater fish from Australian region (Langdon and Humphrey 1987). Later, it was isolated from farm-raised rainbow trout (*Oncorhynchus mykiss*) (Langdon et al. 1988). While red fin perch is most vulnerable to the viral infection, this virus also infects many other fish species. The geographical range of this virus is restricted and it is mostly endemic to south-east Australia (Victoria, South Australia and New South Wales).

The varying level of infection and mortality was reported in mosquito fish (*Gambusia affinis),* Australian bass (*Macquaria novemaculeata*), Atlantic salmon (*Salmo salar)* and other species of perch like Macquarie (*Macquaria australis)*, golden (*Macquaria ambigua)* and silver perch *(Bidyanus bidyanus)* raised under laboratory conditions (Langdon 1989).

Clinical signs and symptoms

Pathology of the disease involves necrosis of hematopoietic tissues, as the name suggests. Infected fish displays multifocal necrosis of the liver, spleen, renal hematopoietic tissue, atrial trabeculae and gastrointestinal epithelial cells. Hyperplasia of epithelial cells of gills can also be seen after viral infection. Infected cells have intra cytoplasmic inclusion bodies, which are

Figure 1: Rainbow trout infected with IPNV. Fish at the top shows normal belly, while fish at the bottom is infected with IPNV, showing swollen distended belly. Taken from Tamer et al. (2020). "Pathogenicity trials regarding Turkish isolates of infectious pancreatic necrosis virus and viral hemorrhagic septicemia virus in rainbow trout" Used with permission: courtesy C. Tamer.

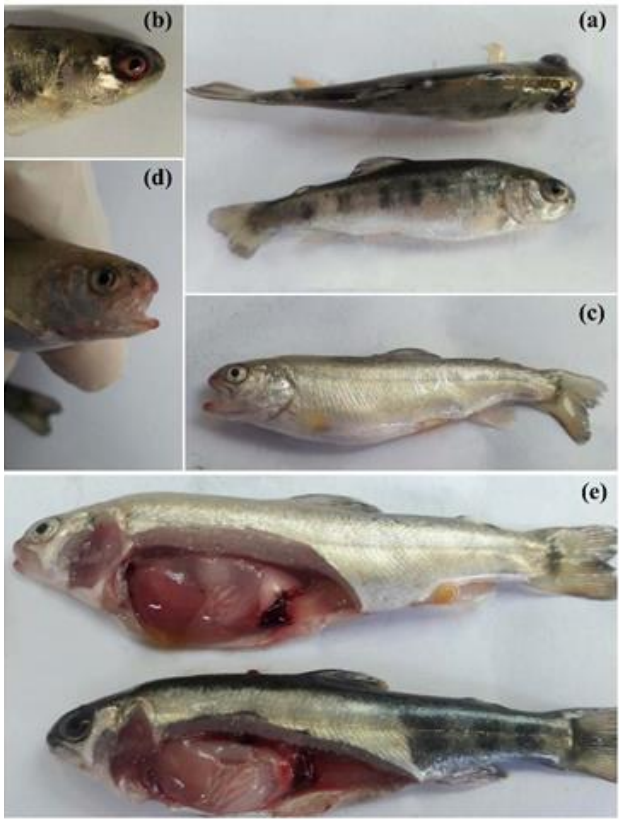

Figure 2: Gross pathological signs of IHNV infected fry trout showing skin darkening (a and b), abdominal distension (a & c), exophthalmia (a & b), hemorrhage of the eye (a and b), pale gills (e), ulceration of the snout (c and d) and visceral pallor with yellowish fluid in the intestine. Taken from Ahmadivand et al. (2017). "Infectious hematopoietic necrosis virus (IHNV) outbreak in farmed rainbow trout in Iran: Viral isolation, pathological findings, molecular confirmation, and genetic analysis". Used with permission: courtesy S. Ahmadivand.

basophilic in nature and are a distinct microscopic characteristic of EHNV infection. These structures are virus assembly sites, where para-crystalline arrays can be seen by electron microscopy (Whittington et al. 2010). Swim bladder oedema and ulcerative dermatitis can only be seen in rainbow trout after EHNV infection (Reddacliff and Whittington 1996).

Prevention and control

EHNV is highly resistant to drying and may live for months in water and may survive for at least a year in the frozen fish carcasses and tissues (Langdon 1989). There are currently no vaccines available for the management of EHNV. Control of infection and spread on-farm is managed by active surveillance of fish health, good husbandry practices, minimizing physiological stressors and up to the mark bio-security measures. Destocking and disinfection according to OIE protocols (Wolf 1988) help to avoid reinfection on contaminated properties.

Infectious Salmon Anemia

A single-stranded RNA virus of the *Orthomyxoviridae* family causes infectious salmon anemia (ISA). The viral infection was first reported in Norway in 1984. Since then, it has been reported from Chile, Canada, Ireland, Scotland, the Faroe Islands and the United States of America (Geoghegan 2002). Infection with this virus results in a systemic and lethal disease, characterized by extreme anemia, necrosis in multiple organs and variable hemorrhages (Geoghegan 2002, Godoy et al. 2008). Even though the disease takes a long time to develop and has low daily mortality (0.05–0.1%) rate, but overall mortality and economic loss increase as the virus spreads. Exposure to seawater or rearing fish in sea cages increases the chances of exposure to virus. Farmed Atlantic salmon is more susceptible to natural outbreaks of ISA, but the virus has the potential to infect variety of farmed and wild salmonid species (Kawaoka et al 2005).

Clinical signs and symptoms

Infectious salmon anemia is a systemic disease, which mainly affects the fish circulatory system. Fish infected with ISA becomes sedentary, swim near the surface and losses appetite. Internally, liver and gut become dark in color due to hemorrhaging, while gills become pale due to anemia (Figure 3). Blotted kidney, pop-eye, swollen belly, and blood spots in the eye chamber are common in infected fish. In the early stages of infection, hemorrhages, circulatory failure and severe anemia are common.

Prevention and control

The main mode of disease transmission is horizontal transmission of the virus. In Norway and elsewhere, well boats have been linked to the spread of the disease. The ISA virus is resistant to a variety of disinfectants, including ozone, sodium hypochlorite, formic acid, and ultraviolet light (Snow et al. 2009). Vaccine against ISA is used in the Faroe Island, Chile and United States of America. Europe has been declared ISA free and therefore, use of vaccine is not allowed.

Figure 3: Fish infected with infectious salmon anemia. Hemorrhagic liver and spleen, pale gills. Taken from Health situation in Norwegian aquaculture. Used with permission, Courtasy Geir Borno.

Figure 4: Clinical signs of VNN: Inflation of swim bladder (left) and abdominal extension (right) in infected *Liza aurata* in Caspian Sea. Taken from Zorriehzahra (2020). "Viral Nervous Necrosis Disease". Used with permission: Courtesy MJ Zorriehzahra.

Spring Viraemia of Carp

Spring viraemia of carp virus (SVCV) is the causative agent of spring viraemia of carp (SVC) disease. It is a Rhabdovirus that causes significant infection in carps, cyprinids and ictalurid fish species (Dixon 2008). This virus belongs to genus *Sprivivirus* in the virus family *Rhabdoviridae*. This virus is highly resistant at low temperature and can tolerate a wide range of pH (Ahne 1976).

The virus can enter the fish through gills and abruptly affects other vital organs such as spleen, liver, kidneys and gut. Virus can be physically spotted in fish urine, faces and in a water body. Small fish are most vulnerable to the disease; however, virus can affect fish of all age groups. Intensity of infection may vary with age and age-related innate immunity. This virus can easily transfer through crustacea and from members of annelids and hirudinea. Virus can simply transfer from infected to healthy fish (Ahne et al. 2002). Virus is usually not transferred through eggs, but it is necessary to disinfect eggs with iodophor. Disease is particularly spread in winter season, when the temperature is between 15-17°C.

Clinical signs and symptoms

Spring viraemia of carp is difficult to identify because symptoms are non-specific and not all fish show all symptoms. Most commonly observed signs are distended abdomen with hemorrhages. Fish infected with SVCV swim erratically and slowly. Loss of balance is another common sign of infection. Abdominal distention may be due to accumulation of fluid which may be clear or bloody. Oedema of internal organs and enlarged spleen is usually observed. Hemorrhages on gills, skin and eyes are common. Farms with SVC outbreaks have rapid onset of fish mortality.

Prevention and control

The research trial showed that DNA vaccination can save fish from serious theatres of SVC virus (Emmenegger and Kurath 2008) but further research is required to implement this idea in carp culture at commercial level. Ponds and handling equipment should be disinfected at regular intervals. High density and poor management increase the chance of infection. In 2002, it was reported that virus caused loss up to 70 percent population of young carps in Europe and 20 percent population died in USA (Ahne et al. 2002).

Channel Catfish Viral Disease

Channel catfish virus disease (CCVD) is caused by *Ictalurid herpesvirus,* which affects the Channel catfish species (*Ictalurus punctatus).* The virus was reported first time in southern United States of America and infection spread in the fish industry in mid1960 (Fijan et al. 1970). However, status of CCVD infection in other countries is unknown, though some cases were reported in Honduras and Russia following shipment of fry from USA (Plumb 1994).

CCVD can be easily spotted from infected fish and carrier bodies, however, it is difficult to find out the chain of coming off infection. The virus is sufficiently present on kidneys, gills, skin, spleen and intestine during the dominant stages of infection (Kancharla and Hanson 1996). Moreover, virus genetics analysis showed that it has capacity to transform in to new strains (Vanderheijden et al. 1999). Transmission of virus can be vertical and horizontal, but horizontal transmission is the most obvious and pathway of vertical transmission is vague, as no signs of infection were detected in the sex organs. Hedrick et al. (1987) explained that chance of infection is greater in fish which are less than one year or four months in age. The second most important factor is the temperature of water body; it has been noticed that tendency of infection is severe and mortality rate was high at 27°C and dramatically reduced below 18°C.

Clinical signs and symptoms

The necropsy examination of infected fish revealed the presence of excessive blood in peritoneal cavity with yellow or light reddish fluid. Liver and kidneys may be paled and spleen is narrowed and darkened in color. Yellow-coloured mucus is present in intestine instead of food. Oedema, necrosis, hemorrhages, accumulation of macrophages are noticed in hematopoietic tissues, nephrons, liver, kidneys, intestinal tract and in cardiac tissues (Major et al. 1975).

Virus can be easily detected by immunosorbent assay (ELISA) or through polymerase chain reaction (PCR). As during infection new proteins and virus are not produced, antigen-antibody (ELISA) test is not suitable; it is better to do PCR for genomic DNA test.

Prevention and control

Virus outbreaks can be minimized through adopting some precautionary measures, such as low stocking density and avoiding stressful handling. The fish should be totally isolated during developmental stages from carrier fish or infected fish. Epizootic capacity of disease increases in stress and overcrowded environment (Thompson et al. 2005). Currently, no vaccine is available in the market. This virus drastically affects the economy and is responsible for closure of many catfish farms in USA.

Pike Fry Rhabdovirus Disease

Pike fry rhabdovirus disease, which is abbreviated as PFRD, mainly affects the pike ((*Esox lucius*). This virus caused severe infection in fry and juvenile stages of Netherlands hatchery in 1965. The disease was named as hydrocephalus' and red disease by Bootsma (1971). The virus is geographically limited to Europe, especially in Germany and Hungary.

Nucleotide sequencing revealed that the virus which affects the pike belongs to Geno-group III and other three geno-groups of this virus affect the other fishes (Stone et al. 2003). Haenen and Davidse (1993) described that early age stages of fish make them more susceptible for the infection, while sexually mature fish have complete resistance against the viral infection.

Pike fry rhabdovirus (PFR) is closely related to spring viraemia of carp virus (SVCV); because of this closed resemblance, it is difficult to isolate the PFR from SVCV through serology. Enzyme linked immunosorbent assay (ELISA) can be used to separate these two pathogenic strains, however nucleotide sequence analysis is the best method for identification of PFR (Stone at al. 2003).

Clinical signs and transmission

The pike fry rhabdovirus causes hemorrhages in connective tissues, with accumulation of fluid in the ventricle. Proximal tubules of kidneys show severe degeneration, with petechiae in the spinal cord and optic tectum (Bootsma 1971).

The most obvious and significant symptom is that fish schooling behavior is disturbed; the affected fish move very slowly at surface or present at the bottom motionlessly. Slight swelling behind the eyes, pale gills, popped eyes, distended belly and hemorrhages on fins and skin can easily be observed.

The sources of pike fry rhabdovirus are indefinite, while transmission can be possible through injection, and when injected to eggs it can cause hundred percent infection. The virus also be transferred from one organism to other organism through predation or cannibalism (Ahne 1985). The previous reports do not show any significant economic loss recorded due to pike fry rhabdovirus infection.

Koi Herpesvirus Disease

Common carp (*Cyprinus carpio)* is a global aquaculture species. Many strains are also raised for ornamental industry, where it is known as koi. Koi herpesvirus disease is caused by a highly infectious koi herpesvirus (KHV), also known as cyprinid herpesvirus 3 (CyHV3). First case of KHV was reported in 1996 from UK (Haenen et al. 2004). Since then, many cases have been reported from almost all the countriesthat culture koi carp, except Australia (Pokorova et al. 2005).

Viral genome is double stranded DNA that belongs to family *Alloherpesviridae.* Temperature of the water body affects the virulence of the KHV. Virus infects fish of all ages at the water temperature between 16-25°C, with the mortality range of 80-100% (Haenen et al. 2004). Like other herpesvirus, KHV can survive in infected fish for a long period of time. Fish that survive the KHV infection carries the virus and acts as carrier (Petty and Fraser 2005; Eide et al. 2011).

Clinical signs and transmission

The symptoms of KHV infection are mostly non-specific. Behaviorally, infected fish gather at the water surface, gasping, disorienting, and swimming erratically, and mortality rates begin to rise. In the early stages of KHV infection, there is a lot of mucus secretion. Externally, the most noticeable symptoms are white necrotic patches, light coloration and hemorrhages in the gills. During clinical infections, KHV is most prevalent in the gills, spleen and kidneys. Infected populations can experience rapid mortality, which starts within 24-48 hours of the onset of clinical signs. Under experimental conditions, high mortality rates up to 82% were recorded within 15 days after virus exposure at temperature of 22°C (Ronen et al. 2003). The most common clinical signs of infection by KHV are gill lesions that can be severe, resulting in gill mottling with red and white patches. Necrosis of the gill tissue results in the white patches. Pale patches on the skin and sunken eyes are some other external symptoms of KHV infection. A notched nose is also seen in some koi carp infected with KHV (Goodwin 2012). The KHV infection is mostly accompanied with secondary parasitic and bacterial infections, which can exaggerate/hide and overlap the viral symptoms.

Prevention and control

KHV does not have any medication. KHV and other viral diseases in cultivated fish are not treatable with antiviral drugs. According to the previous studies, rising water temperature of the fish holding bodies to 30°C can improve survival rate of fish (Ronen et al. 2003), but the increasing water temperature can increase the incidents of other parasitic and bacterial diseases. Treatment of the

water with 200 ppm of chlorine for one hour is usually effective. Quaternary ammonium compounds (QACs) are another choice for disinfection of aquaculture systems and equipment. Treatment of 500 ppm of QAC for one hour is effective against KHV infection (Noga 2000).

Viral Nervous Necrosis (VNN)

The etiological agent of viral nervous necrosis is Betanodavirus (Ball et al. 2000), one of two genera that make up the *N*odaviridae family (viral encephalopathy; VNN & VER retinopathy). Betanodavirus are small, non-enveloped virus. The capsid is present which is octahedral in shape. Their genetic material is single stranded, positive senses RNA.

This disease was first observed in Australian farmed barramundi (Munday et al. 2002). This virus infects almost 40 species of freshwater and marine fish in different parts of the world (Munday et al. 2002). South and East Asia, UK, Ngorway, Europe, Oceania and North America are all affected by viral nervous necrosis. To date, no cases of the virus have been recorded in South America.

Betanodavirus has the following four different species of virus (ICTV 2009), characterized by the species they infect.

i. Redspotted grouper nervous necrosis virus (RGNNV), causing disease in red spotted grouper
ii. Barfin flounder nervous necrosis virus (BFNNV)
iii. Striped jack nervous necrosis virus (SJNNV)
iv. Tiger puffer nervous necrosis virus (TPNNV)

Nervous necrosis virus (NNV) virions are small in size, with diameter ranges from 25-30 nm, non-enveloped, spherical; their genome is made up of double positive sense RNA molecules, complete sequences of which have been regulated (Tan et al. 2001).

Clinical signs and transmission

This disease is characterized by vacuolating the necrosis of neural cells in the retina, brain, and spinal cord.The virus can kill larval and juvenile fish up to 100% of the time, as well as cause major losses in fish. Their pathology is well defined (Munday et al. 2002; Tanaka et al. 2004), but viral characterization analysis was delayed until the virus was isolated and expanded in the SSN-1 cell line (Frerichs et al. 1996), and our knowledge regarding the biology of virus has rapidly expanded since then. Horizontal transmission of NNV is through maternal fluids on the egg surface (Kai et al. 2010). Figure 4 shows inflation of swim bladder and abdominal extension of *Liza aurata* suffering from VNN.

Prevention and control

Good husbandry practices and various necessary biosecurity protocols can limit the chances of viral infection. Inactivation of virus resulted after treatment with a solution of sodium or calcium hypochlorite (50 ppm), iodine, or benzalkonium chloride. A short exposure for around 2.5 minutes with ozone (0.1 µ g/mL) was also helpful in inactivating the virus (Kasai et al. 2002).

Conclusion

With the expansion of aquaculture and the use of new techniques for fish breeding and culture, the production of animal-based proteins has increased tremendously. Stocking densities in semi-intensive and intensive cultures are high. If culture system is not managed properly, it can lead to stressful conditions and the fish under stress are more prone to infectious diseases. Viral diseases are responsible for huge economic loses to the fish industry worldwide. Some important viral diseases were discussed in this chapter. Viruses are transmitted both vertically and horizontally. Best practice to avoid infection is its prevention. Use of sanitary conditions, proper quarantine and hygiene is the key to prevent viral infections. Chemical disinfectants and UV radiations, as discussed above, can limit the spread of the virus. Vaccines are used for the prevention and control of viral diseases. The most common way of vaccine administration is through intraperitoneal injection. However, injecting larval fish is not feasible, but quite laborious. Another way of vaccine administration is bathing which is used to vaccinate large numbers of fish without stressing them. Protection provided by bath vaccines is for limited time, which is the important limitation of this method. Moreover, vaccines against some viruses are yet to be developed. Recently, use of medicinal plants having anti-viral properties has been evaluated for the treatment of viral diseases. This approach is eco-friendly with minimum foot prints; however, more research is needed in this regard.

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EPIDEMIOLOGY AND IMMUNOPATHOLOGY OF RABIES VIRUS

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INTRODUCTION

Rabies virus (RABV) belongs to the family *Rhabdiviridae*, and genus *Lyssavirus*. This pathogen is oftent cited in literature as a 'cunning virus' because it produces severe neurological signs and symptoms, that ultimately lead to encephalitis and death (Jackson 2010; Mani and Madhusudana 2013). Rabies is an ancient zoonotic disease and a highly underestimated public health problem of the developing world. It is also the most feared infectious disease and a top priority neglected tropical disease of poor and marginalized areas (Hampson et al. 2010; Mani and Madhusudana 2013). Research studies have focused on the host-cell response of RABV, using *in vitro* and *in vivo* models to explain how RABV causes neuronal dysfunction instead of cell death. The molecular interface between RABV and the host-cell immune system is relatively complicated as compared with other viruses of different genera. Specific genes of RABV play key roles in attaching the viral coat to the host-cell surface, followed by transcription, translation, and release of new viral particles via clathrin-mediated endocytosis with the help of an actin-microtubule cytoskeleton. Hence, understanding of the virology and specific features associated with RABV genes are integral to envisage the immunological properties and the pathways to produce pathogenesis. In this chapter, epidemiological characteristics of RABV are described to understand spatio-temporal distribution and clinical profiles of this virus. Additionally, important host-cell responses, such as production of interferon, neuronal and mitochondrial dysfunctions, apoptosis, and production of Negri-bodies through evasive strategies of RABV are also discussed.

Historical Perspective of RABV

The word 'rabies' has been originated from the Greek word 'Lyssa', and it has also been often written in literature as *Lussa*, *Lytta*, or *Lutta*. Another finding reported that the word 'rabies' originated from a Sanskrit or Latin word called *Rabbahs* or *Rabere,* meaning 'fierce'. Moreover, the word 'Lyssa' has also been derived from 'Lykos', meaning 'wolf'. This idea was dominated during the 30th century BC, which is also known as the Indian Vedic period (Geison 1978; Schneider et al. 1988).

Interestingly, rabies is the most ancient zoonosis of humans and there are many variations and controversies in delineating the actual and precise history of RABV in scientific literature and encyclopedias. The ancient etymologists have reported that the basic origin might have been from 'Lysis', meaning decomposition. However, early scientists used to describe lyssa in various forms, such as furious expressions, madness, or lyrical songs. Hence, an authentic study has not been able to define the literal origin of the word 'rabies'. Hippocrates were the first, who used the already existing word (rabies) to explain the term 'hydrophobia' or fear of water (Steck et al. 1982).

In ancient periods, people believed in many superstitions, such as considering furious dogs as a 'god of death' (Geison 1978; Schneider et al. 1988). A person infected with RABV would eat shearwater liver, gentian, or river crabs as an attempt of postexposure prophylactics to prevent rabies. These bizarre and ignorant remedies were considered as treatment of rabies. In severe cases of dog bites and suspected rabies, cauterization of the victim's skin was also a thoughtful treatment. In brutal and outlandish acts examples, the tongue of a rabid dog was removed and pinched under the foot or toes of an infected victim so that he/she may get rid of this disease. In addition to these, chicken feces were also offered as a remedy to rabies in suspected dogs. If any of these methods had not proved successful, then the victim had to be thrown in shallow water to let him/her drink plenty of water to overcome the signs of rabies (King et al. 2004). Various strange, but unsuccessful, treatments had developed that usually included the manipulations of blood samples from the dog bite wound. The fear of rabies was overwhelming because of unknown multiple reservoirs and the absence of credible treatment (Steck et al. 1982; Dunlop 1996; King et al. 2004).

The first major rabies epizootic was reported among foxes and dogs in North America in 1768. By 1771, the disease had spilled over from the canids to domestic animals and swines (Dunlop 1996). Within a few years, the outbreak sprawled to the regions of France, the West Indies, and eventually penetrated throughout North America. The earliest reliable rabies data were found in the Eshnunna Mesopotamian Codex during 1930 BC. It stated that the suspected owner of a pet dog was supposed to acquire control measures to stop the infection. If a rabid dog caused death to other individuals, then the owner of that rabid dog had to pay for the price of the dead victims. During the late 19th century, almost 1,112 humans died of rabies in England (Dunlop 1996; George 2011).

George Zinke demonstrated for the first time in 1802 that rabies might possibly be transmitted from saliva of an

infected dog to a healthy dog (Zinke 1804). In 1879, Pierre-Victor Galtier reported the transmission of an actual case of rabies from one animal to another (Galtier 1879). The fear of horrifying deaths began to decline by the end of 1885, when two French scientists, Emile Roux and Louis Pasteur, successfully introduced the first vaccine against RABV on Joseph Meister, a victim of a rabid dog bite. The vaccine had attenuated or weakened RABV particles, which were harvested from the brain of a rabbit that died of rabies. The components of the vaccine were also weakened by keeping the vials under dry conditions for five to ten days (Geison 1999).

Global Epidemiology

In 2009, raccoons, skunks, and bats were reported to be responsible for 98% of rabies cases in America (Blanton et al. 2009). At present, dogs are the principal reservoir of RABV and account for 99% of rabies cases in Asian and African states (Hampson et al. 2015). Globally, rabies causes approximately 59,000 human deaths, with the highest death toll in Asia (59.6%), followed by Africa (36.4%) and America (0.05%). The economic and social cost values of the prophylactic and control measures are also considerably higher in the developing countries, as shown in Fig. 1 (Hampson et al. 2015). Fig. 1 also shows the highest death rate in Asia, followed by Africa and other countries of the world. Different percentages of costs pertaining to the vaccination, surveillance, animal losses, travel cost, income losses, and premature deaths have also been shown in Fig. 1.

Rabies has influenced almost 150 countries of the globe, and the underdeveloped countries are at the front list. However, few states (such as New Zealand, Japan, Britain, and Australia) have almost eradicated canine rabies by adopting strict serosurveillance and control measures, together with increasing availability of postexposure prophylaxis (Hudson et al. 1996; Mani and Madhusudana 2013). The Caribbean and Latin America have substantially decreased cases of rabies in animals and humans by following the recommended guidelines of the World Health Organization (WHO 2013).

A total of 8,384 rabies cases were recorded in dogs and humans in the United States, which included 33 indigenous cases reported in 1946 (Talbi et al. 2009). In later periods, annual human rabies cases decreased from hundreds to less than a dozen by the end of 2010. Some countries, including Paraguay, Uruguay, Belize, Colombia, Chile, Ecuador, Panama and Costa Rica have not reported any rabies cases in animals nor in humans for the last ten years. Large-scale dog vaccination programs have reduced considerable number of rabies deaths in animals and humans in Indonesia, Sri Lanka, the Philippines and India (Youwen et al. 2016).

The major reasons behind higher death tolls due to rabies in Asia and Africa are climatic conditions, lack of awareness, ignorance towards the use of prophylactic measures, and deficient integrated control strategies at domestic and national levels. Human rabies-related death rates in China are mainly concentrated in the south (Gunagxi, Gunagdong, Hunan, Henan and Hubei provinces), which is a relatively hot region (Yin et al. 2013). In China and neighboring Pakistan, the number of dog bite cases burgeoned higher during summer months (April, May and June). Multiple surveys in these two countries have concluded that the dog bites and incomplete follow-up of prophylactic measures are the major barriers in reducing the concurrent rabies cases (Yin et al. 2013; Zaidi et al. 2013). Kathmandu has previously reported 200 human rabies deaths annually and recorded up to 35,000 stray dogs (Hampson et al. 2015; Tenzin et al. 2015). In Sri Lanka, 100 people die of rabies each year, while in Bangladesh 3,000 human deaths were reported during 2009 (Smith and Rupprecht 2008; Tenzin et al. 2015). In Pakistan, the highest numbers of dog bites were routinely reported in Karachi during 2013, and there were nine people out of one million suffer and die from rabies (Parviz et al. 2004; Zaidi et al. 2013). Figure 2 shows the distribution of rabies cases and costs of various parameters associated with these cases worldwide. Deaths due to rabies may be due to failure in a range of important factors, such as limited dog vaccination, unavailability of travelling cost, animal losses and non-existent surveillance tools etc. The gray area of the Figure also shows countries free from canine rabies, while perhaps some variants of RABV may still have caused sporadic outbreaks of the disease among these canine rabies-free countries (Hampson et al. 2015).

Transmission

The transmission of RABV has been reported through horizontal and vertical (mother to fetus) routes in domestic and wild animals (Jackson 2000; Jackson 2010). It may normally occur through animal bites, whereas transmission through human bites, non-bite exposures such as open wounds, mucous membranes contaminated with RABV-infected saliva, abrasions, or scratches, has also been reported (Srinivasan et al. 2005; Schnell et al. 2010). Microscopically, the transmission of RABV occurs through transcytosis, which is a “cell to cell” spread at the neuromuscular junction. The saliva contains the RABV particles that contaminate the wound site in animals or humans, and the RABV attaches with the neuromuscular junction through receptors (Mani and Madhusudana 2013). The preparation of the raw meat (for example butchering) poses a greater risk for transmission than eating it. However, consuming poorly cooked and RABV infected meat can also potentially cause rabies in healthy individuals, hence, ensuring properly cooked meat also depletes the chances of rabies transmission in certain cases (Srinivasan et al. 2005). Furthermore, corneal and organ transplants have been documented for the transmission of RABV (Javadi et al. 1996).

Animals or dog bites are the primary causes of rabies transmission in humans and animals, involving the neuromuscular junction (myoneural junction). This junction is a specialized synapse that connects a motor neuron (a neuronal cell in which cell body resides in the spinal cord and lengthy axon projects outside the spinal cord) to muscles for the transmission of electrical impulses, as shown in the diagram (Fig. 3).

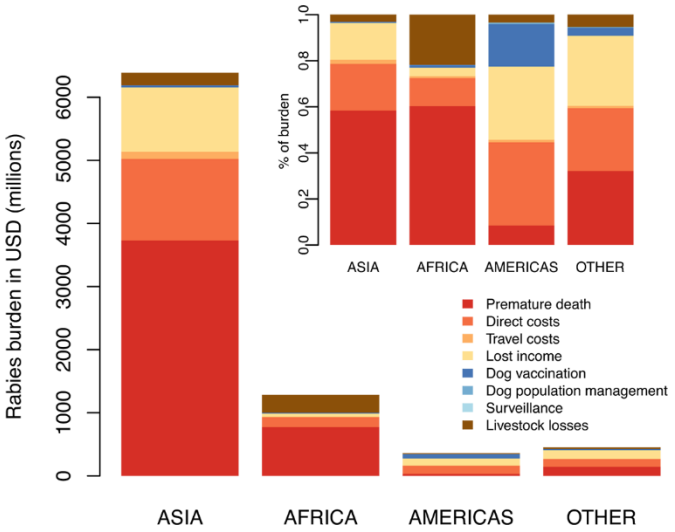

Fig. 1: Rabies burden in USD (millions) across different regions. Inset image shows proportional expenditure in different continents (adopted from Hampson et al. 2015)

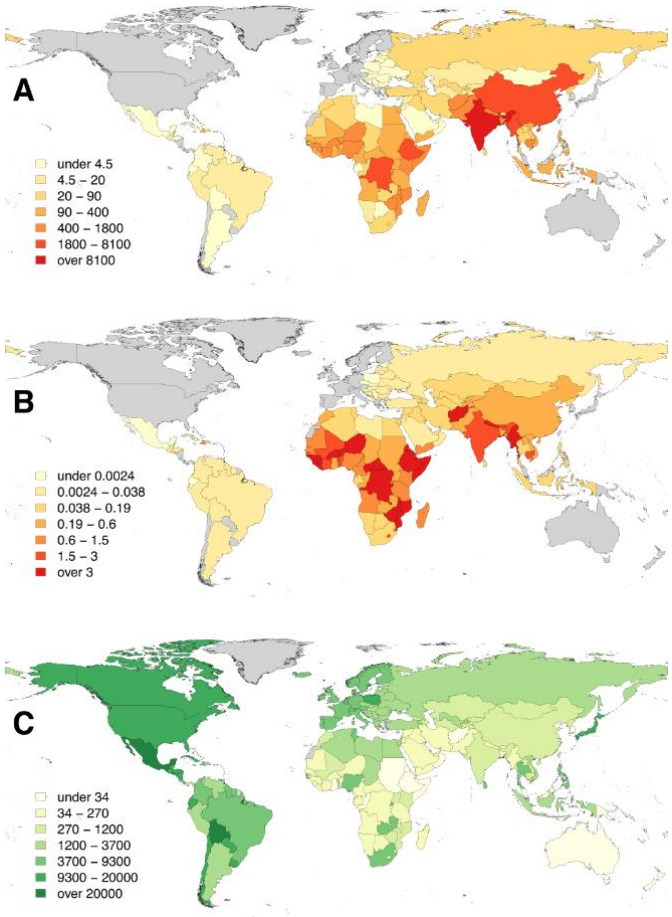

Fig. 2: Rabies burden worldwide: (A) Human deaths due to rabies, (B) Death rates per capita (100,000 persons), and (C) cost for dog vaccination (per 100,000 persons). Gray color shows countries where canine rabies has been eliminated (adopted from Hampson et al. 2015)

Reservoirs of RABV

In the early nineteen-nineties, Dr. Joseph Lennox Pawan first revealed that vampire bats could be the potential source of rabies transmission between animals and

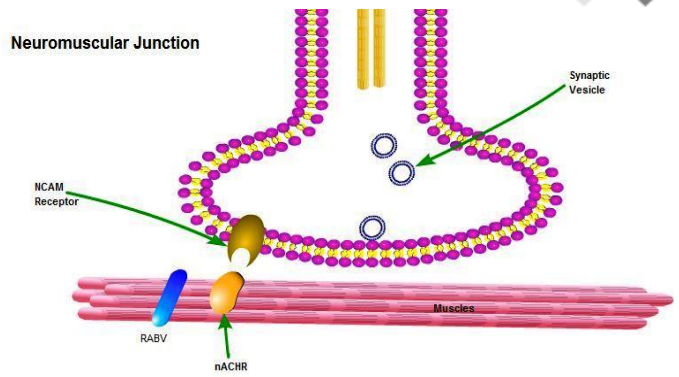

Fig. 3: Schematic representation of neuromuscular junction, showing synaptic terminal, muscle fibres and the corresponding RABV receptors.

humans (Pawan 1936; Pawan 1959). Any warm-blooded mammal can possibly be infected with the RABV by showing the specific symptoms, and is capable of transmitting the disease to humans via bites. Infected bats, skunks, ruminants, caprines, and canines are examples of the wide range of reservoirs disseminating rabies to large territories of the world. Potential exposure to infected farm animals, wild carnivores, and bears are high-risk species, while groundhogs and weasels may also put the host at relatively low risk. Other high-risk groups may include scientists or workers in a rabies laboratory, international travelers, hunters, veterinarians, and pet lovers. Poultry species, small rodents (rats, mice, gerbils, chipmunks, hamsters, squirrels, and guinea pigs) and lagomorphs (rabbits and hares) usually die of bite wounds before they become rabid, but these species can be infected with RABV (Jackson 2000; Jackson 2010; Schnell et al. 2010).

Clinical Profiles

The incubation period of the RABV depends upon the location of the suspected animal-bite and varies from a few days to weeks, and even months or a year. Generally, it is 20 and 90 days in animals and humans, respectively (Jackson 2000; WHO 2005). In dogs, characteristic symptoms exist with variable intensity, like excessive salivation, tremors, hydrophobia, pharyngeal paralysis, aggression, licking, and restlessness. In addition, sexual excitability, imbalanced movements, hyperexcitibiltiy, excessive urination, and bellowing are commonly seen in domestic and wild animals (Vural et al. 2001; Brookes et al. 2007; Zhang et al. 2011). The clinical signs and symptoms in animals can be divided into three major stages of RABV infection. These are: i) Prodromal phase, ii) Furious/Classical phase, and iii) Numb/Paralytic phase.

Prodromal Phase

This is the initial stage of the symptoms that appears as soon as the RABV reaches the brain. Intense drooling of saliva and undefined neurological signs appear, leading to death within 10 days. Priapism, numbness, tingling in limb, discomfort or pain at the site of bite, elevated

temperature, and malaise are the significant clinical signs in humans.

Furious/Classical Phase

Electrophysiological studies have shown that the furious stage in animals is probably associated with the dysfunction of anterior horn cells of the gray matter of spinal cord and an animal in this phase may die within 1-5 days after onset of symptoms. This phase is characterized by increased viral count in saliva, cardiac arrhythmias, hydrophobia, excitation, convulsions upon exposure to light, air currents, or sound, intense thirst, stages of excitement and hallucination, and extreme aggression. Almost, 80% of RABV infections in dogs exhibit this phase (Tepsumethanon et al. 2005; Jackson 2010).

Numb/Paralytic Phase

This form of presentation is characterized by weakness, flaccid paralysis of the limbs and chewing muscles that further spread to all body muscles, resulting in an inevitable death. Almost 20% of RABV infections in dogs exhibit this phase (Tepsumethanon et al. 2005; Jackson 2010). Clinical signs in the furious and paralytic phases are mainly observed among wild animals (especially dogs). However, rabid animals or humans also show signs of either furious or paralytic phase (sometimes overlap between the two phases have also been observed).

Diagnostic Techniques in Animals and Humans

Several ante-mortem and post-mortem diagnostic techniques for RABV infection are based on the following three main categories.

Viral Antigens

This category includes enzyme linked immunoassay, fluorescent antibody test, immunohistochemistry, rapid rabies enzyme immune diagnosis, staining techniques (such as Hematoxylin & Eosin and Seller's staining for the detection of Negri bodies), direct rapid immunohistohemical test, latex agglutination test, and rapid immunodiagnostic test. Rabies antibodies are used in these tests to detect viral antigens in a suspected sample (Woldehiwet 2005; Stein et al. 2010; Faizee et al. 2011).

Serological Assays

Fluorescent antibody virus neutralization, virus neutralizing antibody test, and rapid fluorescent focus inhibition test are included in this category (Woldehiwet 2005).

Detection of Viral Genome

Cell culture inoculation tests, nucleic acid sequence-based amplification, reverse transcriptase polymerase chain reaction, TaqMan RT-PCR, loop-mediated isothermal amplification, and viral isolation through mouse inoculation test are commonly used for detection of RABV infection (Lumlertdacha 2005; Clement et al. 2013).

Prevention and Treatment

There is no globally accepted treatment to avoid death against rabies once the symptoms of disease appear in humans and animals. Therefore, pre-exposure and post-exposure vaccination is highly recommended for humans and animals of high-risk group. Life-support therapy is sometimes practiced in medical wards that contain antagonistic receptors for NMDA interferon-α, ketamine, and antiviral drugs (ribavirin) (Jackson 2000). Thus, the use of reliable pre and postexposure prophylactic measures is the last hope to countercheck the pathogenic spread of RABV in animals and humans.

It is worth mentioning here that there are three categories of animal or dog bites inflicted to humans. In the first category, the dog or animal may lick the intact human skin and there is no breakage in the skin. The second category includes abrasions or scratches in the skin without bleeding, while the deep subcutaneous tissue remains intact. The third category of bite involves deep subcutaneous tissue with bleeding, and this type is more serious compared to the previous two categories. These categories of bites do not really 'matter' and anyone with a potential exposure should receive post-exposure treatment for rabies. However, third category bite from a rabid animal is very likely to expose patients to a large viral load, but people have had undetectable small bites from bats or scratches and still succumbed to rabies. In general, the wound site should be immediately washed with soap, and a dose of equine rabies immunoglobulins or human rabies immunoglobulins must be administrated in the third category or severe animal bite for passive immunity. It is mandatory to ensure the complete follow-up of post exposure vaccination and rabies immunoglobulins, prescribed by the World Health Organization (WHO 2005; Slate et al. 2009).

To produce quality vaccines against rabies, multiple attenuated strains of RABV are used. Amongst these, attenuated rabies vaccine strain SRV9, Pasteur Virus (Dietzschold et al. 2003) and Street-Alabama-Dufferin are well-known (Faber et al. 2002). Pasteur was the first scientist, who isolated RABV from the brain tissue of rabid cow in 1882; this strain of RABV is also used in a parent vaccine of the laboratory-based Challenge Virus Standard (CVS) (Dietzschold et al. 2003). The CVS strain is mostly used in serological assays for the analysis of the antibody titer against different strains of RABV. Currently, different kinds of anti-rabies vaccines are being used in developing and developed countries, such as live attenuated vaccines (oral vaccination) (Borisov et al. 2002), inactivated vaccines (purified chicken embryo cell), human diploid cell rabies vaccine (Dietzschold et al. 2003; WHO Survey of Rabies), DNA vaccines (Bahloul et al. 2003), vector vaccines containing oral vaccines for wildlife (Faber et al. 2002) and plant-derived antigens (Loza-Rubio et al. 2007).

Table 1: Taxonomic classification of lyssaviruses (adopted from the webpage of 'WHO-Rabies-Bulletin-Europe')

Virus	Potential vector(s)/reservoirs	Distribution
Rabies virus (RABV)	Carnivores (worldwide); bats (Americas)	Worldwide (except several islands)
Lagos bat virus (LBV)	Frugivorous bats (*Megachiroptera*)	Africa
Mokola virus (MOKV)	Reservoir unknown	Sub-Saharan Africa
Duvenhage virus (DUVV)	Insectivorous bats (Microchiroptera)	Southern Africa
European bat lyssavirus 1 (EBLV-1)	Insectivorous bats (*Eptesicus serotinus*)	Europe
European bat lyssavirus 2 (EBLV-2)	Insectivorous bats (*Myotis daubentonii, M. dasycneme*)	Europe
Australian bat lyssavirus (ABLV)	Frugivorous/insectivorous bats (*Megachiroptera/Microchiroptera*)	Australia
Aravan virus (ARAV)	Insectivorous bats (*Myotis blythi*)	Central Asia
Khujand virus (KHUV)	Insectivorous bats (*Myotis mystacinus*)	Central Asia
Irkut avirus (IRKV)	Insectivorous bats (*Murina leucogaster*)	East Siberia
West Caucasian bat virus (WCBV)	Insectivorous bats (*Miniopterus schreibersi*)	Caucasian region
Shimoni bat virus (SHBV)	*Hipposideros commersoni*	East Africa
Bokeloh bat lyssavirus (BBLV)	Insectivorous bats (*Myotis nattereri*)	Europe
Ikoma virus (IKOV)	Isolated from *Civettictis civetta*	Africa
Gannoruwa bat lyssavirus (GBLV)	Isolated from *Pteropus giganteus*	Asia
Lleida bat lyssavirus (LLEBV)	Insectivorous bats (*Miniopterus schreibersi*)	Europe (Spain)

Differential diagnosis of RABV can include encephalitis, lockjaw, and epilepsy in the initial phase of infection, while poliomyelitis, belladona poisoning, delerium tremens and acute polyneuritis are with the paralytic phase in humans. Symptoms in hepatitis, distemper epilepsy, brain tumours, poisoning, and head injury can be clinically confused with those in rabid dogs (Dietzschold et al. 2003; Tepsumethanon et al. 2005; Lumlertdacha, 2005).

Taxonomic Classification

According to the International Committee on Taxonomy of Viruses, RABV falls in *Mononegavirales* order, *Rhabdoviridae* family, and *Lyssavirus* genus. This family further contains *Lyssavirus, Ephemerovirus,* and *Vesiculovirus* as genera, while the genus is further subdivided into 11 genotypes based on their antigenic characterization and phylogenic lineage (Table 1). Among these genotypes, classical RABV is commonly found worldwide (Fauquet et al. 2005).

Structure of the RABV

RABV has a bullet-shaped structure, with a measured length and diameter of 100-300 nm and 75 nm, respectively (Schnell et al. 2010). The virion measures 12 kb, with two fundamental subunits. The first subunit is the ribonucleoprotein (RNP), which is also associated with phosphoprotein (P segment) and viral polymerase, making the internal compact core. The RNP complex comprises nucleoprotein (N segment), P segment, and RNA-dependent RNA polymerase (L segment); these are all collectively associated with viral RNA. The matrix protein (M segment) forms the bridge between virion membrane and capsid. The second basic subunit is glycoprotein (G segment), which wraps around the outer surface of the genome and contains 400 trimeric spiky projections (Tordo et al. 1986; Schnell et al. 2010).

RABV is single-stranded, non-segmented, and negative-sense RNA genome of approximately 12,000 nucleotides. The N segment consists of 450 amino acids and helps in the transcription of RNA. The P segment contains 297 amino acids and is linked to the L segment to serve as a non-catalytic cofactor for RNA polymerization (Schnell et al. 2010). The M segment comprises 202 amino acids and is responsible for viral assembly and attachment with the host membrane (Mebatsion et al. 1999). An extensively studied protein of RABV is G segment that contains 524 amino acids. This protein is primarily responsible for inducing pathogenic response in the host (Benmansour et al. 1991).

Immunopathology

The interaction between RABV and the host cell immune system is complicated as compared to other viruses of different genera. Various factors have been discovered to understand the mechanism of rabies immunology. Distinct details about the life cycle and proposed mechanism of rabies endocytosis are discussed below to explain the immunology and pathogenesis of RABV.

Brief Overview of Life Cycle

It is well known that RABV has three receptors for cellular uptake, namely, nicotinic acetylcholine receptor (nAchR) located at the post-synaptic muscle membrane, neuronal cell adhesion molecule (NCAm) located at the post synaptic membrane of the neuron for the attachment of the RABV, and nerve growth factor p75NTR (BeX3 and NGFR) which is a molecular structure to provide entry sites for RABV. The life cycle of RABV recirculates the following steps: binding, un-coating, production, and assembly of the viral components inside the host cell (Tuffereau et al. 1998; Thoulouze et al. 1998). Various studies have shown that RABV follows clathrin-mediated endocytosis for the internalization within the endothelial cells (Sun et al. 2005; Piccinotti et al. 2011; Weir et al. 2014).

Internalization

The precise mechanism to follow the pathways of the clathrin-mediated endocytosis is not yet clear. However,

many research studies have speculated that viral proteins of RABV are usually internalized through clathrin-coated pits during clathrin-mediated endocytosis, and at later stages in lysosomes using late endosomes. The G segment of RABV and pH-dependent membrane fusion are responsible for the membrane fusion and attachment with the host cell (Superti et al. 1984; Pulmanausahakul et al. 2008).

Intracellular Transport

After the attachment and entry into the host cell, either a single capsid or whole virion is transported to the neuronal cell body of the host cell in the form of a vesicle. During this transport, RABV also interacts with the dynein light chain (LC8), using the support of microtubule along the entire length of axon, but deletion of LC8 alone do not affect the transport of RABV. Another study demonstrated that capsids and enveloped-labeled viruses enabled the transport of the whole virus in endosomal vesicles. The transport mainly depends upon the G segment of RABV, which is embedded inside the vesicle. Thus, the nature of the vesicle determines the transport strategy of RABV (Tan et al. 2007; Klingen et al. 2008).

Replication, Transcription and Translation

The regulatory mechanism of RABV has evolved for efficient, but limited production of viral particles to avoid recognition by the immune system of the host. Negri bodies, containing ubiquitylated and heat shock protein (Hsp70), are the entities for the replication and transcription of RABV, and are formed by hijacking the toll-like receptors (TLR3) (Whelan et al. 2004; Pauline et al. 2009). The interaction of Hsp70 has also been observed with the N segment of RABV. Viral proteins, mRNAs, and viral particles were reduced as gene expression of Hsp70 protein was down-regulated (Cheng et al. 1983). These findings indicate that Hsp70 has a close association with one or many life stages of RABV assembly, or it may also have a proviral role in the mechanism of RABV pathogenesis. The M segment of RABV also facilitates the process of translation. RABV follows 'stop-start model' to complete its life cycle by starting the transcription at the 3′end of the RNA and then sequentially synthesis at 5′end to prepare a genomic ladder of viral protein (Schnell et al. 2010). It has been observed that whenever adequate contents of N segment are formed through 'stop-start model', the encapsidation of leader RNA forms a complete genome and thus, the phenomena is converted from transcription to replication (Schnell et al. 2010).

Budding

In the last phase of RABV infection, the M and G segments play their role in the budding, and both viral proteins are interdependent to facilitate the process of budding, as shown in Fig. 4 (Mebatsion et al. 1996). In this stage, the newly formed segments of RABV are assembled and subsequently join to form new virions and release through plasma membrane in the form of budding. Experimental studies in skunks have shown that the budding occurs mostly from synapse or plasma membranes of the dendrites, and less commonly from the plasma membrane of perikaryons (Charlton and Casey 1971). The M and G segments of RABV facilitate the process of budding, and the budding efficiency of RABV decreases 30-fold in the absence of its G segment (Schnell et al. 2010).

Overview of Clathrin-Mediated Endocytosis

The internalized vesicle formation follows through the five bulging steps in the clathrin-mediated endocytosis, which proceeds to fuse with early and late endosomes. Figure 4 represents different stages, such as the nucleation, selection of cargo, assembly of coating, scission, and ultimately uncoating of the vesicle. The adaptor protein-2 (AP-2 complex) and Fes/CIP4 homology domain (FCH domains), together with additional factors, accumulate clathrin proteins into a bunch. The clathrin membrane forms curvature to develop a well-defined clathrin coated pit. These changes are also stimulated with the addition of phosphatidylinositol 4, 5,-biphosphate (PIP2) and the whole process is called nucleation (Koh et al. 2007; Dannhauser and Ungewickell 2012).

In this process, the scaffolding protein also plays important role to join the two proteins simultaneously to stimulate the process of molecular signaling. Scaffolding proteins, such as epidermal growth factor receptor substrate 15 (EPS15), and intersectin are also involved in clathrin-meidated endocytosis (Koh et al. 2007; Łyszkiewicz et al. 2020). The process of endocytosis is highly complicated, as demonstrated by *in vivo* studies, while evidence of these scaffolding proteins and other factors has also been described. The adaptor protein-2 also recruits intersectin and EPS15 that systematically assist the process of clathrin-coated pit formation (Koh et al. 2007; Dannhauser and Ungewickell 2012; Łyszkiewicz et al. 2020). The appendage domain of adaptor protein-2 also binds the corresponding adaptors for the selection of cargo. In addition, the μ and σ sub-units of adaptor protein-2 engage many receptors. This protein forms the clathrin coat around the newly formed clathrin-coated pit. For the scission of the pit, the actin polymerizes at the constricted neck of the clathrin pit (Piccinotti et al. 2011; Weir et al. 2014).

The process of uncoating occurs through the cyclin G-associated kinase or auxilin. It reveals the clathrin-coated vesicle through heat shock cognate 70, and releases an independent endocytic vesicle. The clathrin assembly is again ready for the intake of another cargo at the plasma membrane. Different effectors are involved for the induction of curvatures in the flat plasma membrane to form a well-defined invaginated membrane (McMahon and Boucrot 2011).

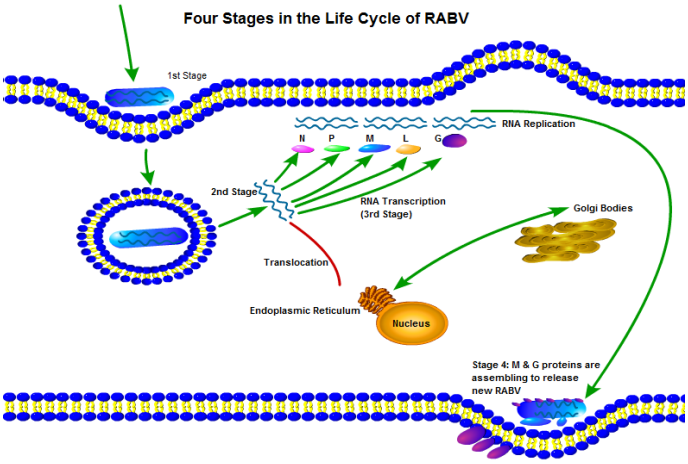

Fig. 4: The first stage includes the attachment and internalization, followed by the genetic expression of different segments of RABV. The third stage is RNA transcription, while the fourth stage includes the assembling of M and G segments of RABV that is again ready to bud out from the cell.

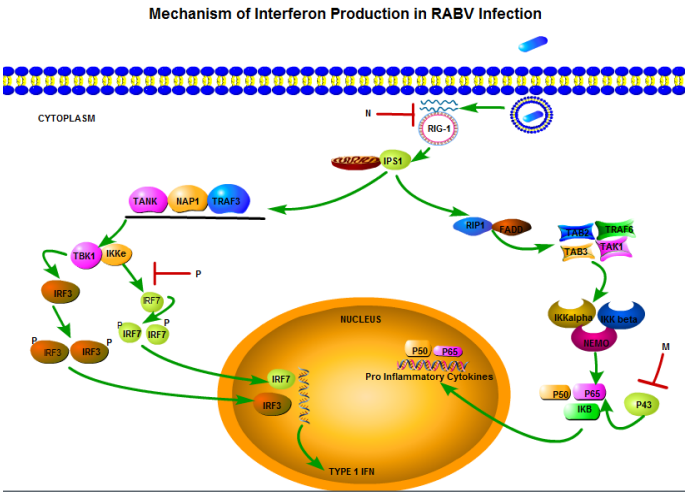

Fig. 5: Schematic mechanism of downstream signaling pathways that start from RIG-1 and further pathways are up-taken by IPS1 and cystosolic proteins, effector molecules and scaffolding proteins to finally produce type 1 IFN and pro inflammatory cytokines in RABV infection.

Mechanism of Interferon Production in RABV Infection

The retinoic acid-inducible gene-1 (RIG-1) generally detects the viral RNA, which is a potential activator for the production of type 1 interferons. The RIG-1 is among the most widely observed pattern recognition receptors. It binds to the caspase activation and recruitment domains (CARDs) that facilitate the downstream signaling phenomena. IFN-β promoter stimulator I (IPS-1) is usually present on the mictochondrial membrane. The cytosolic proteins detect the presence of RABV segments and bind to the IPS-1 (Fig. 5) to mediate receptor interacting protein-1 (RIP-1), which is also an adaptor protein. The adaptor protein, in turn, phosphorylates with interferon regulatory factor 3 and 7 (IRF7/IRF3) through TANK-binding kinase 1 (TBK1)/IκB kinase (IKKε), nucleosome assembly protein 1 (NAP1), and tumor necrosis factor-receptor associated factor 3 (TRAF3). This phosphorylated IRF3/IRF7 is translocated into the nucleus to induce the expression of Type I IFN genes. The adaptor protein complex-1 (AP-1 complex) also interacts with receptor-interacting protein 1 (RIP1) and a death domain called fas-associated protein with death domain (FADD). The latter initiates the activation of the 'nuclear factor kappa-light-chain-enhancer of activated B cells' (NF-κB) pathway that is a combination of hetero-and homodimeric complexes of the Rel family members and controls cytokine production and cell survival. Mitogen activated protein kinase kinase kinase (MAPKKK) are important set of signaling proteins for cell differentiations and other intracellular functions. It has cofactor proteins TGF-β-activated kinase1 (TAK1) and its associated binding/activating partners TAK1-binding protein-2 (TAB2) and TAK1-binding protein-3 (TAB3). The TAK1 is an integral protein to regulate interleukin and activate NF-κB pathway. The TAB2 is a binding or scaffolding protein of TAK1 that connects TAK1 to TRAF6. The p65/p50 heterodimers are the finest rated subunits of NF-κB pathway. A new member of the NF-κB family, p43 (RelAp43), has also been recently recognized. IκB kinase (IKK) is a complex that consists of regulatory subunit NF-κB-essential modulator (NEMO) and catalytic kinase subunits (IKKα and/or IKKβ). Its activation results in the phosphorylation and subsequence degradation of IκB. This phenomenon helps to release free NF-κB in translocating to the nucleus, where it induces target gene expression, as well as pro-inflammatory cytokine encoding genes (Fig. 5).

Phosphoprotein of RABV Inhibits Innate Immune Response

RABV has developed an escape mechanism to skip the trap of the immune system and to avoid the neutralization of virion within the host defense system (Alandijany et al. 2005). The P segment of RABV counteracts IFN reaction by jamming the response of host cell to type I and II IFN signaling and pathogen-associated molecular patterns (PAMPs). Melanoma differentiation-associated gene 5 (MDA5) and RIG-I are the cytoplasmic helicases that detect the viral RNA. The MDA5 and RIG-1 are also known as IFIH1 and DDX58, respectively (Caillet-Saguy et al. 2015). These innate sensors sensitize or trigger through a TBK1-IκB kinase ε (IKKε) complex and NF-kB activator. The NF-kB activator is associated with TRAF family member, and is also termed as TANK. These triggering responses induce IFN regulatory factor 3 (IRF3) phosphorylation (Brzozka et al. 2005). The resultant IRF3 dimerizes itself and is transported to the nucleus to provoke the transcription of interferon beta (IFNβ) in concurrence with nuclear factor-κB (NF-κB) and activate transcription factor 2 (ATF2). Furthermore, the induction response is also sent by TLR3 receptor. The P segment of RABV inhibits the IFNβ by blocking the phosphorylation of IRF3 in the cytoplasm, and it may also block the signal transduction pathways (type I and type II). Normally, Janus kinase

(JAK) carries out phosphorylation of transcription 1 (STAT1), but P segment of RABV attaches the phosphorylated STAT1, and inhibits its antiviral transcriptional response and transports to the nucleus (Vidy et al. 2005). Smaller versions of P segment bind the STAT1 and STAT2 heterodimers complexed with IRF9 in the nucleus. This result in the formation of two complexes, STAT1 homodimers and IFN stimulated growth factor 3 (ISFG3). These complexes prevent the activation of IFN-stimulated response element (ISRE), type I and II IFN-dependent immune response, and γ-activated sequence (GAS)(Vidy et al. 2007).

Immunopathology of RABV

Rabies pathogenesis is diverse and covers a wide range of diverse lesions, as described comprehensively in the following sections.

Specific Immunopathological Lesions

Negri bodies are the granular and oval-shaped cytoplasmic inclusion bodies, which are observed in the stained tissue sections processed through paraffin embedded tissue sections. Negri bodies are mostly seen in the RABV-infected purkinje cells of the hippocampus, and often termed as the pathognomonic or characteristic intracytoplasmic bodies to confirm the presence of RABV. These inclusion bodies appear pinkish and magenta red in tissue sectioning and Seller's staining techniques, respectively (Jogai et al. 2000; Schnell et al. 2010; Stein et al. 2010; Faizee et al. 2011). Gross lesions of the brain tissue include congestion, edema, and petechial hemorrhages during post-mortem examination of animals. Likewise, Hematoxylin and Eosin staining technique has shown congestive changes, perivascular cuffing surrounded by multiple layers of inflammatory cells, Babès nodules, vacuolation, rod-shaped neurons, satellitosis, and spongy (cavernous) lesions in naturally and experimentally infected tissues of animals. Similarly, variable proportions of RABV antigens have also been reported in different parts of the brain tissue during diagnosis through immunohistochemistry (Stein et al. 2010; Faizee et al. 2011).

Neuronal Dysfunction

The RABV causes neuronal dysfunction by manipulating the proapoptotic factors and reducing the threshold level of corresponding gene expression in host cells (Schnell et al. 2010). Disruption in ion channels has already been interpreted in the neuroblostoma cells. The functional impairment in sodium and potassium ion channels is associated with the loss of action potential and membrane polarization (Iwata et al. 1999). The functional proteins of ion channels and the ability to maintain homeostasis are also altered (Dhingra et al. 2007). The Glu81 and Arg77 amino acid sequences of M segment of RABV regulate the survival of infected neurons, and these amino acid sequences induce apoptosis (Bauer et al. 2015). The RABV also causes cell death, either by producing necrotic changes or apoptosis. The CVS strain of RABV caused neuronal apoptosis in mouse neuroblastoma cells, while attenuated viruses caused apoptotic changes in primary neuronal cultures as compared with virulent strains (Theerasurakarn and Ubol 1998; Morimoto et al. 1999; Fazakerley and Allsopp 2001).

RABV Induces Apoptosis

Many strains of RABV induce apoptosis, but pathogenic viral strains are not included in this category (Jackson 1998). Human lymphocytes and mice showed apoptotic changes with the highly attenuated RABV strain called ERA, but these findings have not been reported in naturally infected humans (Thoulouze et al. 1997). Highly attenuated RABV strains produce more apoptotic changes as compared to the pathogenic strains because of increased production of G segmented protein of RABV (Hemachudha et al. 2005).

Degeneration of Neuronal Processes

RABV is also responsible for the degeneration of neuronal processes, as well as disintegration of synaptic structures, in the hippocampus of mice (Li et al. 2005). The MRV strain of RABV induces dendritic injuries and depolymerization of actin filaments in the hippocampal cells of mice (Yan et al. 2013).

Configuration of Negri Bodies

There are almost 10 different kinds of TLRs in humans. These are present on the cell surface, act as innate immune receptors, and respond to PAMPS program (Rock et al. 1998). In the formulation process of these intracytoplasmic Negri bodies, RABV hijacks the TLR3 receptor. These are present in the central nervous system and high levels are also found in glial cells (Jack et al. 2005). The Negri bodies are in fact composite of 'aggregated TLR3' found in an inner core that is enclosed by a cover of genomic material and proteins. Negri bodies are not observed in TLR3 deficient experiments, hence the rate of RABV growth decreases significantly. Furthermore, they are also located in late endosomes in human's neuronal cells, as shown in Fig. 6 (Pauline et al. 2009).

Mechanism of Mitochondrial Dysfunction and Oxidative Stress

The CVS infection results in mitochondrial dysfunction that further induces degeneration of neuronal processes, oxidative stress and over-production of reactive oxygen species. In mitochondria of RABV infected neurons, the activities of electron transport chain (ETS) complex I and IV are enhanced. This could be due to three distinct reasons; a) direct or indirect interaction with RABV proteins, b) consequences produced by the up-regulation of sirtuin activity, c) higher $NADH/NAD^+$ ratio. The sirtuins are group of protein family that control metabolism, stress responses, longevity and development.

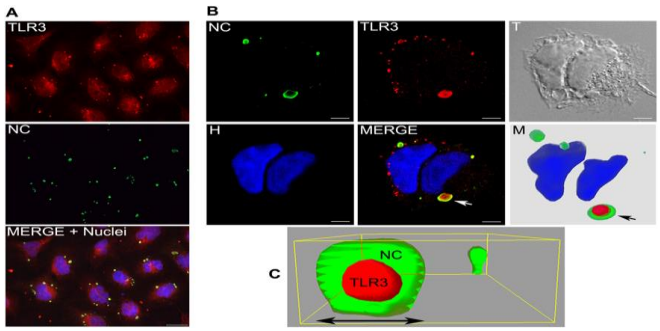

Fig. 6: Diagram showing the TLR3 and N segment of RABV in the perinuclear structures. Nucleocapsid (NC) is green, TLR3 is red and nuclei are blue; The insets (M & C) show the 3D image of Negri bodies with an internal aggregation of TLR3 (red) enclosed by NC with a diameter of 2.7 µm (adopted from Pauline et al. 2009).

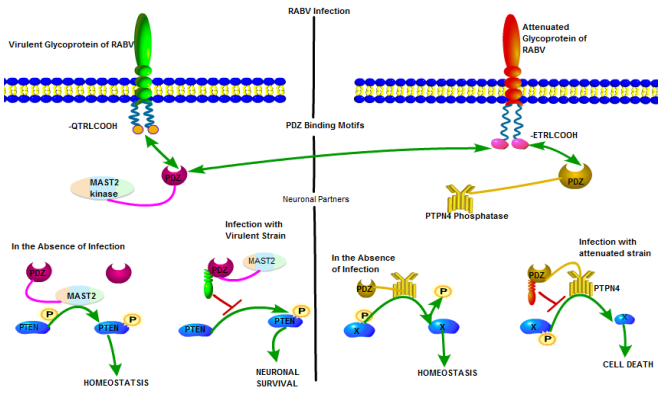

Fig. 7: Schematic diagram showing different neuronal factors and cellular signaling pathways to hijack PDZ domain by virulent (left) and attenuated (right) strains of RABV.

The prompt propelling of protons across the mitochondrial membranes produces a high mitochondrial membrane potential. Electron leakage during forward and reverse ETS complexes also results in superoxide formation which is dismutated by mitochondrial superoxide dismutase into hydrogen peroxide. Superoxide and hydrogen peroxide reduce the levels of intracellular ATP by raising hydrolytic levels of ATP molecules. Mainly, the accumulation and excessive production of superoxide and hydrogen peroxide cause oxidative stress that ultimately leads to degenerated neuronal processes (Alandijany et al. 2005).

Oxidative stress also plays a vital role in dendritic beading, impaired axonal growth, and injury of dorsal root ganglionic neurons due to CVS-11 strain of RABV. The immuno-staining showed that axonal outgrowth was reduced at certain spots of neurons, and modified shapes (kidney and circular) of axonal swellings were seen (Jackson, 2010).

Interferon Stimulated Genes (ISGs) Inhibits RABV Life Cycle

IFN-inducible transmembrane proteins inhibit RABV entry into host cells. These are diverse proteins that have been remarkably efficient in blocking entries of Influenza and Dengue viruses (Siegrist et al. 2011). The cholesterol-25-hydroxylase is an endoplasmic reticulum-linked enzyme that manipulates the action of viral fusion with the cell by inducing changes in the cellular membrane (Holmes et al. 2011). MxA is a gene product of two human genes Mx1 and Mx2 that blocks primary transcription (Verhelst et al. 2013). The ISG20 is a 20kDa protein that plays a key role in host defense, whereas ProMyelocytic Leukemia (PML/TRIM19/Motif protein-19) induces protein degradation and antiviral defense (Nisole et al. 2005). Both of these ISGs inhibit secondary mode of transcription. Protein kinase and IFN-inducible proteins with tetratricopeptide repeats of proteins inhibit viral translation, while virion cellular budding is inhibited by Tetherin (Danielle et al. 2015).

Evasive Strategies in RABV Infection

RABV promotes neuronal survival and prevents premature cell death of the infected neurons through three important mechanisms. First, the TLR3 prevents axonal growth by residing within the nucleo-core of RABV and in this way, enables survival of infected neurons. Secondly, a study investigated the attenuated or virulent strain of RABV and unleashed the underlying involvement of PDZ domains that represent a family of protein-protein domains interaction in cells. The PDZ is an acronym made up from three diverse domains, namely the Post synaptic density protein, Drosophila disc large tumor suppressor, and Zonula occludens-1 protein. The PDZ domains regulate multiple vital functions, like ionic transport, signal transduction pathways and cellular signaling, in various species and their number vary from specie to specie. These domains mainly control the cellular functions, such as neuronal signal transmission, and recognize PDZ-binding motifs (PBM) that are usually found at the carboxyl terminus of proteins. The RABV mimics the host PBM and hijacks the PDZ proteins to manipulate host cellular functions to complete one or more stages of its life cycle. The survival or death ratio in infected neuroblastoma cells is regulated by a single mutation Q (-3) E in the C-terminal cytoplasmic tail of G domain, which is termed as CytoG (Prehaud et al. 2010). This mutation is found in a PBM at the C-terminus sequence of CytoG and abundantly influences the nature of the neuronal allies interacting with G domain. In case of virulent (left side in Fig. 7) and attenuated strain (right side in Fig. 7) infection, the viral G segment with the-QTRL$_{COOH}$ PBM and ETRL$_{COOH}$ PBM interacts with the PDZ domain of microtubule-associated serine and threonine kinase-2 (MAST2). However, -ETRL$_{COOH}$ PBM interaction also involves other cellular partners, such as tyrosine-phosphatase (PTPN4). In uninfected neurons, PTEN phosphorylates by MAST2, but the G domain interacts via its PBM with the PDZ domain of the MAST2 in virulent strain infection. This contact may possibly stop MAST2-controlled phosphorylation of PTEN, which changes the homeostasis of the RABV-infected cells and survives the infected cell. The cellular partner X (as shown in right side of Fig. 7) is dephosphorylated by

PTPN4 in the absence of infection, but the viral PBM interacts with the PDZ domain of PTPN4 in case of an attenuated viral strain infection. This interaction controls proficient dephosphorylation of cellular partner X that modifies the homeostasis of the infected cell and triggers apoptosis, as shown in Fig. 7 (Prehaud et al. 2010).

Thirdly, the interferon beta production, TLR signaling, and RIG-I induce the expression of FasL, HLA-G and B7-H1 proteins in RABV infection. These biological components move to the surface of non-infected astrocytes or infected neurons. At these sites, these molecules interact with their respective receptors on T cells (CD8 for HLA-G, Fas for FasL and PD-1 for B7-H1). These complex molecules or ligands further trigger the death of $CD8^+$ T or $CD3^+$ cells that facilitate the entry of RABV in the nerve tissue. It is the critical step for the progression of the disease in the nervous system of the host. Due to these three strategies, it is a relatively complicated fight against the well-automated evasion mechanism of RABV (Larrous et al. 2010; Lafon 2011).

Conclusions

Rabies has remained as the top priority neglected tropical disease of humans by the World Health Organization since ancient period. Historical findings have proven that lack of awareness, ignorance and weird curative methods have created an everlasting fear in poor communities of the developing world. Knowledge regarding epidemiology, zoonotic potential, and pathogenesis of RABV are imperative ways to understand the biology and mode of viral actions in different species. Rabies endemic countries have to spend designated budget on control programs, such as national mass dog vaccination, to attain the goal of rabies elimination by the end of 2030. It would definitely require highly coordinated and collaborative efforts of multiple stakeholders and health professionals of the respective countries.

Insights into immunology, pathobiology, and evasive techniques of RABV are useful to understand the real time infectious pathways of RABV and to design effective vaccines or chemical ligands to block the receptor-mediated endocytosis inside host cells. RABV has shown well-known pathological changes inside host cells that include, specific pathognomonic lesions, neuronal dysfunctions, apoptosis, neuronal degeneration, alteration in the neuronal cytoskeleton, and formation of Negri bodies. All these pathological alterations enable the RABV to survive inside the complex host-cell environment and produce featured clinical signs and symptoms in multiple species. RABV is a cunning virus that adopts sophisticated pathways to dodge the immune system of the host cells. Latest tools of real time live imaging and intrinsic factors of immune system may help to solve the mystical pathogenesis of RABV.

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REVERSE ZOONOSIS AND ANIMAL HEALTH

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INTRODUCTION

When pathogens are capable of transmission from human to non-human hosts, such phenomenon is referred as reverse zoonosis. It is also known as anthroponosis or zooanthroponosis (Hubálek 2003). Whereby, Anthroponosis is a term used to describe human to non-human animal transmission, as well as human transmission, zooanthroponosis is exclusively used to explain human to animal pathogenic transmission (Joint and Organization 1967; Hubálek 2003). Humans transmit the pathogenic agents to the animals, where they sometimes mutate and spread through different species of animals. Depending upon the nature of pathogens, reverse zoonosis is categorized as reverse zoonosis by bacteria, viruses, protozoa/parasites and fungi. Infection potential of a pathogen in a population, either humans or animals, depends upon the mode of its transmission. Direct human to human, animal to human or human to animal via aerosol or feco-oral route is easy and quicker than the involvement of any other vector. This is because in a geographical distribution, presence and survival capabilities of different vectors are different. If a vector is abundantly present in an environment, then disease spreading rate will be higher and arthropod vectors are the most notorious in this field. Attempts have been made to compile the incidence of reverse zoonosis occurred around the world. Most of the focus is on the bacterial and viral pathogens, as they are mainly involved in interspecies transmission. Since transmission of pathogens is either direct or vector involved, both of these aspects are considered in discussion. Human pathogens can affect variety of species of domesticated animals, as well as wildlife. Evidence of reverse zoonosis can be found in domestic animals or captive wildlife, but it is difficult to ascertain in the free-range wild animals (Fig. 1). It is also observed that drug resistant pathogens are also involved in interspecies transmission, which is alarming. Mutation is also one of the factors in the reverse zoonosis that is discussed in this chapter.

Reverse Zoonosis by the Bacterial Pathogens

Bacterial zooanthroponosis has been documented since long, affecting wide range of animals including farm animals, companion animals and the wild life. The mechanisms involved in the transmission vary among various species of animals. However, generally the mechanisms involved include the direct contact, feco-oral route, fomites, direct inoculation and aerosol route (Hackendahl et al. 2004; Messenger et al. 2014; Barasona et al. 2017). There are certain bacteria which are involved in the reverse zoonosis, causing the outbreak of diseases of human origin in animals with possible outcome of severe ailment, drug resistance and mortalities.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus (*S. aureus*) is considered as a bacterium of substantial importance in human medicine. It is also proclaimed as a serious public health concern. *S. aureus* imparts adverse effects and conditions in human beings, ranging from minor skin and soft tissue infection to serious ailments including meningitis, bacteremia and pneumonia (Tong et al. 2015). The non-human hosts (mostly livestock) are focused as the reservoirs of infections and antibiotic resistance related to humans. Now, the interest has been shifted to investigate the role of humans as the source of infection for animals (zooanthroponosis) (Fluit 2012; Price et al. 2012).

Anthrozoponosis is more common than the zooanthroponosis by the *S. aureus*. However, it is now obvious from the studies determining the potential of *S. aureus* to shift the host species, with resultant adaptation in new host and further transmission in newly adopted host species (Shepheard et al. 2013).

This phenomena of adapting in the new host after switching and interspecies transmission is now very well known to occur between humans and animals. Therefore, *S. aureus* associated with the human origin causes rapid colonization and infection in the host as compared to non-human primates (animals), either domesticated or in the wild (Schaumburg et al. 2012).

The premiere driver of host adaptation following the inter-host transmission is addition or deletion of the genes directly associated with mobile genetics of the bacterium (Spoor et al. 2013) . Therefore, non-human host provides suitable environment to the bacterium to acquire the novel virulence and antimicrobial resistance (Sung et al. 2008). The antimicrobial resistance and acquisition of unusual virulence have been observed in the clones of MRSA from humans that have been reported in livestock and vice versa (Sung et al. 2008; Köck et al. 2013). Reverse zoonotic potential of the *S. aureus* is adversely affecting many livestock species. One such example is from the equines, where 11 horses from

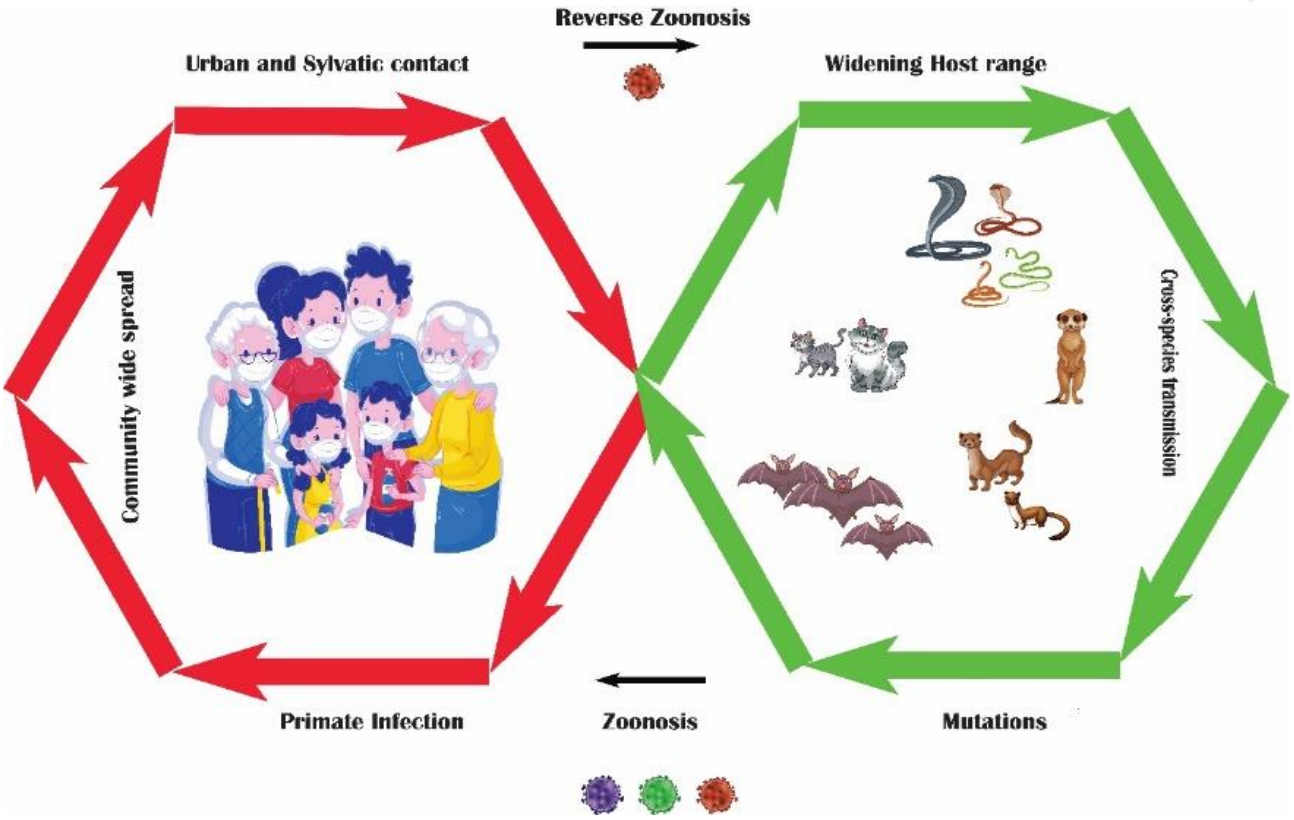

Fig. 1: Showing transmission possibility of pathogens between human and animals, mutation and it's spread through community

different farms exhibited MRSA infections. As the MRSA is extremely rare in horses, the outbreak was acquired at the hospital, which is known as nosocomial infection (Seguin et al. 1999). However, potential of the *S. aureus* to affect non-human primates is not restricted to equines, as it also affects other species like cows, turkeys, and pigs (Hasman et al. 2010; Price et al. 2012). Although, mutation is continuously reducing the zoonotic threat for humans, but it augments the *S. aureus* resistance capabilities, which will ultimately enhance the occurrence of MRSA infections (Price et al. 2012). Human to livestock transmission of MRSA has also been documented in advanced countries of the world,including USA (Smith 2015) , UK (Graveland et al. 2011), Canada (Armand-Lefevre et al. 2005), China (Zhou et al. 2018), Germany (Köck et al. 2009) and France (Price et al. 2012).

Tuberculosis

Bovine tuberculosis (TB), caused by the *Mycobacterium* (*M.*) *bovis*, *M. tuberculosis*, or rarely by *M. caprae,* is an important and significant disease of public health concern due to its zoonotic potential and resultant considerable economic losses (Torgerson and Torgerson 2010, Bayraktar et al. 2011a, 2011b). In humans, majority of the TB cases happened either due to utilization of the contaminated milk or through aerosols from the infected animals (Moda et al. 1996). However, situation has become complex with the emergence of reverse zoonosis. Sharing of the micro environment and premises by the human and non-human primates has significantly potentiated the disease (Shitaye et al. 2007). Previously, it has been reported that humans are preliminary the cradle of *M. tuberculosis* infection for animals (BhanuRekha et al. 2015). It is important to consider that *M. bovis* can infect urogenital system in humans and secretions from such patients can affect the animal health through reverse zoonosis (Ocepek et al. 2005). An extraordinary pervasiveness of zoonotic TB in humans is due to its significantly high occurrence in the cattle (Asiak et al. 2007). Nonetheless, transmission of the TB can occur mutually and previous studies have supported this phenomena of reverse zoonosis, where isolation of *M. tuberculosis* has been done from cattle and that of *M. bovis* from humans (Ocepek et al. 2005; Jenkins et al. 2011; Adesokan et al. 2012). The canvas of TB is expanding and many animal species other than the cattle have also been affected by the human infection. Lesions of TB have been reported in wild red deer and boars, where they had been confined under the intensive care management conditions such as large game fences and auxiliary feeding locations (Schöning et al. 2013). Spread of the same strains of TB found in humans to these wild deer and boars suggests that the possible spread was either through direct human contacts or their excretions (Barasona et al. 2017). The reverse zoonotic potential is also affecting the free range wild life species and in such an incidence the necroscopic findings of the African elephant revealed seriously damaged lungs due to the strain of the *M. tuberculosis*. This reverse zoonosis phenomenon was the culprit behind this infection that might be due to aerosolization of the agent either

through contaminations by the surrounding human community or tourists (Miller et al. 2019). There is significant evidence of direct or aerosol transmission of the *M. tuberculosis* from humans to companion animals (Hackendahl et al. 2004).

Escherichia coli

Multidrug resistant strains of pathogenic *Escherichia* (*E.) coli* are important in public health concern because of their speedy spread through the large population and also due to their supposedly perpetual evolution (Ewers et al. 2010). Transmission of the zoonotic pathogens has increased between the humans and animals due to sharing of the same habitat. There is strong evidence of transmission of E. *coli* from humans to livestock including mountain gorillas, which revealed that habitat sharing is affecting the human to animal transmission of this pathogen through the reverse zoonosis (Rwego et al. 2008). *Escherichia coli* DSM22664 is a human pathogen which showed the similarity index of >85% with six B2-O25b-ST131 CTX-M-15 strains detected in animals in five different countries of Europe. This is an indication of its potential of distant transmission (Nicolas-Chanoine et al. 2008). The possible spread of virulent serotypes of cephalosporin-resistant *E. coli* from human to companion animals, especially dogs, has been previously investigated in Germany (Ewers et al. 2010). These strains were previously considered exclusively for human population but emergence of the CTX-M-15-positive B2-O25:H4-ST131 strain in a dog infection is alarming (Coque et al. 2008; Nicolas-Chanoine et al. 2008; Pomba et al. 2009). Presence of the clonally related human pathogenic multi-resistant strains of *E.coli* in the pet animals across different European countries has pointed out its potential of inter-species transmission, humans to other animals and vice versa (Ewers et al. 2010).

Helicobacter pylori

Helicobacter pylori is an important pathogen of human gastrointestinal tract, causing serious and prolonged ailments. Animals in the captivity are also among those affected by this pathogen of human origin through reverse zoonosis phenomena. In such an example in Australia, the stripe faced marsupial suffered repeated and multiple outbreaks of the *H. pylori* while under captivity; the causative strain had 100% association with the strain of the human origin. Transmission of *H. pylori* to these animals was from the handlers of these animals, which further emphasizes the importance of the reverse zoonosis (Every et al. 2011).

Salmonella spp and Shigella spp

Salmonella and Shigella have significant potential to cause morbidity and mortality in captive gorillas (Stetter et al. 1995; Mundy et al. 1998). It has been found that free-running mountain wild gorillas are affected by these bacteria in human adjusted territories. Some of the Shigella species isolated from these wild creatures are *S. sonnei, S. boydii, and S. flexneri* (Nizeyi et al. 2001). These were found in sub-adults and grown-up gorillas aged from 6.0 to 11.9 years. The sharing of Salmonella spp. diseases among humans and gorillas has increased during previous years. The isolation of Shigella spp. interestingly from mountain gorillas may show upgraded reverse zoonotic transmission (Nizeyi et al. 2001).

Transmission of Zoonotic Viral Diseases

Viral infections such as dengue fever, yellow fever and zika fever, which mostly spread through arthropod vectors, can be justified by both anthroponosis and reverse zoonosis/zooanthroponosis, provided which host (human or mosquito) is considered the primary source of infection (Alekseev and Dubinina 2001). Conversely, infections such as Human Immunodeficiency Virus (HIV) which instigated in primates (LeBreton et al. 2007) and influenza virus attributed to avian species (Nelson and Vincent 2015), despite being originally identified as cases of zoonotic transmission, can now be termed as anthroponosis. Certain important viral pathogens of reverse zoonotic importance have been discussed in this chapter. Ironically enough, wild animals rarely come into direct contact with humans and due to their improbable presence in close vicinity, most cases of reverse zoonosis emerge between humans and wild animals as a consequence of contact with abiotic substrate contaminated with sapronotic agents (Hubálek 2003).

Arboviruses

Arthropod-borne viruses are called as Arboviruses. Several of these viruses, namely Zika virus, Dengue fever virus and Yellow fever virus belonging to family *Flaviviridae,* exhibit reverse zoonosis. Moreover, Chikungunya virus, which has been classified into Alphavirus genera, is also considered an arbovirus (Kuno et al. 1998; Forrester et al. 2012). These arboviruses have been reported to involve urban, as well as sylvatic transmission cycles. In case of Dengue fever, the virus has been known to jump from human population to primates through the help of *Aedes aegypti* mosquitoes. Similarly, certain arboviruses endemic in wildlife populations have the potential to infect human hosts (Figueiredo, 2019). Therefore, consequent intermingling of sylvatic and urban cycles is quite common due to a myriad of mosquito species capable of viral transmission.

Zika Virus Fever

Aedes mosquitoes are responsible for the spread of this single stranded RNA virus in human and other animal populations (Nah et al. 2016). In 1947, Zika virus was isolated from primates in Uganda, West Africa. In following years, most of the outbreaks of Zika virus were seen in temperate regions of Asia and Africa. The virus, for the most part, cycled between monkeys and arboreal mosquitoes but instances of human infections were

sporadically reported. Zika virus experiences propagation in distinct sylvatic and urban cycles. However, in most cases zone of disease emergence for humans are areas where these cycles could overlap and co-exist. Previous investigations have postulated possible involvement of 47 animal species belonging to three orders (reptile, avian and mammal), other than primates and arthropod vectors (Singh et al. 2016). Another animal model studied antigenic response of Zika virus in pregnant rhesus macaques. To this end, Zika virus isolates from confirmed human cases were inoculated into pregnant rhesus macaques, resulting in propagation of viral bodies and detection up to 105 days after administration corroborating the probability of reverse zoonotic transference of viruses (Coffey et al. 2017).

Yellow Fever

Yellow fever is a flavivirus disease transmitted by arthropod vectors. It is an endemic to tropical regions of Africa and South America despite ample availability of vaccine. The spread of this disease across continents separated by vast oceans of water has been deliberated for several years now (Monath and Vasconcelos 2015).
However, Yellow fever is generally believed to have been introduced into American continent from West Africa approximately 400 years ago (Chippaux and Chippaux 2018). The disease was first reported amongst Europeans involved in the illicit slave trading business (Hamrick et al. 2017). It has been hypothesized that most probable source of the Yellow fever on the shores of new world may be attributed to the arrival of shackled infected individuals from West African countries, who were subsequently bitten by indigenous mosquitoes, thereby spreading virus to other humans upon next feeding (Yen et al. 2018). Regarding the concept of Reverse zoonosis, hypothesis suggesting anthroponosis of Yellow fever virus to un-infected Aedes species after feeding on diseased human is extremely noteworthy (Goenaga et al. 2012). In recent years, Yellow fever has been reported in non-human primates and other sylvatic communities living closely with un-vaccinated human populations (Figueiredo et al. 2018). An example of such a scenario was reported in Espirito Santo, Brazil, where PCR and immune-histologic examinations were performed on 22 deceased non-human New World primates and Yellow fever lesions were detected in 21 primates (Fernandes et al. 2017).

Chikungunya Virus

Since its emergence in Asia during 1950s, Chikungunya virus (CHIKV) has caused severe, debilitating arthralgia, with severe morbidity in humans. It is believed that this disease probably spread across Europe and the Americas due to genetic mutations that resulted in the development of a protective glycoprotein envelop (Tsetsarkin et al. 2016). These revelations led to the rational conclusion that Asian strain of the virus propagating in urban cycles originated from ECSA progenitor, which was introduced in the environment 50–430 years before 2000 (Volk et al. 2010).
Chikungunya virus, being a single stranded RNA alphavirus commonly referred as arbovirus, is typically spread by *Aedes* mosquitoes amongst humans and non-human primates, exhibiting high potential for spillover and spillback pathogenic events (Tsetsarkin et al. 2016). Chikungunya virus has been reportedly found capable of involving sylvatic mosquito species such as *Haemagogus leucocelaenus* and *Aedes terrens* for pathogenic transmission (Weaver 2014). Several *Aedes* species have been found to be enzootic transmitters of the disease. These vectors may be responsible for disease transmission between non-human primates and other vertebrates serving as amplification hosts (Tsetsarkin et al. 2016). Data confirming cross species transmission or anthroponosis with regards to vector borne Chikungunya virus is scarce, however in experimental studies *A. furcifer* has successfully transmitted virus from infected humans to African green monkeys (Mcintosh et al. 1964). Moreover, Senegalese baboons (Monlun et al. 1993) and Congolese chimpanzees (Osterrieth et al. 1960) have also been found seropositive for this virus without clinical manifestation of any malady. Seroprevalence of this virus in non-human primates of Bahia state residing in peri-urban areas also suggests Reverse zoonosis (Forrester et al. 2012; Ndenga et al. 2017).

Dengue Fever

Dengue virus belongs to genus *Flavivirus,* which is also known as an arbovirus. Mosquito, such as *Aedes* spp., are vectors responsible for spreading this virus. Not unlike other arboviruses, Dengue virus and its reservoir vector concurrently arrived the Americas probably as a consequence of slave trade (Hanley et al. 2013). In 2009, a French study undertaken at one of its colonial territories in Guiana reported endemicity and seroprevalence of Dengue virus types 1 to 4 amongst all kinds of mammalian hosts including primates, rodents, bats and marsupials (Fouque et al. 2006). The rationalization for suggesting cross carrying of viral strains between sylvatic and urban vectors has been reinforced by studies conducted in Brazil, Ecuador, Peru and Colombia, where a common backyard mosquito *Aedes albopictus* found in urban and peri-urban areas was identified as a carrier for Dengue virus type 3 (Usme-Ciro et al. 2008). Likewise, *Haemogogus leucocelaenus,* a mosquito purely feeding upon wildlife, was discovered to carry Dengue virus type 1 in the state of Bahia (De Figueiredo et al. 2010). Presence of antibodies has also been used as an indicator to confirm the presence of sylvatic lifecycles in the case of Dengue virus. A study in the *Sapajus xanthosternos* and *Leontopithecus chrysomelas* primates indigenous to Atlantic forest of Bahia revealed the presence of antibodies against Dengue virus type 1 and 2 (Catenacci et al. 2018). Similarly, Dengue virus type 3 antibodies have been reported in Sloths (*Bradypus torquatus*) as well (Catenacci et al. 2018).

Influenza Virus

There have been reported cases of Influenza A virus subtype H1N1 being transmitted from humans to wild, companion and domesticated mammals due to contact through abiotic elements of environment (de Jong and Hien 2006; Morens and Taubenberger 2010; Messenger et al. 2014). This phenomenon is justified by the fact that the said virus is extremely resilient and capable of mass infections without any reservoir host or biological vector (Zambon 2014). Most viruses have a narrow host range, however, antigenic shift, drift and point mutations could be responsible for cross species infections (Zimmer and Burke 2009; Ma et al. 2018). In 1990s, a wild seal admitted to Dutch seal rehabilitation center was reported to be infected with human influenza B virus (Osterhaus et al. 2000). The close proximity, with humans cohabiting in close quarters with companion animals, increase the probability of Reverse zoonosis several folds, especially in case of Influenza virus that may be transmitted through contact with soft, porous surfaces (Mukherjee et al. 2012). An incidence confirming this deduction was reported in 2009, when household pet ferrets were naturally infected with human H1N1 strain (Swenson et al. 2010). Viral infections jumping across species are not exclusively seen only in companion animals, but several studies have reported such incidences in livestock as well (Janke 2014). However, the underlying factors, such as contact with contaminants, remain the primary cause of such instances. In Norway, a flock of turkeys contracted Influenza type A H1N1 virus from a diseased farm hand. Phylogenetic analysis of isolates from both species revealed identical set of strains infecting both of them (Kulberg Sjurseth et al. 2017). The transmission of H1N1 from humans to pigs is extremely widespread and cases have been identified throughout the South-East Asia (Song et al. 2010), Europe (Chastagner et al. 2019) and Canada (Howden et al. 2009).

Coronavirus

Wild animals in captivity and sanctuaries have reportedly been victims of Reverse zoonosis, especially in the case of coronavirus. Contrary to popular belief, coronavirus existed before COVID-19 outbreak in both humans and animal species (Ng and Hiscox 2020). Previously, symptoms and genetics of coronaviruses afflicting human and animal hosts were distinct. However, it was vexing when coronavirus isolates from a 2007 outbreak in Alpacas was evolutionarily quite similar to that of human coronavirus. This discovery has suggested the human to alpaca mode of transmission. Moreover, these findings have become succinctly significant in the light of recent events when a genetic relative of the same coronavirus is wreaking havoc on human population (Crossley et al. 2012). In 2016, a similar instance of human coronavirus (HCoV-OC43) transmission to wild chimpanzees housed at Taï National Park, Côte d´Ivoire was reported to cause common cold (Mackay et al. 2012). Through the years, coronavirus has been detected in all classes of the animals, ranging from ungulates to carnivores, iterating plausibility of coronavirus host switching (Patrono et al. 2018).

COVID-19

Recent COVID-19 pandemic has caused scientists to investigate the ability of household pets to serve as carriers or reservoirs of this virus. Experimentation has suggested the transmission and clinical incidence of the disease in cats and ferrets (Shi et al. 2020). The water droplets contaminated with SARS-COV-19 virus can be transmitted to cats. The fecal samples from inoculated cats exhibited viral shedding within 3-5 days. Moreover, viral RNA samples were also collected from upper and lower respiratory tract. Severe lesions were observed in lungs of kittens and mucosae of upper respiratory tract (Shi et al. 2020). Some instances of natural transmissions were reported in domesticated cats, while most experimental investigations were conducted in laboratory settings (Bosco-Lauth et al. 2020). In addition to cats, ferrets have also been observably prone to reverse zoonosis on account of COVID-19 virus (Monchatre-Leroy et al. 2021). In experimental settings, ferrets were inoculated with samples obtained from Huanan Seafood Market in Wuhan, China and clinically positive humans causing subsequent shedding of virus. Virus successfully replicated in the upper respiratory tract mucosae and remained detectable in rectal samples for prolonged period without causing death. Thirteen days post inoculation of viral isolates, mild peri-bronchitis along with other marked symptoms associated with COVID-19 were observed. Serum antibodies against SARS-CoV-2 were also detected (Shi et al. 2020). Contrarily, low susceptibility was seen after inoculation of virus into Beagle dogs whereby, only 50% of them seroconverted after 14 days (Shi et al. 2020). However, such findings were not observed when chicken, ducks and pigs were inoculated, as these species remained seronegative even 14 days after infection (Shi et al. 2020).

Measles

Measles is a common pediatric infection of viral origin caused by measles virus (MeV), which is a member of the genus Morbillivirus. Phylogenetic evidence has suggested that MeV has evolved from Rinderpest virus (RPV) around the 11th to the 12th century and is commonly associated with infections in cattle (Furuse et al. 2010). Since then, measles has wreaked havoc on human populations, causing high morbidity and mortality in infected individuals (Hilleman 1992). The outbreak reported in 1996 amongst 94 primates has since then been identified as an instance of Reverse zoonosis following urine and serum analysis were found positive for measle specific IgG and IgM antibodies. A reverse transcriptase-polymerase chain reaction was also employed for confirmatory virus isolation (Willy et al. 1999). The incidence of measles outbreak in humans precisely during the same period legitimized the said claims (Willy et al. 1999).

Rhinovirus-C

Rhinoviruses, which belong to the family Picornaviridae and genus Enteroviridae, are responsible for causing common cold in humans (Matsuzawa 2020). Interestingly, human rhinovirus-3 was never thought to be capable of jumping species. However, several reports in non-human primates withsevere flu-like symptoms have indicated a spillover effect into sylvatic communities (Velayudhan et al. 2006). Outbreak of human rhinovirus-C (HRV-C) in 2013 amongst chimpanzees has been identified by the presence of universal homozygosity for the 3 CDHR3-Y529 allele (cadherin related family member), which is a receptor that drastically increases susceptibility to rhinovirus-C infection and asthma in humans. Susceptibility of chimpanzees to a highly pathogenic virus implicates inevitable spillback into human hosts (Scully et al. 2018).

Pneumo Viruses

Human metapneumovirus (also known as MPV, Pneumoviridae, Metapneumovirus) and a human respirovirus-3 (also known as HRV3, Paramyxoviridae, Respirovirus, or formerly as parainfluenza virus-3) have been found as causative agents for spread of respiratory infection amongst Ugandan Chimpanzee community (Negrey et al. 2019).

Human respiratory viruses have become a major threat to wild non-human primate communities (Dunay et al. 2018). These respiratory maladies have become predominant causes of the disease and mortality amongst apes in wildlife sanctuaries of Uganda (Emery Thompson et al. 2018) and Tanzania (Lonsdorf et al. 2018). Reverse zoonosis of human respiratory pneumoviruses has also been reported in gorillas from Congo (Grützmacher et al. 2018) and Central African Republic (Grützmacher et al. 2016).

Parasitic and Fungal Anthroponosis

In a similar fashion of other infectious agents, parasites and fungi also make their way between humans and animals. Most of the time parasitic cycle of transmission is slower than that of bacteria and viruses due to involvement of intermediate transporters and nature of slow growth in case of parasites**.** However, parasites are able to share different hosts, including humans and animals. The fungal kingdom contains as many as 6 million species and is important in terms of the breadth and depth of its effects in areas of public health, animal health, environment, biomedical and agriculture (Taylor et al. 2014). Fungi can be naturally transmitted between animals and humans in both directions and in some cases cause significant public health problems. A large number of fungi associated with zoonotic and reverse zoonosis transmission are among the group of the most common fungal diseases worldwide. It is, however, notable that some fungal diseases have failed to attract sufficient attention in international one health efforts, leading to insufficient attention on their preventive strategies (Seyedmousavi et al. 2015).

Cryptosporidium parvum

A parasitic disease in the intestinal tract of mammals, called cryptosporidiosis, is caused by *Cryptosporidium* (*C.*) *parvum*. The infection of *C. parvum* is mainly manifested by watery, non-bloody acute diarrhea. The presence of cryptosporidiosis disease in livestock has become a considerable glitches both for animal wellbeing (together clinical and subclinical) and commercial harms (Santín 2013). These effects are due to growing veterinary amenities and work expenses, aggregate animal health-carefulness rate, and deteriorating the growth of animals along with high mortality. *Cryptosporidium parvum* was one of the most prevalent parasitic species in animals, infecting 30% of buffaloes at farm level (Inpankaew et al. 2014).

The parasite can be transmitted by two routes, including direct and indirect methods of transmission. Direct transmission occurs through feco-oral route by unintended ingestion that leads to the excretion of *Cryptosporidiu*m oocytes in feces. A parasite adopts various routes for its transmission from humans to animals or vice versa and for human to human transmission (Hubalek 2003). In humans, it causes infection by living in the epithelial exteriors of the intestines, distributed from stool or feces, and/or filthy in the atmosphere (environmental contamination). There are only a few studies for animals that demonstrate the influence of nitazoxanide drug contrary to clinical contaminations of *Cryptosporidium* spp. in which this nitazoxanide drug might reduce *Cryptosporidium* oocysts in defecation. Nevertheless, this drug is not being used commonly in animals (Ghazy et al. 2015). There is an extensive need to practice the preventive measures to reduce the spread of *Cryptosporidium* spp. particularly in livestock. By mitigating different factors, namely congestion in farmhouses, cross contamination by workers and contact with animals which bear greater risk of infection (Robertson et al. 2020).

Trichuris trichiura

Trichuris (*T.*) *trichiura* is a species that can be found in the tropical and temperate areas around the globe, however, mostly found in the dampness of the tropics (Roberts and Janovy Jr, 2000). This parasitic specie is also named as whipworm and infects animals and humans. There are two situations, namely deprived sanitation and poor environment, required for dispersion of *T. trichiura* and for the development of worm. These environmental conditions comprise a moisturizing moderate climate, wet soil, low lights and lot of rainfall (de Silva et al. 2003). The eggs of *T. trichiura* have been detected in the feces of some pigs, dogs and cats in tropical areas with poor sanitation, raising the possibility of reverse zoonoses. *T. trichiura* was also identified in mountain gorillas, and there are possibilities that these parasites

belong to human origin (Sleeman et al. 2000). A recent study in Thailand found that more than half of the *Trichuris*-positive fecal samples from dogs contained the human parasite *T. trichiura*, as identified by PCR. Likewise, more than half of the fecal samples from dogs and cats in Malaysia were found to contain eggs identified genetically as from *T. trichiura*. Whether these animals were infected with adult worms or simply shedding eggs acquired from the environment is currently unclear. (Phosuk et al. 2018; Mohd-Shaharuddin et al. 2019).

Blastocystis spp

Blastocystis is a genus in the Stramenopile group that is very diverse, comprising brown algae, slime nets, diatoms and group of water molds. These *Blastocystis* are eukaryotic organisms that are unicellular and anaerobic in nature (Hoevers and Snowden 2005). It is the most common parasitic protest globally and also a pluralistic enteric parasite found worldwide (Ahmed and Karanis 2018). The virulence mode of action of *Blastocystis* is not fully described yet. Although, it has pathogenic potential but its capability to cause gastrointestinal and various other infections has been questioned (Mohamed et al. 2017). A vast variety of animals, such as invertebrates, reptiles, amphibians, birds, mammals and humans, are infected by *Blastocystis* (Ahmed and Karanis 2018). One of the most common parasites that infect humans globally is the *Blastocystis,* as it is extensively spread worldwide. The commonly acknowledged pathway of transmission is the feco-oral diffusion and current investigations have revealed that spread occurs merely through the cyst form of the parasite (Yoshikawa et al. 2004). However, when ingestion of the cyst takes place, its life cycle is started. Different forms of cyst develop after ingestion, which possibly will turn into cyst forms sequentially. The transmission cycle is repeated when cysts are released into environment through humanoid feces to infect the humans and other animals. The contamination caused by *Blastocystis* parasites can take place by different species either through zoonosis or reverse zoonosis (Noël et al. 2005). However, studies found zoonotic isolates having large potential and stay problematic in recognizing the host roots and communication ways. *Blastocystis,* whether commensal, pathogen, or part of human microbiota, is still ubiquitous parasite cycling among humans, animals and environment. Evolution of this parasite is increasingly reported almost everywhere worldwide involving animals, humans, and even water. However, *Blastocystis* still remains underestimated and underreported in comparison to other protozoa (Ahmed and Karanis 2018).

Encephalitozoon intestinalis

Encephalitozoon (E.) intestinalis is one of the most predominant *Encephalitozoon* species found in humans around the world. This specie has been found to occur in numerous, principally native, mammalian species, for example cattle, pigs, goats, donkeys, dogs or gorillas (Hinney et al. 2016). Animal to human and human to animal transmission has been proposed to be an important source of infection. Human associated *Encephalitozoon* spp. was found in waterfowl in Slovikia (Malčeková et al. 2013). Several case reports of this parasite transmission in captive wild birds have been described. Subsequent studies on larger populations showed that asymptomatic infections in captive wild birds are widespread (Phalen et al. 2006). Close interaction between humans and non-human primates can create routes for the transmission of this zoonotic disease in both directions (Graczyk et al. 2002).

Candida albicans

Species of Candida, a yeast like fungus, cause a localized mucocutaneous disease called “Candidiasis” (Hani et al. 2015). Candidiasis is prevalent all over the world in humans and animals. Many animal cases were reported that had the same isolates as those of humans (Wrobel et al. 2008). Canine isolate was obtained from a graduate student-owned dog and two feline isolates were obtained from animals at the Champaign County Humane Society analysis of the domestic animals. *Candida albicans* isolates showed that the cats and dogs were colonized with common human isolates. The pets tested were all indoor animals that were in close contact with humans (Wrobel et al. 2008). Parakeet (bird) was found infected with *C. albicans*. It was also a case of reverse zoonosis (Kumar et al. 2017). Ingestion of contaminated food or drinking water is the usual means of transmission. Contaminated environment, such as litter from diseased and game bird rearing in the same amenities and areas contaminated with human waste, are suggested as sources for Candidiasis exposure to birds (Kumar et al. 2017). The consensus drawn from various texts is that *C. albicans* is associated mainly with humans, other mammals, and avian species (Wrobel et al. 2008).

Trichophyton rubrum

Trichophyton (T.) rubrum belongs to phylum Ascomycota. It is dermatophytic, anthropophilic, saprotrophic fungus and exclusively clonal, which colonizes the upper layers of dead skin cells (Gräser et al. 1999). *Trichophyton rubrum* is a cosmopolitan, anthropophilic species and is the most usual causative agent of human dermatophytosis across the planet. Despite being anthropophilic, this dermatophyte is also infrequently found to be reason of dermatological disease in dogs. Therefore, *T. rubrum* can also be considered as an anthropozoonotic and zooanthroponotic pathogen (Overgaauw et al. 2020).

Microsporum gypseum

Microsporum gypseum is a soil-associated dermatophyte that is recognized to colonize and infect the upper layer of skin in mammals (Samanta, 2015). Tinea or ringworm is the fungal infection, which affects external body parts. It

is one of the fungal diseases which are transferred from humans to animals and humans to humans (Summerbell and Howard, 2003). People engaged in experimentation and handling with laboratory animals can also be at risk of contracting the infection, if the animals were predisposed to a certain region (Ranganathan and Balajee 2000). Animals such as cattle, which repeatedly come in contact with soil, are predominantly affected by this fungus, but equines, rodents, apes and canines have a disposition to acquire it (Messenger et al. 2014). A dog was found positive with *M. gypseum,* which is a case of zooanthroponosis (Sharma et al. 2009); though the soil is usually considered as the prime reason of infection. Analytically, at least 10 fungal spores must deposit on a keratin rich skin surface of animals or humans to manifest a fungal infection (Messenger et al. 2014). Diagnosis is usually made on the shape of the fungus on the host through a sample culture.

Conclusion

Reverse Zoonosis is a reality and is playing an important role in the sufferings of the animals. During the past few decades, human animal interaction, especially with the wild life, has increased. Transmission of interspecies pathogens could be direct or through vectors. All types of pathogens i.e., bacteria, viruses, protozoa, and fungi have capability of reverse zoonosis. Although it is difficult and labor extensive work to consider animal factors while studying a disease outbreak in humans, yet it is need of the time, as the evidences are strong for inter species disease transmission. The countries having higher population density like Pakistan, have higher chances of human to animal transmission of pathogens, as they are residing in close vicinity. There is need to focus on this aspect of research and study in Pakistan. In the light of COVID-19 disaster, the thinking horizon of the scientist has now changed. There will be more focus on 'One Health' for one globe rather than only human health. Because health is important, host specie doesn't matter now.

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Section D

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AQUATIC FUNGI AND IMPORTANT FUNGAL DISEASES OF FARMED FISH

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INTRODUCTION

Fisheries and aquaculture are the most important sources of animal protein and this sector provides employment to around 31.1 million people globally (Gozlan and Britton 2014). Disease outbreaks in animals, including fish, result in significant economic losses. Many infectious agents like bacteria, virus, fungi and parasites cause diseases in fish. Bacterial and fungal diseases are two top reasons for economic losses in aquaculture (Gonçalves and Gagnon 2011). Fungal diseases are becoming one of the most important emerging problems with increasing incidences, mainly because of their wide geographic range and increase in virulence (Loo 2009; Peeler *et al.* 2010). Many infectious fungi have now been discovered and many of them have found new hosts (Kim and Harvell 2004; Frick *et al.* 2010). The exact reason behind the increase spread and virulence of fungi is unclear, however, their resilient nature (Mitchell *et al.* 2008), opportunist behavior (Fisher *et al.* 2012), active and long life cycle (Andreou *et al.* 2009) and spread of invasive and infectious species can be the major contributing factors (Gozlan *et al.* 2010). Outbreaks of fungal infections are increasing rapidly and are responsible for local extinctions of many species, including bees, amphibians, turtles and other reptiles, bats and fish (Gozlan *et al.* 2005, 2009; Fisher *et al.* 2009; Frick *et al.* 2010; Ratnieks and Carreck 2010; Sarmiento-Ramírez *et al.* 2010).

Fish are more susceptible to fungus and fungus like micro-organisms. Despite the huge mortalities of fish species caused by fungal and fungal like pathogens, it is difficult to detect infections primarily due to lack of direct observations and also due to absence of external signs and symptoms (Gozlan *et al.* 2010; Gozlan and Britton 2014). Hence, a healthy fish without external signs may be carrier of fungal pathogens that may result in massive infection and high mortalities (Andreou *et al.* 2011). Fungi causing infections in fish are mostly saprophytic that get nourishment from dead and decaying organic matter. The saprophytic fungus infects almost all stages of fish, including eggs, fry, fingerling and adult stage. Fungi may be the secondary invaders, as they attack the host that is already injured mechanically or suffering from an infection other than fungi (Roberts 2012).

This chapter focuses on true fungal pathogens of fish and also highlights the diseases caused by fungi and fungal like pathogens and their impact on fish populations. Moreover, the detection methods and their limitations have also been discussed.

Fungi and their Classification

Fungi are eukaryotes and like other living organisms, they are identified on the basis of their morphological characters like shape, structure and behavioral properties. In early 1990's, stains were used to identify fungi but with advancement of technology, various other methods like pattern of nutrient utilization, growth temperature and rate testes were used for fungal identification (Raja *et al.* 2017).

Traditionally, fungi are grouped as single independent cell *i.e.* yeast or fungi that have thread like hyphae called molds or hyphal fungi. Asexual reproduction is common in fungi. Sexual reproduction also occurs in almost all fungi, except deuteromycetes previously known as *fungi imperfecti*. Sexual reproduction results in the formation of spores and spore bearing hyphae, called as mycelium. Spores have different names, such as zygospores produced by fungi belonging to Zygomycota. Motile diploid spores are produced by Chytridiomycota, basidiospores are produced within basidia by fungi belonging to Basidiomycota, while ascospores are produced within asci of fungi belonging to Ascomycota (Wyatt *et al.* 2013).

Traditionally, fungi were classified into:

i. Ascomycota
ii. Basidiomycota
iii. Zygomycetes
iv. Chytrids

This classification has now been revised. Fungi now include true fungi, fungus like organisms and molds. True fungi are most diverse group of organisms classified into six phyla, 35 classes and 129 orders (Fig. 1).

Chytrids are most primitive and simple form of true fungi. Their cell wall contains chitin, except for one group that contains both cellulose and chitin. Most members of phylum Chytridiomycota are unicellular but some hyphal forms also exist, which have aseptate hyphae. Spores produced by chytrids are diploid with a single flagellum.

Phylum Zygomycota, also known as conjugated fungi, includes bread molds which propagate on bread, fruit and vegetables. The important genus of this phylum is Rhizopus. Asexual reproduction is through sporangiospores, while sexual reproduction is through formation of diploid zygospores that undergo meiosis and develop into new organisms.

Most of true known fungi belong to phylum Ascomycota, also known as sac fungi. Members of this phylum are

economically important. The phylum includes morels and truffles, which are used as food, for example yeast is used in baking fermentation and brewing, *Aspergillus oryzae* is used to produce sake, while some members are parasitic. Asexual reproduction is through conidiospores, while sexual reproduction is through ascospores that are produced in fruiting body called ascocarp.
Basidiomycota, also known as club fungi, are easily recognizable under light microscope due to the presence of club shaped fruiting body called basidia. Members of Basidiomycota are mushrooms, smuts and rusts.

Deuteromycete, also known as *fungi imperfecti* and belonging to Deuteromycota, are the only fungi that reproduce asexually. They are closest to ascomycetes and the mycelium spread on substrate like a mold. Members of this phylum include some economically important fungi. Some are used in cheese making, others are used to make antibiotics (like penicillium), while some produce toxic metabolites like mycotoxin producing fungi, aspergillus.
Most of the fungal species responsible for infection in fish belong to Ascomycota (Hibbett *et al.* 2007) and some belong to phylum Zygomycota orMesomycetozoea (Glockling *et al.* 2013). Various species in these phyla are opportunist and not exclusive to fish. Some cause infections in plants *e.g., Phoma herbarum* and *Penicillium corylophilum,* while others cause infections in immunosuppressed humans *e.g., Ochroconis humicola* and *Exaphiala xenobiota*.
Fungal like parasites belong to oomycote, also known as water molds, and include more than 500 species (Beakes *et al.* 2012). Oomycetes resemble morphologically to fungi, as they have filamentous growth and similar feeding behavior. The main difference is the composition of cell wall. The cell wall of fungi is made of chitin, while that of oomycetes is made of glycan and other cellulosic compounds. Moreover, the nuclei of fungi are haploid, while those of oomycetes are diploid. Important genera of order Saprolegniales that cause infections in fish are Saprolegnia, Aphanomyces, Achlya and some are from genus Pythium. Two most important species are *Saprolegnia parasitica* and *Aphanomyces invadans,* causing infections in 12 and 42 fish species, respectively.
Hyphochytriomycota are fungus like zoospore producing organisms. They are most closely related to algal groups. They have been previously classified with Protiscta and Protoctists (Whittaker 1962; Margulis 1990).
Slime molds are other fungus like organisms and have two main groups; cellular slime molds and plasmodial slime molds. Cellular slime molds (Dictyosteliomycota) are singular ameboid cells but on getting a signal they start aggregating to form a giant amoeba or slug that starts acting as single cell. Plasmodial slime molds (Myxomycota) are formed when many flagellated cells fuse together. The resulting giant mold is a big bag of cytoplasm with thousands of nuclei (Volk 2013). Plasmodiophoromycota are endoparasites and don't have cell walls in their assimilative states.

Important fungal disease in aquaculture

In order to reduce environmental footprints, increase biosecurity and to have better control on culture conditions, aquaculture practices are shifting towards intensive aquaculture. High stocking densities in intensive aquaculture systems may lead to stress in fish and increase incidents of disease outbreaks (Peeler *et al.* 2010). Fungal spores are present everywhere, and poor water quality and stress in fish are two main factors that contribute to the disease outbreak in aquaculture system. Other factors contributing to fungal infections are; poor hygiene, low immunity and high amount of decomposing organic matter at the pond bottom (Verma 2008). Important fungal diseases and their respective hosts are listed in Table 1.

Saprolegniasis

Saprolegniasis, commonly known as cotton wool disease, is caused by *Saprolegnia* sp. (*Saprolegnia parasitica* and *Saprolegnia diclina*), belonging to family Saprolegniaceae (Table 1). These two species are most commonly associated with saprolegniasis infection and, hence often written as *S. parasitica–diclina* complex (Rezinciuc *et al.* 2014). Saprolegnia is a filamentous fungus with branched and aseptate mycelium. It is a classical opportunist saprophytic fungus that feeds on dead decaying matter.
Saprolegniasis is the most common infection of freshwater and estuarine fish present in warm tropical water. Temperature plays an important role in saprolegniasis infections. Chances of infection increase as water temperature decreases below the physiological end point for fish (Roberts 2012). This may be due to the fact that many oomycetes are active in cold water. Severity of infection increases when water temperature hits 15°C, resulting in high fish mortality which is also called winter kill. Stress in fish resulting from high water temperature also results in disease outbreak.
Unfertilized fish eggs are infected with Saprolegnia and infection spreads to fertilized and healthy eggs, resulting in the complete loss of the batch.

Diagnosis

Saprolegniasis is characterized by the presence of white cottony growth on the fish. Skin tissue is the main infected organ. However, eyes and gills may also be infected with the fungus. Saprolegnia, a water mold, is a fungus like pathogen not a true fungus (Latijnhouwers *et al.* 2003). The hyphae of saprolegnia are aseptate, while hyphae of true fungi are septate. The common diagnosis is based on the presence of cotton like growth on gills and skin. Microscopic observation of aseptate and broad hyphae that can be stained with hematoxylin and eosin and also with Gomori methenamine silver stain is also an indication of saprolegnia infection (Noga 2010). Fish appear lethargic, stop responding to external stimuli, show loss of balance and die.

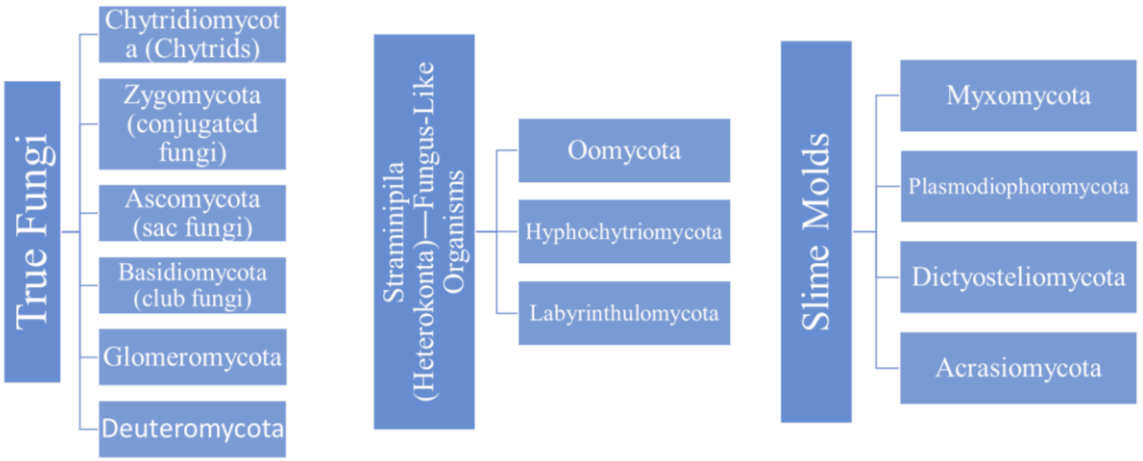

Figure 1: Classification of fungi based on scheme presented by Deacon (2006).

Table 1: List of fungal pathogens of fish and their reported hosts

	Fungal species	Order	Reported host
True Fungi	*Exophiala angulospora*	Chaetothyriales	Atlantic cod
	Exophiala pisciphila		Zebrafish shark
	Exophiala xenobiotica		Striped jack
	Paecilomyces lilacinus	Eurotiales	African catfish, Nile tilapia
	Penicillium corylophilum		Red snapper
Fungus	*Dermocystidium cyprini*	Dermocystida	Common carp, Eurasian ruffe
Like	*Dermocystidium fennicum*		European perch
Organisms	*Dermocystidium koi*		Common carp
	Dermocystidium percae		European perch
	Dermocystidium branchiale		Brown trout, Arctic char
	Sphaerothecum destruens		Common carp, common bream, sunbleak, rainbow trout, Atlantic salmon
	Ichthyophonus hoferi	Ichthyophonida	speckled sanddab, Atlantic herring, surf smelt, coho salmon, chinook salmon, rainbow trout, brown trout
	Ichthyophonus irregularis		yellowtail flounder
Oomycetes	*Achlya bisexualis*	Saprolegniales	Grey mullet
	Achlya racemose		Pejerrey
	Achlya americana		African catfish, Nile tilapia
	Achlya oblongata		European white fish
	Achlya ambisexualis		Rainbow trout
	Aphanomyces frigidophilus		Brown trout, European whitefish
	Aphanomyces invadans		European eel, barbs, African catfish, channel catfish, Channa sp, common carp, Indian major carps, rainbow trout, grey mullet, breams
	Aphanomyces irregularis		
	Aphanomyces laevis		African catfish, Nile tilapia
	Saprolegnia brachydanis		Zebrafish
	Saprolegnia diclina		Persian sturgeon, brown trout, red drum
	Saprolegnia shikotsuensis		European white fish
	Saprolegnia ferax		Brown trout, rainbow trout, pejerry, goldfish
	Saprolegnia furcate		Brown trout
	Saprolegnia hypogyana		Rainbow trout, brown trout
	Saprolegnia parasitica		Rainbow trout, brown trout, Channel catfish, Atlantic salmon, masu salmon
	Saprolegnia polymorpha		Common carp
	Saprolegnia salmonis		White spotted char, brown trout, rainbow trout, red salmon, masu salmon
	Pythium aquatile	Pythiales	European white fish
	Pythium pulchrum		
	Pythium thalassium		
	Pythium torulosum		

Treatment and control

Once infection is started, it is usually progressive and terminal. Treatment of saprolegnia has gained much attention and many chemicals have been tested against fungal growth *in vitro*. External disinfectants are usually used but most of the legally approved disinfectants have limited effect. While many fungal diseases are difficult to treat, the best preventive measurements are maintaining good water quality parameters, husbandry practices, improved feeding and avoiding overcrowding. Localized treatment of wound with 0.1% malachite green or bath treatment (0.2 ppm for an hour or indefinite with 0.1 ppm) is usually recommended. Use of malachite green is

prohibited in many countries due to its mutagenic and teratogenic effects. Environment with high salt concentration limits saprolegnia growth, that's why the infection is rare in marine fish. Bath treatment with sodium chloride (30 g/l for half hour or 1-3 g/l for indefinite time) is helpful in treating saprolegnia infections. Bath treatment with potassium permanganate for 30-90 minutes at the rate of 1g per 100 liters of water is effective. Other treatments include bathing with copper sulphate for 10-30 minutes (5-10 g per 100 liters of water) or with 10-25 g sodium hydroxide for 10-20 minutes (Dorcas *et al*. 2015).

Branchiomycosis

Branchiomycosis, commonly known as gill rot, is another important fungal infection of fish. Similar to saprolegnia, two species of *Branchiomyces* are responsible for gill rot. *Branchiomyces demigrans* causes infection in bass (striped and largemouth bass), pike and tench, while *B. sanguinis* causes infection in carps.

Environmental stressors, like low dissolve oxygen, low pH (5.8-6.5) and high algal blooms, are responsible for the disease outbreak. Fungal spores are carried through detritus on pond bottom and dispersed through pond water. Fungus can grow at water temperature range of 14-35°C but grows fast when water temperature ranges from 25-32° C.

As the name indicates, this fungus causes pathology of gills. Both species of *Branchiomyces* cause ischemia and thrombosis of gills and gills becomes marbled or striated in appearance. Infection of *B. sanguinis* is restricted to blood vessels of gills, while that of *B. demigrans* spreads from blood vessels to the lumen. Fish becomes lethargic and swim near water surface to gulp air (El-Sayed 2020).

Diagnosis

Externally, the gills of infected fish may appear pale with brown area because of hemorrhage, or grayish due ischemia. As the infection progresses, the narcotic gill tissue may slough off and fish might get a secondary infection of saprolegnia.

Fungal hyphae penetrate in the gills and obstruct the blood vessels, resulting in necrosis. As gill damage progresses, fish mortality starts due to anorexia. Disease outbreak results suddenly with a mass mortality up to 50% in 2-4 days.

Histopathology and wet mount analysis of the lesions are used to diagnose branchiomyces infection. Hyphae penetrated deep in the gill lamellae are characteristic of the infection (Yanong 2003).

Treatment and control

Branchiomycosis infection can be treated with 0.3 ppm of malachite green for 24 hours. One hour exposure of 1-4 ppm of benzalkonium is also used for the treatment. Common chemicals used for treatment are 3-5% sodium chloride or 100 ppm copper sulphate for 10-30 minutes.

Reducing water temperature below 20°C and reducing organic load of the pond is helpful in slowing fungal growth. Treatment with formalin can also help in lowering mortalities. All the water holding sites, raceways and aquaria must be dried and treated with calcium oxide (quick lime) (Noga 2010).

Ichthyophoniasis

Fungal infection 'Ichthyophoniasis' is caused by fungus Ichthyophonus. This species infects cod, herrings, groupers and trout.

Diagnosis

Clinical signs and symptoms usually vary according to the fish species. Infected fish show erratic swimming pattern, swollen belly, anemia along with loss of appetite. Internally, swelling of vital organs like liver, kidneys and spleen are reported with white nodules. Such white nodules are also apparent in the muscles of infected fish.

Treatment

No specific treatment is reported for the affected fish.

Epizootic Ulcerative Syndrome (EUS)

Aphanomyces invadans or *A. piscicida* is the causative agent of Epizootic Ulcerative Syndrome (EUS). This fungus causes the disease in more than 30 freshwater species. Catfishes and snakeheads are most commonly infected.

Diagnosis

Infected fish show loss of appetite and swim below surface water or with head protruding out of the water surface. Occasionally, fish may show jerky movements and appear very hyper active. Darker skin discoloration, along with ulcerative lesions on the body, is indicative of EUS. Lesions may be small with rosacea appearance and restricted only to sides of jaw and head or deep lesions may be present anywhere on the body.

When the disease progresses, lesions become very deep and visceral organs or the vertebral column may be exposed. Tail erosion is also common in infected fish. Internally, the fungus penetrates fish muscles, resulting in inflammation and severe tissue necrosis. Mortality occurs when fungal hyphae reachcranium and kidneys.

Treatment and control

Outbreaks of EUS usually occur in cold weather from December to February. Therefore, special care should be taken in these months to avoid the disease outbreaks. Disinfecting the pond and water supplies and reducing fish stocking density can also help in limiting the disease outbreaks.

Treatment of infected fish with chelated copper compounds, like 5 ppm of control or with 0.1 ppm of malachite green, may be helpful in treating the EUS infection.

Current detection techniques and future perspective

Pathogens are traditionally characterized by their morphological, serological and phenotypic properties. Lesions are normally developed in fish after infection. Swab sampling from the area of lesion, isolating and culturing of the causative agent is the usual practice to identify the pathogens and to understand the disease etiology and host-parasite relationship. However, the procedure is time consuming with a chance of contamination and requires technical expertise.

Grocott's methanamine silver stain is used to stain the fungal species isolated from the lesions. With advancement of science, fluorescent probes have been designed which are species specific and used for direct visualization of the infectious agent. Immunofluorescent identification of *Aphanomyces invadans* and *A. astaci* is made using a monoclonal antibody, MAb 3gJC9, specific for antigen involved in pathogenicity of these species (Miles 2003). This approach has proved to be better over traditional staining identification of the fungus. Moreover, with the use of immunofluorescent detection, infections are detected at very early stages. Fluorescent *in situ* hybridization (FISH) is also used to identify fungal infections (Sosa *et al.* 2007a&b). Improvements in molecular techniques have led to a more accurate identification of fungal pathogens compared to previously used staining methods. Species, sub species and strains level identification is now possible with the use of polymerase chain reaction (PCR) (Phadee *et al.* 2004; Tsui *et al.* 2011). Use of similar DNA fragments to identify the species offers benefits of understanding phylogenetic relationship among species. Primers specified for 18S rRNA are used to identify large number of fungal species, as well as mesomycotozoans; however, oomycetes are more accurately identified using Internal Transcribed Sequence 1 (ITS1).

There is a huge gap of availability of genetic data present in public data bases, such as Genbank. Therefore, it is need of hour that all the regional cases of disease outbreak should be identified at molecular level, sequenced and data be submitted to public data bases, so that more information may become available. Detection of species using 18S rRNA and strains using ITS1 will provides a better knowledge of virulent pathogens. Also, the molecular data will resolve phylogenetic ambiguities among fungal species.

Conclusion

In conclusion, the chapter described the important fungal and fungus like pathogens of farmed fish. It highlighted the existing knowledge about diagnosis and treatment of fungal diseases and also pointed the gaps that need to be addressed in order to prevent epizootic diseases. Most of the fungal infections in aquaculture appear when fish is under stress. Proper farm management is the key to avoid infections and reducing economic loss. Due to the lack of specific treatments for fungal diseases, generalized treatment strategies are opted to control fungal diseases. Chemicals used for treatment of fungal diseases are considered hazardous for human health and are therefore banned in many countries including USA and EU. Maintaining proper water quality, feeding a balanced diet and proper handling of fish can avoid stressful conditions, leading to less disease outbreaks. Recently, use of medicinal plants and herbs in aquaculture has contributed to successful control of fungal infections. Use of plant-based additives are cost effective and eco-friendly, however, further research is required to explore the potential of using plant and plant based materials to control fungal diseases in aquaculture.

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Section E

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RECENT ADVANCES IN BIOTECHNOLOGY IN ANIMAL HEALTH

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INTRODUCTION

The past 30-35 years have witnessed rapid development in the field of biotechnology; the applications which were once only anticipated ideas of technological expertise have finally come to fruition. With the increase in population, changing diet patterns, changing purchase priorities and urbanization–conventional methods of improving food productivity can no longer serve the augmenting consumer demands. It is of no surprise that human health and welfare is directly linked to animal health and welfare, thus where conventional methods are failing to keep up with the rapidly changing trends, modern biotechnological techniques, in contrast, provide pioneering opportunities for enhancing livestock yield, productivity, health, and welfare. These include the deployment of various modern biotechnological tools (Figure 1), which have effectively contributed towards meliorating product enrichment; ensuring food safety; development of powerful diagnostic and therapeutic tools; catering efficient utilization of scarce resources and shrinking the livestock environmental footprint. High end objectives, such as to promote resource sustainability, increase profitability and alleviate food insecurity, are now being widely pursued side by side, however, implications in respect of legal, bioethical and ethno-religious nature continue to pose challenges to the progression of these intense advancements. This paper reviews the multiplicity of biotechnological applications in animal nutrition and health and attempts to evaluate the potential risks involved in projected undertaking of these modern practices.

Utilization of feed additives and other exogenous substances for livestock improvement

As the human population is roughly estimated to surge from 7.6 billion (2017) to 9.8 billion by the year 2050 (Nations 2017), various reports have predicted the need of either doubling up (Tilman et al. 2011; Alexandratos and Bruinsma 2012) or at least grow livestock productivity (termed as "Livestock Revolution") by 25-60% in the coming years (Hunter et al. 2017) to satisfy the predicted increase in demand for milk and meat (Thornton 2010; Alexandratos and Bruinsma 2012). Three major constraining factors are widely known to influence the productivity of livestock sector; these are (i) low quality feed resources, (ii) high feed cost (Sujani and Seresinhe 2015; Aslam et al. 2020), and (iii) presence of anti-nutritional factors (Ramteke et al. 2019), as shown in Figure 1, these result in weight loss, compromised immunity, and natural abortions etc. The quality of meat or its by-products is massively influenced by the type and quality of feed stuff fed to livestock (ruminants and non-ruminants alike). Animal nutritionists have, therefore, developed and practiced various chemical, physical and biological methods of feed improvement over the past few decades. With the growing awareness regarding food safety, biological methods have gained the most prominence and acceptance at large which include efforts to formulate highly nutritional and profitable growth promoters and promising feed additives (Sujani and Seresinhe 2015). The utilization of a variety of nutrient/non-nutrient feed additives, or exogenous health improving drugs, have therefore proved to be effective options for improving the plane of nutrition, enhancing nutrient utilization by animals and reducing food waste. Common examples include immunostimulants, pre-/pro-biotics, essential oils, feeding attractants, antibiotics and other inclusions such as binders, acidifiers, vitamins, enzymes and hormones, which can either be administered as feed additives or subcutaneous dosage. The use of feed additives is a commonly adopted practice for enhancing swine and poultry production and is now gaining recognition for ruminant diets as well. Biotechnological advances have a fair share in adding convenience to the production and availability of these compounds, the following is an account of some major commercially available bio-engineered products manufactured through applications of modern biotechnology.

Enzymes

Enzymes have been used directly or indirectly throughout human history in food manufacturing (Campbell and Bedford 1992; Classen 1993; Perry 1995; Bedford 1996; Bhat 2000; Buchholz and Bornscheuer 2017). The commercially produced exogenous enzymes, such as phytase, gulcanase, and cellulase, are added to feedstuff to improve its nutritive value which is otherwise hampered due to the presence of anti-nutritive and indigestible factors. 'Phytase' is the most commonly used enzyme-additive which when added to monogastric feed, increases the digestibility of phytate (an inositol hexakisphosphate) from around 25% to 50-70% (Ravindran et al. 1999; Ravindran et al. 2000; Kornegay 2001) and, therefor, increases the bioavailability of phosphorus for non-ruminant livestock (Fuller 1989). Phytase is also known to improve the digestibility of amino acids (Zhang and Kornegay 1999) and other nutrients, and improves feed

energy value as well (Ravindran et al. 1999; Ravindran et al. 2000; Kornegay 2001).

Xylanase (also pentosanase), a family of enzymes known to break xylan (component of hemicellulose) into digestible xylose, is another such example used as a feed additive in monogastric diets. Likewise, glucanases and cellulases are widely used to improve the ileal digestibility of non-starch polysaccharides (NSPs) (Hesselman and Åman 1986; Broz and Frigg 1986; Newman and Newman 1987; Campbell et al. 1989; McDonald et al. 2010). The use of fibrolytic enzymes in feeds to improve digestibility of fiber is also a well-adopted practice in livestock farming, especially in monogastric diets. Although, the rumen microbiome has the natural ability to digest the plant lignocellulosic biomass and convert it into high quality milk and meat for human consumption, studies have proved that the use of exogenous fibrolytic enzymes in ruminant diets also renders beneficial effects of increasing the digestibility of neutral detergent fibre in dry matter diets and crop residues to support higher productivity (Oba and Allen 1999; Jung et al. 2004; Tirado-Gonzalez et al. 2018; Refat et al. 2018; Beauchemin et al. 2019). In short, by increasing the digestibility of components like lignin, cellulose, hemicellulose, non-starch polysaccharides and fiber, these enzymes help maximize feed productivity (Prasad and Roy 2018), nutrient digestibility (Salem et al. 2011) and consequentially promote animal growth and production (Sujani and Seresinhe 2015).

Industrial production of bioengineered enzymes is now steadily growing; most of these are extracted from GRAS-status (Generally Recognized as Safe) microorganisms, including bacterial, fungal and yeast sources; a few, however, are extracted from plant or animal tissues as well. Usually, either the production organism or the product enzymes are genetically modified to achieve the desired enzyme yield and functional efficacy. The microbial population is the most exploited source for enzyme production, as it provides easy and high yield harvesting (Filer 2003); *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis* and *B.stearothermaphillus* are the most extensively reported industrial enzyme producers (Aslam et al. 2020). Moreover, the vast amount of microbial genomic information at hand and the increased understanding of microbial gene expression systems, favor the forging for suitable microorganisms from newer microhabitats. Enzyme production method from microorganisms is simple and a general step-wise procedure is outlined in Figure 3. Submerged liquid fermenters are the traditional choice for harvesting microbially derived enzymes. Moreover, solid-substrate fermenters are also used as an alternative (Singhania et al. 2010).

Hormones

Russel Marker, a chemistry professor at The Pennsylvania State College, invented a degradation process to produce affordable steroidal hormones in 1938. This process was named "Marker Degradation" which is also known as "His path-breaking process" and became the founding stone of the steroid industry in Mexico. The process to produce progesterone from diosgenin is shown in Figure 4. It was precisely this process which helped Marker to produce his first kilo of progesterone and within a year the Mexican steroid company, Syntex, was selling progesterone for $50 per gram.

In the following years, due to issues related to profit shares, Marker shifted his work to another company in Mexico, the Gedeon Ritcher Limited, and started producing progesterone under the name Hormonosynth, which was later reorganized as Diosynth (Bohning and Morris 1999). This marked the introduction of synthetic

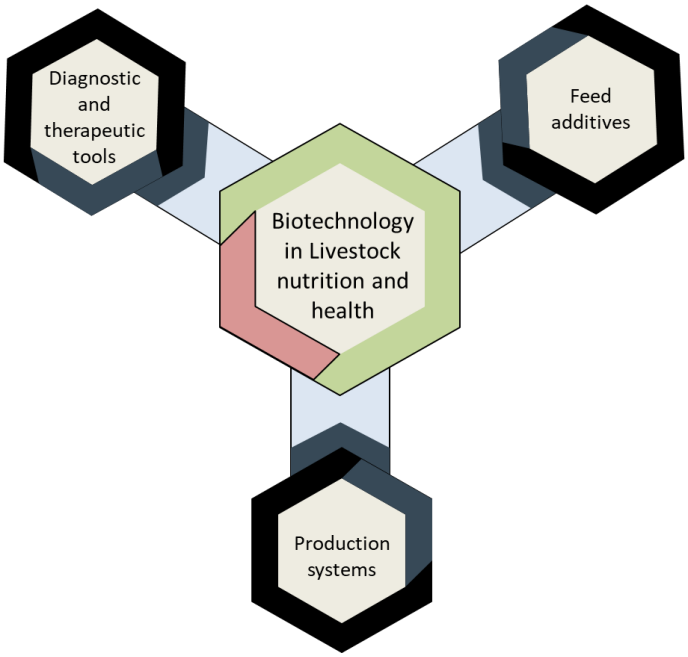

Figure 1: Major biotechnological domains in livestock improvement.

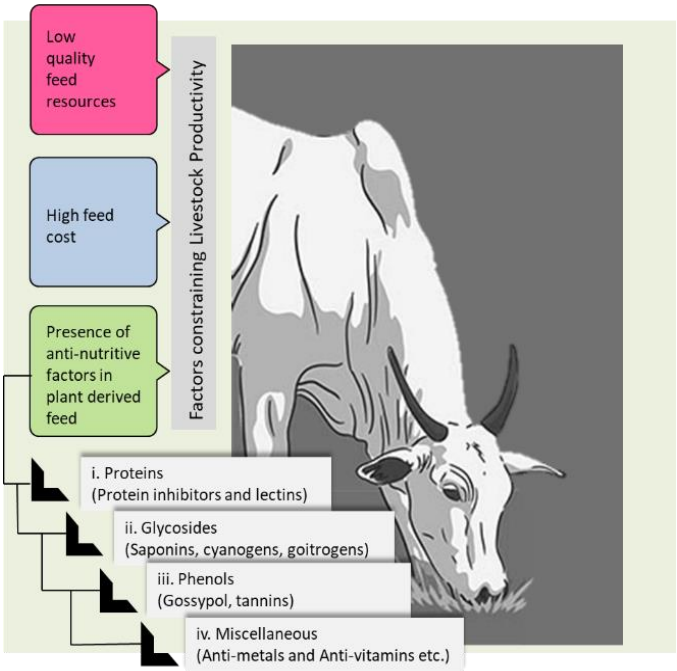

Figure 2: The major constraining factors known to negatively influence livestock productivity.

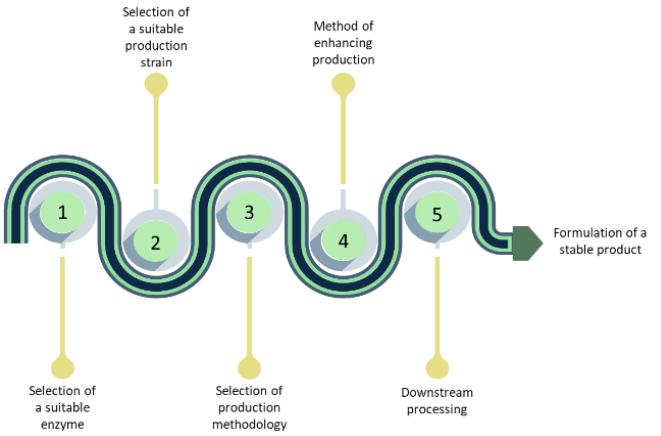

Figure 3: Steps involved in microbially derived commercial enzyme production.

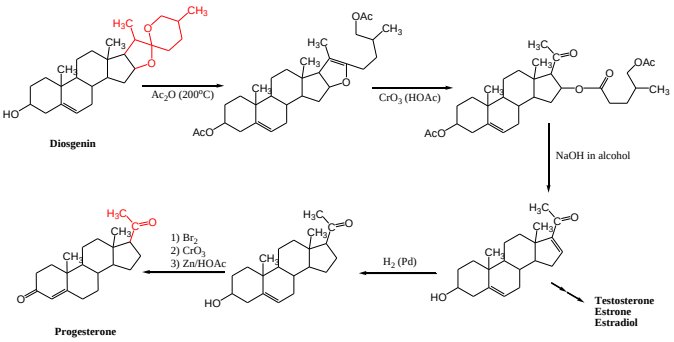

Figure 4: The series of steps involved in production of progesterone from diosgenin, through the process called 'Marker Degeneration'.

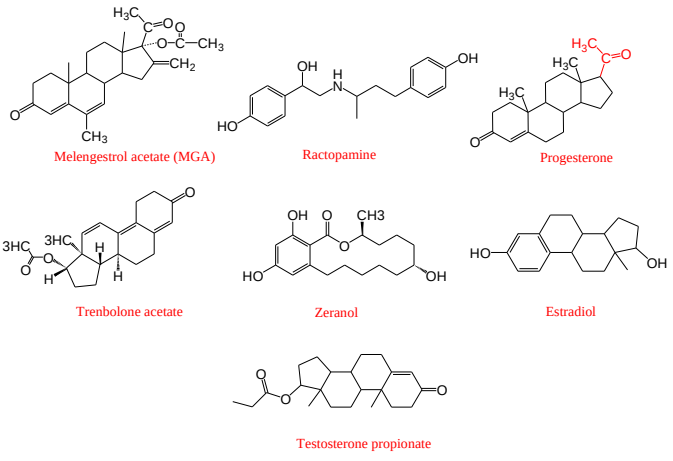

Figure 5: Chemical structures of Melengestrol acetate (MGA), ractopamine, progesterone, trenbolone acetate, zeranol, estradiol and testosterone propionate – the only hormones approved by the FDA for use in food animals. MGA and ractopamine are approved as livestock feed additives, whereas the rest of them are used in the form of 'pellets' which are installed subcutaneously, most probably as 'ear implants' where the pellets are installed at the back of the ear and is discarded before the animal is utilized for its meat or byproducts to avoid health complications. The risk, however, remains even after the pellet region is removed and so use of hormone pellets – no doubt its positive potential – is a rarely adopted method in livestock farming.

hormones in the market; however, the public acceptance grew slowly and the application of exogenous hormones to promote growth is still taken to be the least encouraged practice of all.

Only two hormones, melengestrol acetate (MGA) (Meissonnier and Mitchell-Vigneron 1983) and ractopamine (Marchant Forde et al. 2003), have yet been authorized by the US Environmental Protection Agency and the FDA for use as feed additives. MGA (synthetic progesterone, structure shown in Figure 4) belongs to the most active synthetic gestagens and is used for estrus synchronization or inhibition in cattle or beef animals (Duncan et al. 1964), therefore improving their weight gain and feed efficiency. Ractopamine, on the other hand, is a phenol-based agonist of TAAR1 and β-adrenoreceptors; and is known to promote leanness in meat, along with increasing food digestibility in farm animals (Colbert et al. 1991; Liu et al. 2014). Various other natural and synthetic hormones are also administered to livestock but these are in the form of implants, especially ear-implants, and are directed towards enhancing growth by interfering with the hormonal cycles of recipient animals. Unlike other feed additives, the use of hormones is rather restricted due to the solemn health-associated risk factors. Ractopamine, for instance is approved only in certain regions of America and banned in almost all others. Various other natural/synthetic-hormone-based products, however, are administered otherwise in the form of pellets to increase animal growth rate and reduce lipid deposition. Overall, these hormones are androgenic, corticosteroidal, estrogenic, progestagenic, β-adrenergic and thyreostatic compounds, which can be administered separately or in combinations as required (Stephany 2009). The FDA has approved a total of six hormone drugs, namely; bovine somatotropin (peptide hormone), estradiol, progesterone, trenbolone acetate (anabolic steroidal ester), testosterone propionate (anabolic androgen steroid), and zeranol (non-steroidal estrogen agonist) for use in animals to enhance growth and productivity (FDA 2021), as shown in Figure 5. None of the hormones are approved for use in swine or poultry, however in ruminants the results include weight gain, improved feed efficiency and increased milk production in dairy animals. Table 1 highlights the indications, availability status and livestock species approved for hormone administration.

After Marker's success story, in year 1951, a microbial process of converting oxidized progesterone to cortisone was introduced by scientists at another Mexican company, the Upjohn Co., using Syntex's progesterone (Bohning and Morris 1999). Since then, the industrial production of steroidal hormones and drugs, which are otherwise difficult to obtain through conventional methods, has become the best known application of microbial biotransformation (Fernandez et al. 2003; Fernandez and Cabral 2010; Vom Steeg and Klein 2017; Cano-Flores et al. 2020). Figure 6 shows how bacteria are utilized to produce recombinant bovine somatotropin (rBST) (Shakweer 2008); rBST is administered to increase milk production in dairy cows and improve feed efficiency as well. Likewise,

Table 1: The attributes of different hormones used in livestock industry

Attributes	Hormone						
	BST	E2	T	P	TBA	Z	MGA
Approved for -	Dairy	Steer, Bull, Heifer, Calf	Heifer	Steer, Bull, Calf	Calf, Bull, Steer, Heifer, Cow	Steer, Bull, Calf, Sheep, Lamb, Cattle	Beef
• Weight gain • Feed efficiency • Estrus • Milk production							
Availability -	OTC	OTC	OTC	OTC	OTC	OTC	OTC

**BST*: Bovine somatotropin, *E2*: Estradiol, *T*: Testosterone, *P*: Progesterone, *TBA*: Trenbolone acetate, *Z*: Zeranol, *MGA*: Melengestrol acetate, *OTC*: Over the counter.

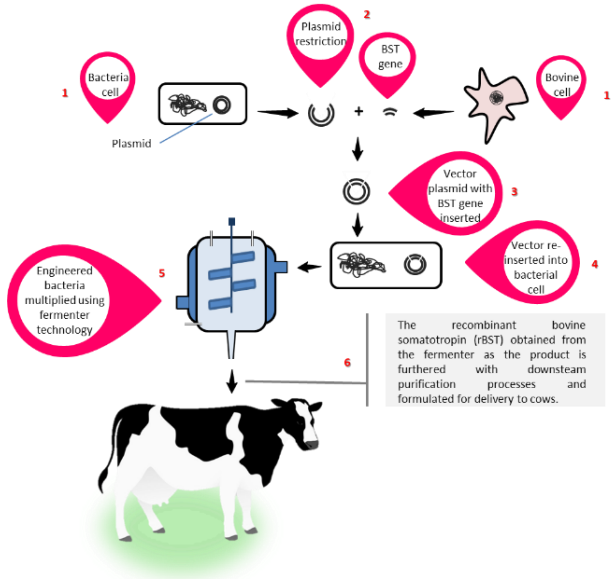

Figure 6: Production procedure of recombinantly derived bovine somatotropin (rBST). The method produces sufficient rBST for commercialization and safe enough to be administered in place of BST extracted from pituitary glands of slaughtered animals.

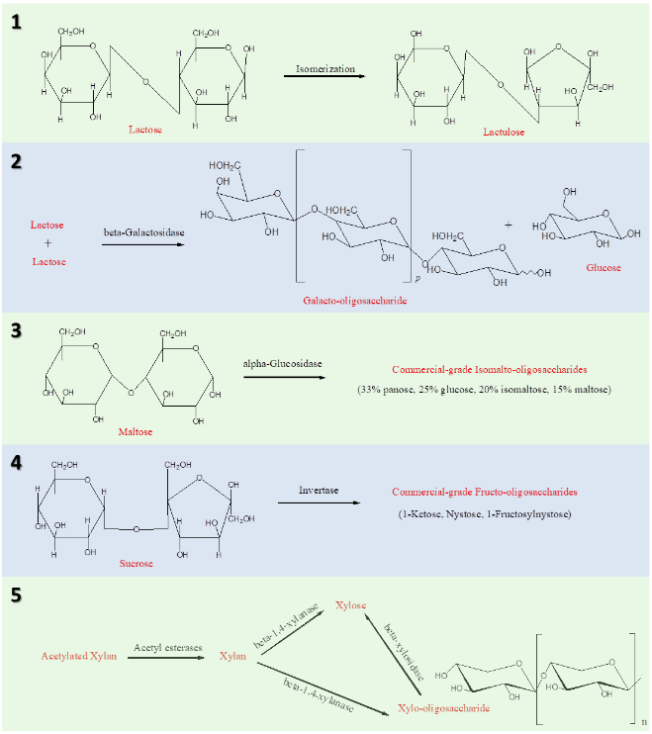

Figure 7: The structures and commercial enzymatic-production of prebiotic ingredients for livestock feed.

recombinantly derived porcine somatotropin (PST) is a variant used to improve leanness, reduce carcass fat content and enhance growth rate in swine particularly. The use of recombinantly derived BST and PST has also been reported to reduce environmental footprint of livestock industry (Etherton et al. 1993).

Prebiotics, probiotics and synbiotics

Prebiotics and probiotics are not drugs; prebiotics are nutritive ingredients (WHO 1994), while probiotics are live microbial supplements which can restore intestinal microbial balance (FEFANA 2005). These do not act directly on the host but functions indirectly by modulating the gut microbiota, either through causing changes to the proportions of its resident microorganisms or by influencing activity of intestinal health-promoting microorganisms (Gibson and Roberfroid 1995). Gut microbiota is known to influence various functions related to the animal health, including fermentation and digestion of nondigestible substrates, trophic effects on the immunity, adding protection against invasion by non-native microbes, better absorption of ions in the intestines, and lastly the production of vitamins (B9, B12, K) and amino acids (Guarner 2007). Thus, the use of prebiotics and probiotics in animal feed indirectly supports improvement of animal health, production and welfare on a significant scale.

Prebiotics are selectively fermented, non-digestible ingredients. These dietary ingredients are intended to be consumed by bifidobacterial and lactobacilli species, which are known to reside in large intestine and have been reported to exert prophylactic and therapeutic influences on animal health.

Commonly used prebiotics for livestock include synthetic saccharides such as lactulose, isomalto-oligosaccharides, transglacto-oligosaccharides, fructo-oligosaccharides (Grajek and Sip 2004) and cereal fibre (Crittenden and Playne 2009; Olveira and Gonzalez-Molero 2016); their structures shown in Figure 7. In order for any ingredient to be classified as a prebiotic, there is a certain criterion for it to befit (Wang 2009; Śliżewska et al. 2013), as shown in Figure 8, and even if the ingredient fulfills all the criteria, its efficacy, effectiveness and efficiency depends on a number of factors, such as the condition of animal's present gut microbiota, the dosage and its frequency, and especially its 'prebiotic index' i.e. "the increase in the absolute number of bifidobacteria expressed divided by the

Table 2: Different prebiotics for livestock feed, their production enzymes, enzyme source and enzyme substrates

Prebiotic	BC	Source of BC	Substrate(s)	OWC		References
				pH	Temp (°C)	
Galacto-oligosaccharide	β-galactosidase	*Bifidobacterium longum*	Lactose	6.8	45	Hsu et al. 2007
		Enterobacter agglomerans		7.5	50	Lu et al. 2007
		Geobacillus-stearothermophilus		6.5	37	Placier et al. 2009
		R109W		7.0	40	Chockchaisawasdee et al. 2005
		Kluyveromyces lactis		6.5	30	
		L. reuteri		6.5	30	Splechtna et al. 2006
		L. acidophilus		5.4	50	Nguyen et al. 2007
		Penicillium expansum F3		6.0	80	Li et al. 2008
		Sulfolobus solfataricus		6.5	40	Park et al. 2008
		Talaromyces thermophillus		6.0	80	Nakkharat et al. 2006
		Thermatoga maritime				Ji et al. 2005
	B- glycosidase	*Rhodotorula minuta* IFO879		5.0	60	Onishi and Tanaka, 1996
Fructo-oligosaccharides	B-fructofuranosidase	*A. Pullulans*	Sucrose	5.0	50	Yoshikawa et al. 2007
		Aspergillus japonicas		5.0	50	Mussatto et al. 2009
	Fructosyltransferase	*Aspergillus aculeatus*		5.6	60	Nemukula et al. 2009
	G-fructofuranosidase	*Erwinia Rhipontici* NCPPB 1578		6.0-6.5	30	Mundra et al. 2007
		Xanthophyllomyces dendrorhous		7.0	-	Chen et al. 2011
		Erwinia sp. D12	Sugarcane molasses	6.4	45	Kawaguti et al. 2011
	Inulinase	*Kluyveromyces marxianus var. bulgaricus*	Sucrose	5.0	50	Santos and Maugeri, 2007
				7.0	37	
		Xanthomonas Campestris	Inuline, tryptone			Naidoo et al. 2009
Isomalto-oligosaccharides	Trans-glucosidase	*Saccharomyces carlsbergensis*	Starch from rice crumbs	-	-	Pan and Lee, 2005
		Saccharomyces cerevisiae				
	Dextranase and dextransucrase	-	Sucrose	6.0	50	Zhang et al. 2010
Xylo-oligosaccharides	B-xylosidases	*Aspergillus nidulans* FGSC A4	Xylose	5.2	40	Dilokpimol et al. 2011
	Xylanase	Paecilomyces themophila	Xylan	7.0	70	Teng et al. 2010
		Pichia pastoris	Cellulose	6.0	65	Jun et al. 2009
		Pichia stipites	Xylan	5.4	50	Yang et al. 2011
		Thermobifidia fusca	Xylan	7.5	60	Huang et al. 2010
		Thermoascus aurantiacus	Sugarcane bagasse	5.0	50	Brienzo et al. 2010

*BC: Bio-Catalyst, OWC: Optimum Working Condition.

daily dose of prebiotic ingested" (Roberfroid 2007). There are various enzymatic and chemical treatment methods to industrially produce prebiotic ingredients from lignocellulosic material; these methods include extraction of substrate saccharides by steam, alkaline solutions and diluted solutions of mineral acids.

Once the substrate saccharide is successfully extracted, various commercially available enzymes (Table 2) are used to catalytically produce the required prebiotic (Garrote and Parajo 2002; Palm and Zacchi 2003; Vazquez et al. 2005; Aachary and Prapulla 2011; Basu et al. 2015; Sorndech et al. 2018). Galacto-oligosaccharides are produced through transglycosylation of lactose (Chen et al. 2003; Panesar et al. 2006), fructo-oligosaccharides are produced through transfructosylation of sucrose or through hydrolysis of inulin (Oscara et al. 2007; Chen et al. 2011); likewise, through hydrolysis xylan, xylo-oligosaccharides are produced (Kubik et al. 2004; Doukyu et al. 2007; Sato et al. 2010) and the debranching and hydrolysis of oligosaccharides result in the production of jsomalto-oligosaccharides (Lee et al. 2002; Zhang et al. 2010).

The use of probiotics to achieve better livestock health and productivity dates back to the 1970's. Intentional addition of specific live microorganisms to livestock feed has shown to promote eubiosis and preclude disruption of intestinal equilibrium (FEFANA 2005). Different types of probiotics exert their effects in different modes of action, these include; (i) Competitive elimination of potentially harmful pathogens through competing for adhesion sites in the gut wall and competing for nutrients or organic substrates (Umesaki et al. 1997; Hughes and Heritage 2002; McDonald et al. 2010; Cho et al. 2011); (ii) Bacterial antagonism – once the probiotic microorganisms establish in the gut, they may produce bactericidal or bacteriostatic substances to ward off unwanted bacterial species from the gut (Hughes and Heritage 2002; Steiner 2009; McDonald et al. 2010); (iii) Neutralization of enterotoxins; probiotic microorganisms produce a range of substances which include antioxidants, organic acids and bacteriocins (McDonald et al. 2010). The presence of these substances in the gut establish anti-microbial environment (Alakomi et al. 2003), which specifically targets the metabolism and toxin production mechanisms of pathogenic bacteria; and lastly, (iv) Immune modulation; probiotics have been studied to support animal's defense against harmful invaders by stimulating the production of antibodies and increased phagocytic activity (Hughes and Heritage 2002; McDonald et al. 2010; Ahasan et al. 2015). Anything about improvement in carcass characteristics or milk yield in dairy cows upon receiving probiotics remains debatable

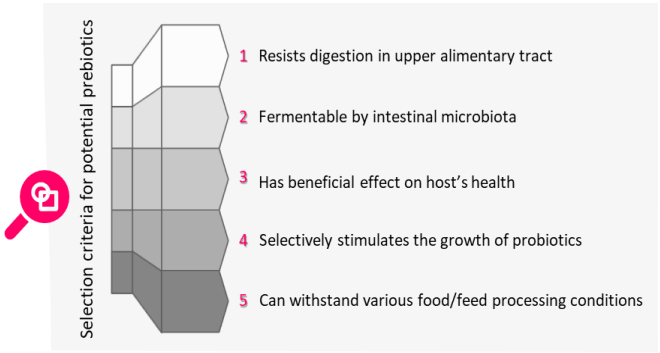

Figure 8: Selection requirements for potential prebiotics.

Table 3: The various microorganisms (by genus and specie) which are regarded as safe and healthy consumable probiotics

Genus	Probiotic species
Aspergillus	*A. Oriza*
	A. Niger
Bacillus	*B. cereus*
	B. licheniformi
	B. subtilis
Bifidobacterium	*B. bifidum*
	B. lactis
	B. longum
	B. pseudolongum
	B. thermophilum
Enterococcus	*E. faecalis*
	E. faecium
Lactobacillus	*L. acidophilus*
	L. amylovorus
	L. rhamnosus
	L. fermentum
	L. brevis
	L. farmicinis
	L. casei
	L. reuteri
	L. plantarum
Lactococcus	*L. lactis*
Leuconosto	*L. lactis*
	L. citreum
	L. mesenteroides
Pediococcus	*P. pentosaceus subsp. Pentosaceous*
	P. acidilactici
Saccharomyces	*S. pastorianus*
	S. cerevisiae
Streptococcus	*S. salivarius*
	S. infantarius

and holds a huge area for research and further study (Yirga 2015). Indications regarding growth and feed efficiency however, are positively reported in heifers and steers, especially with increase in the concentration of feed *propionibacterium* and *L. acidophilus* (Galyean et al. 2000; McPeake et al. 2002). Table 3 enlists the various probiotic microorganisms (Yirga 2015) and Table 4 provides the common benefits of using probiotics in monogastric and ruminant diets.

The substantial evidence of probiotic usefulness is found to be matched with an equal number of denigrators as well (Swinney-Floyd et al. 1999; Rust et al. 2000). However, the use of prebiotics is widely accepted as an effective practice for the above-noted cause. The major consideration while forming an impression on the degree of a pre/pro-biotic's usefulness is the fact that it is a variable factor, as it depends on the nature of the additive (type of prebiotic/strain of probiotic), its stability in the host system, the dosage, frequency of administration, off-target interactions and host-factors i.e. age, nutritional status, health and genetics etc. As human population is expected to grow, the market size for pre- and pro- biotics is also anticipated to increase at ~12% CAGR for prebiotics by the year 2026 (Mordor 2021) and at ~7% CAGR for probiotics by the year 2027 (Transparency 2019). The synergistic combination of pre- and pro-biotics has also evoked high hopes, such a combination is called a 'synbiotics'. As the term itself implies, the prebiotic component serves to selectively favor the activity of probiotic component (Cencic and Chingwaru 2010); the purpose behind this combination is thus apparent to improve the survival tendency of probiotic microorganisms in the gut (Rioux et al. 2005). Considering the superior activity of synbiotics in comparison to individual potential of both pre- and pro-biotics, the application of synbiotics to improve livestock health and productivity seems better promising than its individual component (Scavuzzi et al. 2014), as it helps lower concentration of undesirable metabolites (Bengmark and Martindale 2005) and can guarantee profitability; however, the various possibilities still require further studies.

Antibiotic growth promoters (AGPs) and alternatives

The growth promoting effects of antibacterial compounds were discovered in 1940, as a result of adding by-products of tetracycline fermentation to poultry diet (Phillips et al. 2004). The application of antimicrobial compounds to livestock feed has since increased (Aust et al. 2008; Zuccato et al. 2010; Gao et al. 2012) by as much as 10-20 folds (Brown et al. 2017). Such an intense rise is the consequence of an ever-growing consumer demand for food-animal products, and so AGPs – also interchangeably – 'veterinary antibiotics' (VAs) (Phillips et al. 2004) now represent a large proportion of the global antibiotic consumption. In 1940's and 1950's, AGP administration became an essential component of the 'animal protein factor' (APF) feed supplement, as it deemed necessary for balancing poultry and swine rations. Moreover, pharmaceutical compounds do not bioaccumulate in significant amounts within the body, excessively high proportions of VAs got excreted via milk (Halling-Sorensen et al. 2001; Arikan et al. 2009), eggs (Idowu et al.2010), urine and feces (Ostermann et al. 2013) in non-metabolized form; however, later studies proved the accumulation of VAs in edible tissues as well (Kwon et al. 2011; Kim and Schrenk 2012). Thus, these antibiotic residues get transferred into human diet and pose serious threats to consumer health, either through causing unwanted reactions such as allergies (Phillips et al. 2004), or increasing the risk of transferring antibiotic resistance (Butaye et al. 2003). Figure 9 shows the timeline of AGP acceptance and banning in various parts of the time of their discovery.

Table 4: Some probiotics of potential use in livestock industry with their benefits

Livestock animal	Probiotic microorganism	Common benefits
Veal Calf	*B. pseudolongum*	Promotes weight gain
	L. acidophilus	Limits rumen acidosis
	L. animalis	Improves feed digestibility
	L. paracasei	Improves milk yield
	S. cerevisiae	Limits shedding of human pathogens
		Reduces risk of intestinal diseases
Swine	*B. subtilis*	Decreases stress
	B. cereus	Increases litter size and vitality
	B. licheniformis	Reduces risk of intestinal diseases (diarrhea, constipation)
	E. faecium	Improves feed digestibility
	E. faecalis	Improves milk and meat quality
	L. acidophilus	
	L. reuteri	
	L. johnsonii	
	S. cerevisiae	

Table 5: Biological implications of livestock feed acidifiers

Digestive system	Immune system	Performance and health indices
• Increase villus height	• Increases immunoglobulins	• Improves liver health
• Lower Gut pH	• Enhances antibody production	• Improves lipid profile
• Lowers pathogenic bacteria	• Increases serum albumin	• Antioxidant activity
• Increase gastric retention time	• Stimulates immune system	• Mitigates ammonia emission
• Modifies intestinal microflora	• Enhances immune response	• Mitigates urea excretion
• Improve nutrient digestibility		• Alleviates heat stress
• Improve mineral absorption		• Improves meat and carcass quality
• Improves feed utilization		• Antimicrobial activity
		• Improves reproductive performance

Table 6: The beneficial role and deficiency symptoms for micronutrients in ruminant diets

Micronutrient	Benefit	Deficiency symptom
Boron (B)	Enzyme function	Low conception rates, depressed immunity, brittle bones
Cobalt (Co)	Energy assimilation and vitamin B12 function	Anemia, depressed immunity, infertility, poor growth, exudate from eyes, loss of coat
Copper (Cu)	Hemoglobin formation, enzyme function and pigments	Anemia, poor growth, digestive problems, bone disorders, brain and spinal cord lesions, infertility, discoloration of hair
Iodine (I)	Thyroid gland function	Goitre and reproductive failure
Iron (Fe)	Protein, hemoglobin and enzyme function	Anemia
Manganese (Mn)	Enzyme function	Skeletal abnormalities, stunted growth, reproductive failure, ataxia in newborns
Selenium (Se)	Vitamin E function	White muscle disease, poor growth, infertility
Zinc (Zn)	Enzyme function	Stiff and swollen joints

Understanding the precise mode of action of AGPs has been a challenging task due to the complexity of the intestinal environment and numerous possible interactions therein. In 1963, a study carried out on growth enhancing effects of APGs on germ-free mice led to the observation that growth enhancement was a factor of the presence of microorganisms (Coates et al. 1963); promulgating the idea that gut-microbiota modulation is the primary operating mechanism of APGs (Dibner and Richards 2005). Currently, two primary hypotheses have been worked out i.e. an AGP either acts in a bacteria-centric manner or a host-centric manner. Either way, a further multitude of factors including effects of AMR within the gut, diverse microenvironments, the physiology of the host animal, the environment wherein livestock has been maintained, nutritional status and stress complicate the study due to their strong associations with each other. Figure 10 illustrates the working mechanistic of both hypotheses. Due to the serious risks associated (Chattopadhyay 2014), WHO has strictly prohibited the use of antimicrobials as growth promoters and limited its use to prescription only (WHO 2003; WHO 2004), as the production benefits fail to compensate for the higher costs of AGPs (Graham et al. 2007) and associated health concerns. Today, various potentially active and safer alternative compounds are being explored, examples include antimicrobial peptides (Yoon et al. 2013), pre-/pro-biotics, natural extracts, phytochemicals and polyphenols (Guo et al. 2004a; Guo et al. 2004b; Ohno et al. 2013; Salim et al. 2013; Khadem et al. 2014; Lei et al. 2015; Rostami et al. 2015).

Acidifiers

Acidifiers are organic acids, which have been known for their preservative, prophylactic and nutritional qualities in livestock feed industry since long (Spratt 1985; Partanen and Morz 1999). These specificities are attributed to the

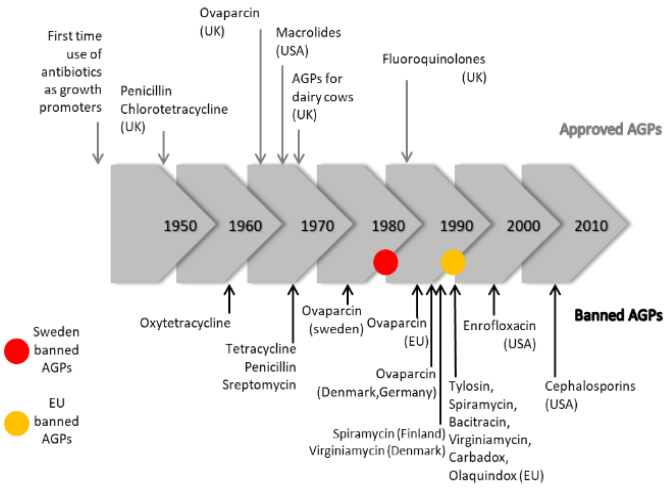

Figure 9: Timeline of antibiotic growth promoters (AGPs).

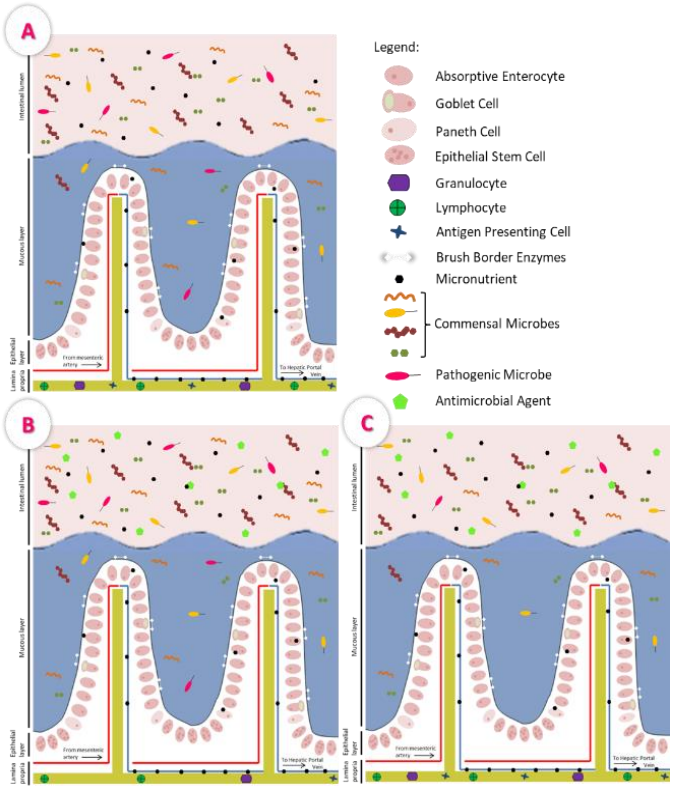

Figure 10: Intestinal immune-metabolism nexus: (A) The normal intestinal homeostasis; (B) bacteria-centric hypothesis – AGPs may alter the composition of microbiota to reduce competition for nutrients; (C) host-centric hypothesis – AGPs act as immunomodulatory agents by shifting resources to metabolic function.

carboxyl functional group (-COOH) of these acids. Various studies have confirmed acidifiers as a cost-effective option for enhancing growth, performance, and productivity in animals (Roth et al. 2017), as they help in diminution of pathogens in the gut, improved nutrient digestibility, enhanced eubiosis in intestinal microbiota (Overland et al. 2009; Ndelekwute et al. 2019) and as a consequence improve the overall livestock production economy.

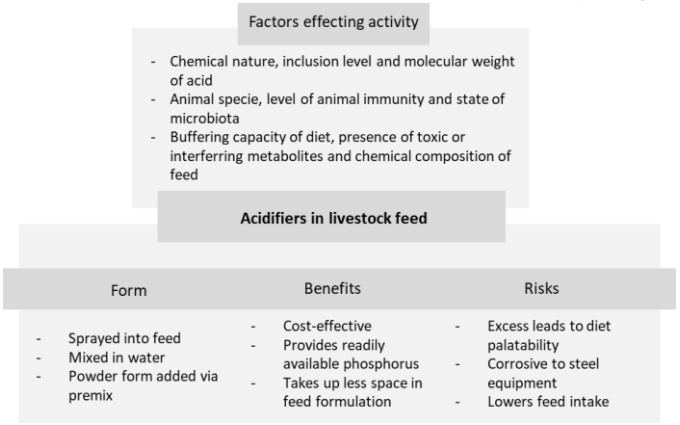

Figure 11: Characteristics, benefits, risks and factors influencing the use of acidifiers in animal feed.

Figure 11 summarizes the characteristics, benefits, risks, and factors influencing the use of acidifiers in animal feed. Table 5 enlists the biological activities of acidifiers when added to livestock feed (pearlin et al. 2019). With the discussed advantages, a few concerns regarding their action mechanism, palatability and neutralization still remain, which demand further study regarding their impact on consumer health and livestock economical footprint.

Other feed additives and growth promoters

Synthetic additives and growth promoters have far-reaching effects in enhancing growth and productivity of animals. The downside to their use, however, can neither be ignored nor avoided. Therefore, alternatives have been sought of organic forms as a means of equally effective biofortification in livestock production. Agronomic influence on livestock feed remains to be the foremost problematic concern, when it comes to compromised nutritional quality of economic produce. Various possible solutions to this include formulation of microelement-enrich feed and use of plant-derived antimicrobial peptides.

Deficiency of micronutrients causes grave restraints on animal health and growth, hence soil enrichment through balanced fertilization has long been practiced widely (Fisher 2008). Today, advancements have brought functional innovations, including the domain of nanoparticles which allows efficient infusion of highly dispersed and scarce micronutrients in feed mixtures. Due to their structure and developed surface, nanomaterials elicit intensified biological activities. Therefore, infusion of nutrients with the help of nanotechnology ensures their uniform distribution in the feed, the later serves as stable and much efficient source of scarce trace elements for animals (Belov et al. 2020). Micronutrients are essential for animal health, and it must be noted that their deficiency leads to the development of various clinical or subclinical implications, such as reduced fertility, browning of hair and compromised immunity (Fisher 2008). The main roles and deficiency symptoms for micro-nutrients in ruminant livestock are shown in Table 6.

Antimicrobial peptides (AMPs) are a family of around 5000 short cationic-peptides which elicit antibacterial or host-defense activities (Ganz 2002; Waghu et al. 2014; Ageitos et al. 2017). Utilization of these peptides is a safe alternative to the use of antibiotics in livestock feed. Studies have shown that dietary supplementation with AMPs for animals can improve rumen digestion, performance, growth, intestinal morphology, nutrient retention, and host immunity (Wu et al. 2012; Yoon et al. 2012; Yoon et al. 2013; Choi et al. 2013; Lee et al. 2014). As AMPs consist of amino acids only, they are easily modifiable and can be produced on an industrial scale through recombinant expression systems (Bahar and Ren 2013). Table 7 enlists the different AMPs applicable to swine feed and their application effects. With several beneficial properties including lowering risk of antimicrobial resistance, enhancing host immunity, excellent disease inhibitory effects and ease of degradation, AMPs represent one of the most promising alternatives to antibiotics. However, their inconsistence efficacy, instability, susceptibility to proteolysis and high cost of production has prevented AMPs from reaching an economically effective market size (Li et al. 2018).

Animal Health and Welfare

After ensuring the provision of best nutritional formulations for animals, the next immediate step is the assurance of disease diagnostic, preventive, and treatment strategies. The broad magnitude of biotechnological advancements today has made it possible to precisely detect and specifically eliminate the developing infection or disease in question. Hence, this section is divided into three parts: Diagnosis, prevention and treatment approaches to animal diseases and infections.

Diagnosis of animal diseases

Detection of previous exposures to potentially harmful pathogens and prompt diagnosis of prevalent diseases has always been a vital part of animal health assurance and maintenance (McKeever and Rege 1999). For several years, this had been executed through application of various macroscopic and microscopic techniques involving different culturing methods and body fluid examinations of the infected animal. Although reliable, these methods had a number of shortcomings and limitations that impeded the progress in the field of animal health. Incorporation of biotechnological techniques in the animal diagnostic studies proved to be immensely beneficial for the scientific community, as it improved the specificity and sensitivity of different diagnostic methodologies along with shortening the required testing time, enabling immediate control of various animal diseases before they could affect a vast population. This fusion also allowed the researchers to extend their approach towards detection and control of various congenital or genetically transmitted diseases that affected animal productivity and health of the whole animal lineage (Myers-Keith 1983; Yanchinski 1983; Jindal and Sharma 2010). Biotechnological techniques employed to detect and identify various disease-causing factors can be generally grouped under five categories: (i) Conventional methods, (ii) DNA-based diagnostic techniques, (iii) Antigen-antibody interaction-based techniques, (iv) Pen-side tests, and (v) Metabolite-based techniques Figure 12). A brief account of these techniques is given below.

Conventional methods

The traditional culturing techniques for isolation and detection of causative agents are still the gold standard methods for detection of disease-causing microorganisms. These include; media cultures to obtain bacterial isolates and cell cultures to assess viral infections. Despite being the gold-standard methods, these methods are more time consuming, labor-intensive, have higher risk of contamination and are less sensitive compared to the advanced diagnostic tools, as they rely on phenotypic-biochemical characterization (Saminathan et al. 2016).

DNA-based diagnostic techniques

Nucleic acid amplification/polymerase chain reaction

Nucleic acid amplification technique, or polymerase chain reaction technique, is a type of DNA-based assay that involves the amplification and synthesis of a distinct nucleic acid sequence present exclusively in the pathogen to be detected. This enzymatic amplification assay of the DNA target molecule comprises a repeated cycle of target molecule denaturation, hybridization to specific primers through DNA polymerases and elongation of the primers, leading to the production of a large number of copies of the particular nucleic acid sequence. This assay has proved to be rather sensitive and specific, enabling rapid diagnosis of an infectious disease (Rodriguez 1997); a titer minimum of 0.01 TCID (tissue culture infective dose) of viral RNA can be identified in the infected tissue culture fluid by a single-step, highly sensitive derivative technique: RT-PCR-ELISA, which proves to be 10,000 times more sensitive than RT-PCR (Rajeev et al. 2011; Sharma et al. 2015). Furthermore, incorporation of fluorescent probes and dyes e.g. Taqman hydrolysis probe or SYBR Green dye, for the simultaneous analysis of the amplified pathogenic sequence through Real-Time PCRs have revolutionized infectious disease diagnosis and control in the veterinary medicine. This technique is also being used in combination with a number of other diagnostic assays, allowing a more far-stretched approach of the diagnosticians towards the detection of pathogenic agents of veterinary significance (Zarlenga and Higgins 2001; Borroto 2009; Balamurugan et al. 2014). Some of the various disease causing agents that are being detected through this technique in animals are Foot and mouth disease virus (FMDV), Porcine parvovirus (PPV), Bovine herpes virus, Pseudo rabies virus, Morbillivirus, Yersinia Entercollitica, Mycobacterium species and Enterotoxigenic *Escherichia coli* (ETEC) (Rodriguez 1997).

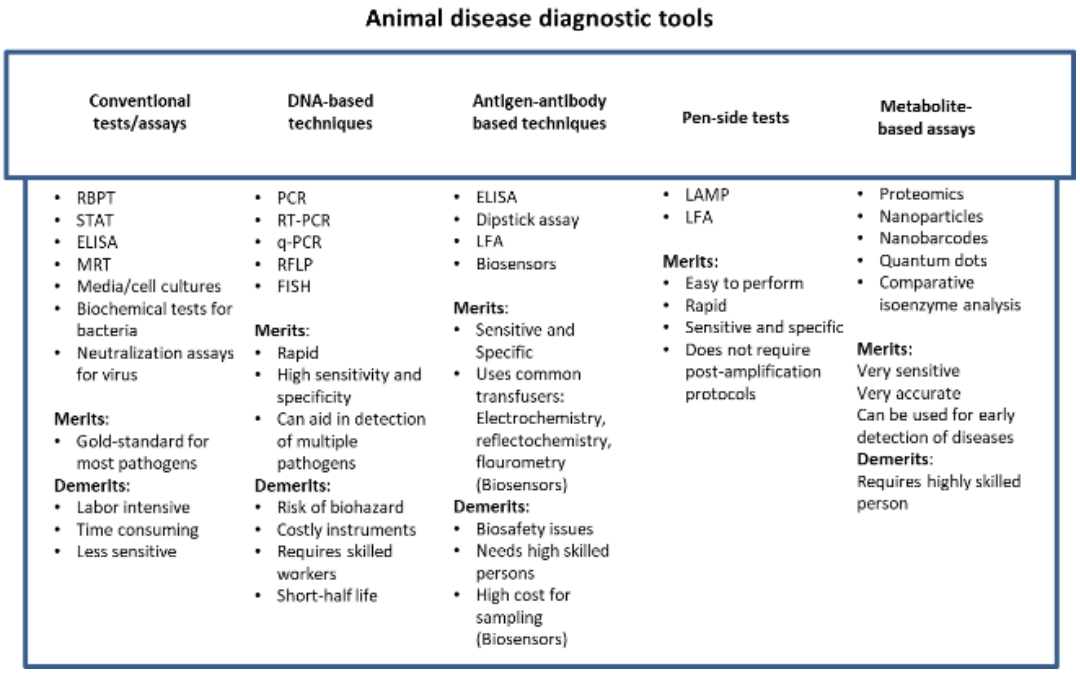

Figure 12: The various biotechnological tools for animal disease diagnosis, their approaches, merits and demerits.

Table 7: AMPs authorized for use in swine feed and their application effects

AMP	Application Effects	Reference
AMP-A3	Improves performance, nutrient digestibility, intestinal morphology, gut microbiota	Yoon et al. 2012
AMP-P5	Reduced coliforms, improves performance and nutrient digestibility	Yoon et al. 2013
AMP cecropin AD	Improves performance, immune status, energy retention, reduces intestinal pathogens	Wu et al. 2012
AMP colicin E1	Reduce probability of diarrhea, improves performance	Cutler et al. 2007
CAMP (Lectoferrin-Defensin-Active yeast)	Improves feed efficiency, intestinal morphology, protein synthesis, intestinal epithelial cell proliferation, antioxidant capacity, lipid and energy metabolism, alleviates organ damage.	Xiao et al. 2013a; Xiao et al. 2013b; Xiao et al. 2015
cipB-lactoferricin-lactoferrampin	Improves absorption of iron, improves performance, reduces incidence of diarrhea, improves performance	Tang et al. 2008
Recombinant Lactoferrampin-Lactoferricin)	Affects serum parameters, improves performance	Tang et al. 2012
Mixture: lactoferrin, defensing, cecropin and plectasin	Reduce probability of diarrhea, Improves performance, increases survival rate	Xiong et al. 2014

Restriction fragment length polymorphism (RFLP)

RFLP is among the earliest molecular techniques used for DNA profiling and gene mapping. This is a DNA-based diagnostic technique that employs the fact that even the homologous genetic makeup of closely related species comprises of variation sequences or polymorphism in their genetic arrangement. This assay begins with the reverse transcription of the pathogenic DNA, followed by digestion of the nucleic acids with the help of specific restriction enzymes. The resultant fragments are then separated and detected through gel electrophoresis and various blotting and dying techniques, respectively. Ideally, the generated pattern is unique to each pathogenic strain, revealing its distinct fingerprint or genetic makeup. Although beneficial in the diagnosis of various veterinary diseases, its use is greatly decreased in the recent years due to introduction of more sensitive and efficient techniques(Jindal and Sharma 2010; Dai and Long 2015).

Nucleic acid hybridization technique

This technique primarily involves synthesis of nucleic acid probes complementary to the genetic sequence present specifically in the genomic or plasmid DNA of the pathogen to be detected. Once paired, the probe-nucleic acid complex is detected through various radioactive or non-radioactive reporter molecules. Employed mainly for the detection of fastidious organisms such as Mycoplasma and Mycobacterium, this technique ensures early diagnoses by surpassing the long culturing time required by such pathogenic species. Some of the various veterinary infections causing entities that are identified through this technique include Adenovirus, Bovine viral diarrhea virus, bursal disease virus, Campylobacter species, Leptospira species, *Anaplasma marginale* and *Histoplasma capsulatum*. Besides being simply a diagnostic technique, this probe-based assay has also been used for genetic mapping of homology among closely related organisms, determination of pathogenic mechanisms on a molecular

level and differentiation of virulent from avirulent strains. Acquisition of such detailed information about the genomics of the pathogens has unfolded a rather meticulous approach towards disease diagnostic studies for the veterinary researchers and diagnosticians (Paul 1990; Sinigaglia et al. 2018).

Antigen-antibody interaction-based techniques

Monoclonal antibodies

The conventional diagnostic measures used for the identification of disease-causing agents were immensely dependent on rather time consuming and hectic culturing techniques. With the introduction of monoclonal antibodies in the field of disease diagnostics, the efficiency of the already existent detection strategies was greatly enhanced. This assay utilizes the basic mechanism of the immune system that involves production of antigen specific immunoglobulins in response to the infectious pathogen or its various constituents. Hence, the presence of such antibodies in the serum of the animal proves the occurrence of the corresponding disease. The antibody titer measurement can further provide insight into the severity of the infection. Use of monoclonal antibodies was initially based of differential diagnosis of the vaccine strains from field strains of the pathogen due to their high specificity. This technique is now used for the identification of various veterinary infectious diseases such as Feline leukemia, Trypanosomiasis and Colibacillosis (Kahi and Rewe 2008; Jindal and Sharma 2010).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA surpasses the previously present precipitation-based antigen-antibody assays by incorporating an enzyme with one of the reactants, most commonly the antibody, for the purpose of detection and quantification. It is an extremely sensitive technique capable of detecting very minute quantities of the target material. It is carried out on a solid phase, to which different components are presented in a sequential manner. The reacting and non-reacting components are separated from each other through washing after each step, making it a heterogenous assay. Due to its high specificity, versatility, precision and reproducibility, this antigen antibody based technique is preferred over other sero-diagnostic methods for the detection and diagnosis of various veterinary infectious diseases (Clark et al.1986). Some of the many such animal diseases against which ELISA kits have been manufactured for are Brucellosis, Rabies, Canine Distemper and Canine Parvo virus, Canine Hepatitis virus, Foot and Mouth disease, New Castle Disease and Egg Drop Syndrome virus-76 (Jindal and Sharma 2010).

Immunoblotting assay

Western Blotting is a technique that provides a mean for identification of various target epitopes related to the infection causing pathogen through application of antibodies from the infected animals, or separately synthesized monoclonal antibodies against the specific antigen. This assay combines the specificity of antigen-antibody reactions with the high resolution of gel electrophoresis for the accurate and effective diagnosis of veterinary infections. It has also been used in combination with other diagnostic methods to increase their precision and efficiency in detection of diseases (Jindal and Sharma 2010). Various researches have been carried out for the effective employment of this technique to detect different chronic and asymptomatic enzootic infections such as Enzootic Bovine Leukemia caused by Bovine Leukosis Virus (BLV) through detection of the hosts humoral response produced against the viral envelop and core proteins (glycoprotein gp51 and core protein p24, respectively) (Gonzalez et al. 1999).

Biosensors

Biosensors are tools which can detect antigens, antibodies and transduce their biological interaction into quantifiable signals through technologies such as fluorimetry, resonance and/or electrochemistry. These tools can read even the slightest variations but need highly skilled personnel to interpret the results. Moreover, the equipment is very costly and have high sample processing charges (Rajeev et al. 2011; Chakraborty et al. 2014; Balamurugan et al. 2014; Sharma et al. 2015).

Pen-side tests

These are also called field tests, which means, they can be done in the field without using expensive laboratory equipment. Developed techniques include loop-mediated isothermal amplification (LAMP–replacement for thermocyclers) (Rekha et al. 2014); simple-dot ELISA, antigen-competition ELISA, immunofiltration, dipsticks and lateral flow test – these tests do not require agarose-gel electrophoresis (Arun et al. 2014). These assays bear high sensitivity and specificity, are user-friendly, rapid and prove better at being economical (Balamurugan et al. 2014; Chakraborty et al. 2014; Dhama et al. 2014; Sharma et al. 2015).

Metabolite-based diagnostic tools

Proteomics

Analysis of the structure and function of proteins, along with the evaluation of various sets of proteins, expressed by the genetic makeup of different organisms under certain conditions is defined as proteomics. For the past few decades, use of proteomics in veterinary diagnostics was limited to detection and quantification of total serum protein of the infected animal through serum protein electrophoresis (SPE). This protein-based investigation included measurement of serum albumin, globulin and albumin to globulin ratio during pathogenesis. With the enhancement in technology, the field of proteomics has

grown significantly and now involves study of protein characterization and post-translational modification. Protein characterization is mainly carried out through the use of two major mass spectroscopy (MS) platforms, including matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). Incorporation of these modified techniques has revolutionized veterinary diagnostic investigations. Proteomics have been frequently researched upon to detect various animal diseases of great economic importance, such as mastitis and other intra-mammary infections caused by *Escherichia coli* and Staphylococcus species. Milk from the infected animal is analyzed before and after infection, to detect differentially expressed proteins through 2D electrophoresis, followed by peptide sequencing or protein characterization with the help of MALDI-TOF. The presence of such unique proteins can help in the diagnosis of certain infections.

Various other animal diseases that are researched upon to be detected via proteome identification include parasitosis, respiratory and intestinal diseases of pigs, articular disorders and equine recurrent uveitis. The study of proteomes and post-translational modifications can further be applied for the identification of potential biomarkers produced during various inflammation conditions and cancerous diseases. Some pathogen specific proteins are also capable of eliciting an immune response from the host body. The detection of infection caused by such protein producing entities through identification of serum antibodies provides a rather sensitive and specific means of disease diagnosis. This is carried out via gel-based and gel free techniques (Gowda et al. 2008; Jindal and Sharma 2010; Ceciliani et al. 2014).

Nanotechnology

According to the National Science and Technology Council of United States of America, nanotechnology has been defined as "research and development (R&D) aimed at understanding and working with -seeing, measuring, and manipulating matter at the atomic, molecular and supra-molecular levels. This correlates to length scales of roughly 1 to 100 nanometers. At this scale, the physical, chemical and biological properties of materials differ fundamentally and often unexpectedly from those of the corresponding bulk materials." Veterinary diagnostics and treatment strategies have been greatly enhanced through the application of nanotechnology, since it carries the capability of early identification and eradication of the disease-causing pathogen. This technology has been applied in the diagnosis of several animal pathogens including Respiratory Syncytial Virus both *in vitro* and *in vivo* through nanoparticle-conjugated monoclonal antibodies. It is also used for early detection of several cancer markers. This technique has proved to be very sensitive and accurate, and a great deal of research is being carried out for studying its use in the detection of various animal diseases (Scott 2007; Num and Useh 2013).

Comparative isoenzyme analysis

Isoenzyme analysis is a direct biochemical assay based on the principle that different isoforms of intracellular enzymes, such as lactate dehydrogenase, glucose-6-phosphate dehydrogenase, purine nucleoside phosphorylase and malate dehydrogenase, possess different molecular structure but similar substrate specificity. When run on agarose gel electrophoresis, these enzymatic isoforms show variation in banding patterns and migration distances relative to one another. The interpretation of the isoenzyme data helps in the direct testing for the presence of various parasites infecting the host population (Drexler 2000; Zarlenga and Higgins 2001).

Prevention and Treatment of diseases

Prevention is the procedure of saving high risk animals by treating in a manner that prevents the occurrence of diseases. Treatment begins when animals show signs and symptoms of a disease. Control of disease is to prevent the transmission of already occurred diseases. The major methods adopted are diet supplementation, use of pre- and pro-biotics (both have been discussed under animal nutrition) and vaccination, which has been discussed below.

Vaccination

Vaccination is an effective way to control and reduce occurrence of a disease in animals and is an important factor in maintenance of animal welfare and health. Vaccines are biological preparations which help with developing immunity against disease causing microorganisms. Today various vaccines are available against many diseases whether they are endemic or exotic in nature. The several types of vaccines can be generally categorized either as non-living vaccines or modified-living vaccines, as shown in Figure 13. Farm animals include different species, thus, a wide range of diseases can be included in this respect. Examples include the eradication of rinderpest - a global disease of livestock in 2011 and control of other diseases like the blue tongue disease and bovine viral diarrhea etc. One most top listed concern associated with high morbidity and mortality rates is the prevalence of food-borne diseases (WHO 2015). Microorganisms like *Trichinella spiralis*, *Taenia solium*, Cryptosporidium spp., *Toxoplasma gondii*, and *Echinococcus granulosus* are top ranking foodborne parasites which are known to affect farm animals and, therefore, enter the food chain. Vaccines which contain live causative agent tend to be more immunogenic than killed or derived vaccines because they can grow and spread, consequently stimulating the immune system in an optimal fashion. Live vaccines cause the infected cells to process the microbial antigens and elicit type-1 response (dominated by cytotoxic T cells). Non-living vaccines consist of killed whole organism or purified antigens which stimulate type-2 response (dominated by

antibodies). Type-2 response does not provide optimal protection and usually requires help of adjuvants, such as ethylene oxide, ethyleneimine, formaldehyde, beta-propiolactone and acetylethyleneimine (Tizard 2020).

While designing a live attenuated vaccine, the level of attenuation stands critical to efficacy of the vaccine i.e., under-attenuation will result in reversion to virulence, whereas over-attenuation will result in an ineffective vaccine. The traditional method of maintaining prolonged tissue cultures can here be considered a primitive form of genetic engineering to carry out microbial attenuation and perform rigorous attenuation-to-reversion studies. This method is mostly famous for viruses; bacterial genomes are too large to manipulate in such a manner. Brucella strain 19 and Sterne strain of anthrax are two most obvious examples of bacterial vaccines developed through tissue culture technique. In extremely rare circumstances, a virulent organism may also be used as a vaccine. This approach is currently only applicable in cases of contagious ecthyma (sore mouth) of sheep, where dried and infected scab material is rubbed into scratches made on inner thighs to cause local infection and trigger immune system. Another simple method of using virulent microorganism for vaccination is to cold-attenuate virulent vaccine virus (i.e. virus adapted to grow at temperature approximately 10 degrees lower than normal body temperature); once administered intranasally, it will grow in the upper-respiratory tract but not spread in the warmer lower tract or organs, thus, causing local infection. Virulent organisms can also be genetically modified to irreversibly attenuate them. This is done by deleting the genes associated with virulence; this approach was first used in swine (against Ausjeszky disease herpesvirus). Antigens encoded genes of the causative agent can also be inserted into an avirulent organism, called a 'vector', in place of its genes instead. Large DNA-viruses are the most widely used vectors for development of vectored vaccines. These vaccines are safe to administer, are effective and stable in the absence of adjuvants, and can be used for mass vaccination purposes (Tizard 2020).

Of the many vaccine platforms and designs, whole-killed vaccines are economical to produce; however, these do contain various components which play no contributing role towards triggering protective immunity and may sometimes carry toxins too. The alternative to these are subunit vaccines, which only consist of the specific critical protective antigens, for example the pili of enteropathogenic *E.coli* and purified (inactivated) tetanus toxin – can be separately used as vaccines. The process of isolating and purifying specific antigens is cost-intensive and hence might not be a suitable approach in every case. For otherwise cases, the production of antigens through gene cloning may surface as a better, cost-effective option; these elicit effective antigenicity and function as effectual toxoids. Viral antigen genes can also be cloned in plants which constitute livestock feed; these plants transfer high concentration of antigens to the animals simply through feeding. This method has been successfully applied against Newcastle disease virus and transmissible gastro-

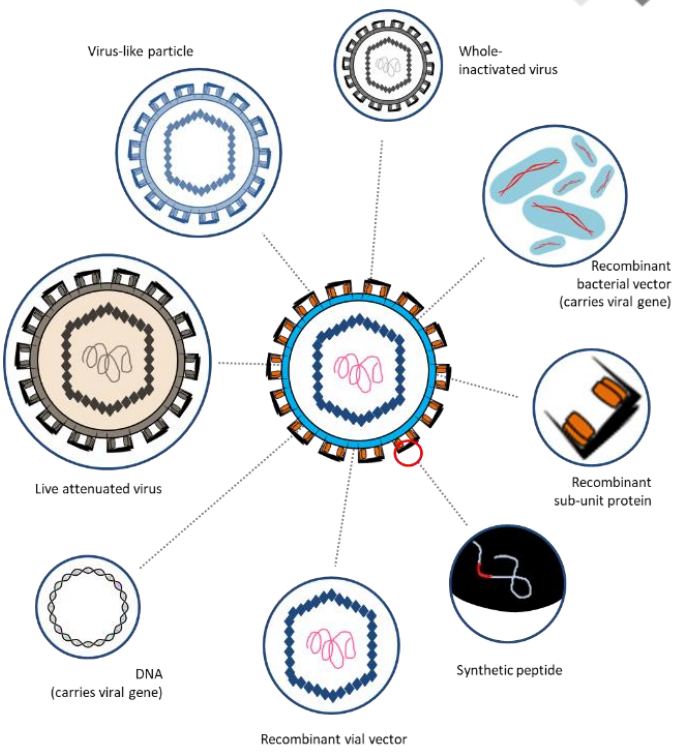

Figure 13: Types of vaccines for animals.

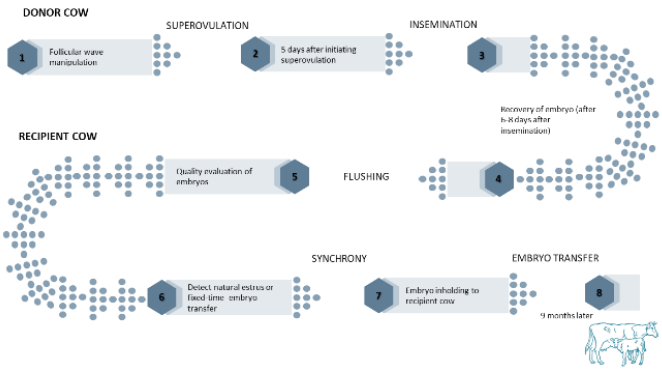

Figure 14: The process flowchart of Embryo transplantation.

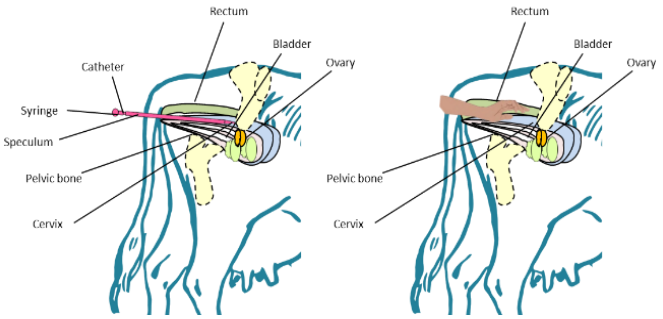

Figure 15: AI for cows. (Left) Speculum method, the semen is deposited through the cervix by vaginal insertion of a speculum; (Right) Recto-vaginal technique, the inseminator holds the cervix through the rectum to guide the inseminating instrument into the opening of the cervix, and then deposits the semen through the inseminating instrument.

enteritis virus. Likewise, viral structural protein can be produced through recombinant technology and assembled into virus-like particles (VLPs). These vaccines act as

potent immunogens in the absence of adjuvants. Similarly, ghost-bacterial vaccines can also be developed in this manner; these are bacterial cells void of their genetic material, thus, only carry surface structures. DNA segments, which are responsible for encoding viral antigens, can also be inserted into a bacterial plasmid (vector) and injected directly into host cells (transfection). The plasmid uses host cell machinery to transcribe and translate the vaccine proteins, which are then expressed by the host cell with major histocompatibility complex class I molecules triggering type-1 and type-2 immune responses (Tizard 2020).

Therefore, the development of veterinary vaccines against known viruses and parasites contribute significantly towards animal health and well-being (Sander et al. 2020). However, as per the current progress of vaccine development, more coordinated efforts are required to increase public compression and acceptance regarding vaccination, and address the various cultural, economic, and political actors which carry influence on disease eradication strategies.

Immunotherapy

Immunotherapy is an emerging way of treating diseases by suppressing or activating immune response in both animals and humans. Immunotherapies that act by activating or amplifying immune system are categorized as activation immunotherapies. Suppression immunotherapies act by suppressing immune response. It involves partial or complete elimination of immune system by chemotherapy. But now it is considered to boost the immune system and protect it. Most useful immune therapy used till now is monoclonal antibodies directed against checkpoint molecules and lymphoma antigens. Blockage of these pathways stimulates cytotoxic ability of T lymphocytes, and also activates immune system responses. Cytokine release and antigen presentation can be activated. Cellular immunotherapy is a type in which T cells are given a chance to fight against disease. Feline asthma has been treated with subcutaneous immunotherapy. Use of monoclonal antibodies is the newer way of immunotherapy these days. Infected animals are given monoclonal antibodies that are specific to some viral antigen or tumor antigen and can neutralize or kill them. These types of antibodies can be administered alone or with some arsenic like toxic compounds. This type of therapy is beneficial, as it can kill the virus or tumor found anywhere in the body that have the particular antigen. This type of treatment makes viral or tumor therapy possible economically and plays an important role in maintaining animal health. This type of therapy is more useful than irradiation or chemotherapy, as it produces local reaction and destroys tumor or virus by the presence of specific antigen.

In veterinary medicine, anti-idiotypic vaccine is another application of monoclonal antibodies. According to this therapy, monoclonal antibody made against idiotype of another monoclonal antibody acts as vaccine. For example, monoclonal antibodies are made against neutralization site of swine causing agent that is pseudo-rabies virus. Then, another monoclonal antibody against first antigen binding cleft monoclonal antibody is made. This anti-idiotypic antibody is administered to pig and it binds to B-lymphocytes that carries antigen of pseudo-rabies virus and then antibodies are made that neutralize pseudo-rabies virus. The anti-idiotypic vaccines are useful as majority of animal vaccines are live; they can divide and cause disease themselves. Monoclonal antibodies are beneficial, as they can be used to diagnose animal diseases. Series of sensitive and quick immune assays identify the disease, and the treatment of animals can be started immediately. Monoclonal antibodies are also used in dipstick techniques. Use of monoclonal antibodies in dipstick methods allows information about level of pesticides or mycotoxins contaminating feed. It is also beneficial in checking whether meat, milk or poultry products are contaminated with antibiotics, harmful drug residues or carcinogens. Future, detection of any organism or substance involves the use of monoclonal antibodies. Monoclonal antibodies are widely used in research as well. They are used to trace synthesis of milk during lactation, purifying compounds and to study role of certain enzymes and protein in cancer. In future, monoclonal antibodies can be very beneficial for animal health.

Cytokine Therapy

Cytokines are regulatory glycoproteins that are small in size. They are secreted by cells of immune system and regulate, as well as boost, immunity. They are produced as messenger by immune cells to attack an entering microorganism. They also communicate with other types of cells of immune system. Cytokines are either membrane bound or secreted, and regulate homeostasis of immune system by acting as intracellular mediators. Cytokines attach through specific receptors to outer membrane of target cell. They induce a cascade of reactions and alter gene expression by signal-transduction pathway activation. This pathway plays a role in both adaptive and innate immunity. Cytokines regulate immune response by inhibiting or stimulating differentiation, proliferation and trafficking of lymphocytes. They also play a role in biological activities i.e. hematopoiesis, adaptive and innate immunity, regulation and development of humoral and cellular immune responses, control of cellular differentiation, proliferation and inflammatory response. They show characteristics of redundancy, pleiotropy, antagonism, synergy, and cascade induction, which altogether regulate cellular functions. They also play an important role in healing of wounds.

Cytokine therapy is now a new method of treatment. Recombinant or purified cytokines are administered during therapy. Drugs are also administered which inhibit harmful effects of increased production of endogenous cytokines. Cytokine-based therapies include colony stimulating factors, hematopoietic growth factors and interferons for progression and stimulation of various processes. Cytokine antagonists have intense effects on inflammatory disorders treatment, such as tumor necrosis

factor inhibitors. Interferons are effective agents used against infectious diseases of horses and cattle. Interferons are antiviral agents, used in controlling viral infections when adaptive immunity is absent. They also act against intracellular bacterial species. They are used both for prevention, as well as cure, of viral infection of animals or act as adjuvant in treatment to decrease manifestations of disease, so that quality of life is improved. Cytokines are used as adjunctive immunomodulators and play important role in treatment of infectious diseases. Ribavirin and PEG-ylated interferons used for hepatitis C and nucleoside analogs and recombinant interferons used for hepatitis B virus are the most common example of cytokine treatment. They induce Th1 immunity. They can also be used in vaccines as mucosal adjuvants such as IL-12, IL-15, and IL-7. Granulocyte stimulating factor, macrophage inflammatory proteins and monocyte chemotactic proteins along with cytokines are used in vaccines. The activity of cytokine therapy depends on three factors that are:

- Cytokine or receptor signaling
- Cytokine or receptor attaching affinity
- Receptor or cytokine endocytic transferring dynamics

Interferons and IL-2 enhance action of T lymphocytes in removing Eimeria in birds, which is a Coccidian parasite. These cytokines used with newly designed vaccines can control experimental Coccidiosis infection in birds. TNFα and gamma interferon are together used in the treatment of mastitis in bovines.

Nanotechnology

Nanotechnology is one of the important factors involved in treatment of animal diseases. Nanotechnology is the field of small things, with chemical and physical modifications in their structure, and higher solubility and reactivity. Natural nano particles are derived from different methods, for example nano-propolis is beneficial for veterinary medicine in relation to food production, health and performance. Nano propolis is propolis particles in nano-size tied together; these are made effective by changing propolis size through different techniques, yet they maintain their properties. Propolis particles show many activities, such as antifungal, antioxidant, anti-inflammatory and anti-cancer. Free form propolis consumption results in low solubility, low absorption, low bioavailability, and untargeted release and restrict the benefits provided by use of non-free nano-propolis. To obtain nano-propolis, different nano-encapsulation methods are used. Due to small size, absorption of nano-propolis is quite easy, and they are easily delivered outside specific barriers, such as subcellular organelles, skin, brain, blood, mucus, eye, extracellular and cellular matric and placenta. Nano-propolis has better antifungal and antibacterial activity than propolis. Nanotechnology is used in human, as well as animal, health, such as the use of nano-propolis in animal nutrition and health. Nanotechnology denotes the developing technology in atomic, molecular and macromolecular fields. It is beneficial to animal health due to its high biodegradability and high bioavailability properties and gives scientific benefit with its specific and rapid moves. These benefits largely affect production, as well as economic losses of animals, and production of healthy feed and food. Nanotechnology devices are used in diagnosis of human, as well as animal, diseases and it's a great accomplishment in health field. Advances in nanotechnology can solve many problems of animal health. It can solve problems related to animal production, reproduction, health and maintenance of food animals.

Reproductive animal biotechnology

One of the quickest growing agricultural sectors is livestock production, comprising more than third of the total agricultural GDP. This is a consequence of increasing global food protein demand, which is shifting from plant protein to animal protein. As discussed previously, biotechnological advancements potentiate animal productivity through improving three factors; improved nutrition, improved health and via increasing animal reproduction through technologies, such as multiple ovulation and embryo transfer (MOET), artificial insemination (AI), sperm sexing, *In vitro* embryo production, embryo splitting, embryonic stem cell technology and lastly by successfully conserving resources of animal genetics through marker-assisted selection (MAS) techniques, which serve various applications including heritability determination, product traceability and trait improvement. The objective of using these biotechnologies, in general, is to improve reproductive efficiency of livestock.

Multiple ovulation and embryo transfer (MOET)

In this process, reproduction is assisted through embryo placement into surrogate uterus in order to establish pregnancy. MOET is done in three steps: (i) hormone stimulated superovulation in selected females, (ii) collection of developed embryos, and (iii) transfer of healthy embryos to healthy female recipients (Figure 14). This technique helps in increasing the number of successful pregnancies and thereby the number of calves produced. As embryos are pre-evaluated (on the basis of their quality) and only selected ones are transferred, this enables preservation and conservation of breeds, maintaining a disease-free herd, and enabling rapid multiplication of superior female breeding stock (Tappa et al. 1994). Moreover, the developed embryos can be transferred across greater distances, making livestock transport significantly economical; an estimated total of 1000 goat embryos, 68,000 sheep embryos and 550,000 bovine embryos were transferred worldwide in year 2004, as reported by the International Embryo Transfer Society (IETS) (Thibier 2005).

Artifical insemination (AI)

This is one of the earliest reproductive technologies through which sperms collected (cryopreserved) from an elite male are planted into the uterus of an elite female without the process of mating (Figure 15). AI remains to be the most simple, economical, and successful assisted-reproductive technology (Jacquelyn and Laura 2008). This technique reduces the transmission probability of venereal diseases, lowers the need of maintaining breeding males, is cost-effective, and facilitates canonical recording of pedigrees (Wilmut et al. 1997).

Sperm sexing

This technology is a derivation of AI with sexed sperms. A tremendous number of calves have been produced through sperm sexing and when used in dairy cows, it is reported to result in a ratio match of 81% (Said et al. 2005; Agung et al. 2016).

In vitro fertilization

This process is used when conditions, such as marginal semen quantity and quality, non-responsive ovaries, blocked female reproductive system or presence of an infection in the tubular genitalia, are encountered. Hereby, fertilization is carried out *in vitro* and the fertilized egg is then introduced into the uterus to establish pregnancy. This system ensures that the resulting zygote is competent for maturation and development in the body. However, the high cost of this approach limits its wide and regularly adopted use in livestock industry (Chang 1959; Bearden and Faquay 2004).

Embryo splitting

With this technology, twins or multiples can be formed through artificial splitting of an embryo. The micro-surgically separated embryos are genetically identical; thus this method is a duplication of the natural process of monozygotic twins (Said et al. 2020).

Embryonic stem cell technology

Stem cells elicit pluripotency and can thereby be manipulated to change their behavior. In this process, stem cells driven from undifferentiated inner cell mass of embryo are harvested from the donor animal and are manipulated *in vitro* to change their behavior and are maintained till the inner cell mass forms egg-like cylindrical structure (Evans and Kaufman 1981).

Marker-assisted selection (MAS)

The discovery of DNA sequences (molecular markers) has opened the possibility of improving traits, determining heritability of traits and catering product traceability. Various genetic diseases or defects can be detected in livestock using these molecular markers. Thus, it can help trace genetic changes and their manifestations; the animals carrying the defect can then be culled from the breeding program to prevent defective genes from getting transferred ahead (Cunningham and Meghan 2001; Womack 2005). These markers are also used to identify livestock parentage and their products (Cunningham and Meghan 2001) and can also be used to select animals which express introgressed genes.

Future implications of biotechnology in livestock industry

With the enormous scale of advancement in livestock biotechnology, the new possibilities introduced for research opportunities will help revolutionize livestock sector and be able to cater the concerns regarding food insecurity in developing and undeveloped countries. As for the growing population, these advancements allow improving health and production potential of animals at as early as the stage of fetus development. Moreover, these techniques will also contribute to lower livestock environment footprint.

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THE CURRENT SITUATION AND PRACTICE OF WILDLIFE MANAGEMENT IN THE UNITED STATES AND CHINA

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INTRODUCTION

COVID-19 has become a serious infectious disease threatening all the people on the earth. It has been the second pandemic in 21st century (Mallah et al. 2021), with over 185 million infections and over 4 million deaths till July 9, 2021 (Chinanews 2021). According to codon usage studies, the novel virus has been transferred from an animal source (such as bats) to humans (Dhama et al. 2020), but there is yet no evidence of direct bat-to-human transmission of coronavirus infection (Frutos et al. 2021). In recent decades (1998-2018), about 421 million threatened (CITES-listed) wild animals were traded between 226 countries or territories (Liew et al. 2021). In the world, many nations and regions have taken measures to prevent and control COVID-19 by declaring a state of emergency, unprecedented quarantine, social distancing, and border closing efforts (Stawicki et al. 2020), but it is still difficult to control the pandemic.

Now-a-days, humans are still suffering from the big disaster of COVID-19, and our life has been totally changed after the new virus came to the world. Therefore, all the governments and scientists need to pay more attention to the efficient wildlife management in order to keep human and wildlife harmony on the earth. Moreover, besides the COVID-19 pandemic, the global climate change and pollution are also threatening the public health. The climate change can influence the distribution, life cycle, and physiological status of hosts, pathogens and vectors, and it can drive novel cross-species viral transmission (Keatts et al. 2021). It should be noted that how to be friendly with the natural environment for humans and how to reduce the conflict between human and wildlife? Some scientific concepts, current threatening aspects for public health, cases for conflicts between human and wildlife, practice for wildlife management in the US and current situation of wildlife management in China are discussed in this chapter.

SCIENTIFIC DEFINITIONS AND EXPLANATIONS

Wildlife

Wildlife includes all free-ranging animals and plants in ecosystems and do not depend directly on humans for their livelihood; this is a broader definition (Abd Rabou 2020; Bailey 1984). For a refined definition, wildlife is limited to terrestrial vertebrates (Fryxell et al. 2014), including mammals, birds, reptiles, amphibians, and fish, which is wildly used (Bailey 1984). In the past, wildlife was considered game animals that were hunted, in another word, "wildlife" was similar with game: the mammals and birds were hunted for sport in 1960s (Bailey 1984). However, the definition of the wildlife is not universally accepted; it could be changed with the viewpoint of the user by the researchers and scientists. In general, the wildlife profession considers that wildlife are free-living, wild animals (excluding feral or exotic species) of major significance to humans. This definition also includes the associated plants and lower animals (e.g., micro-organisms) because wildlife habitats that support wildlife should be considered. Wildlife and their habitats are interlocked and cannot be considered separately (Krausman and Cain 2013).

The above noted different definitions for wildlife from researchers can help us to understand the implication and extension of "wildlife". In fact, all animals had lived in the wild environment for long time, only some were domesticated purposely or unintentionally to meet the requirements in our daily life, such as guarder, food (meat, eggs), warming (fur, skin) for human until now. Dog was the first wild animal domesticated by humans about 15,000 years ago in Eurasia, followed by sheep, goat, humpless cattle, pig and cat etc. as shown in Table 1. Other data about theevolution of wildlife, and the wildlife domestication in the different continents are also available (Figure 1; Table 2).

Wildlife management

Wildlife management involves many aspects in practice. Conservation, sustained yield, and control are three main areas in wildlife management (Fryxell et al. 2014). Bailey defined "Wildlife management as the art of utilizing land for the production of wildlife populations for harvest or other values" (Bailey 1984). Wildlife management includes a series of decisions - whether to have a hunting season (in the US) or not, whether to plant crops and trees, whether to invest in a game-check station or on a forage survey, whether to improve current wildlife habitats or to purchase more lands (Bailey 1984). Moreover, wildlife management also includes controlling the population size, distribution, and quality of wildlife, and manipulating hunting seasons directly or wildlife habitat indirectly. Ideally, wildlife managers would have unlimited budgets and would know all they needed to

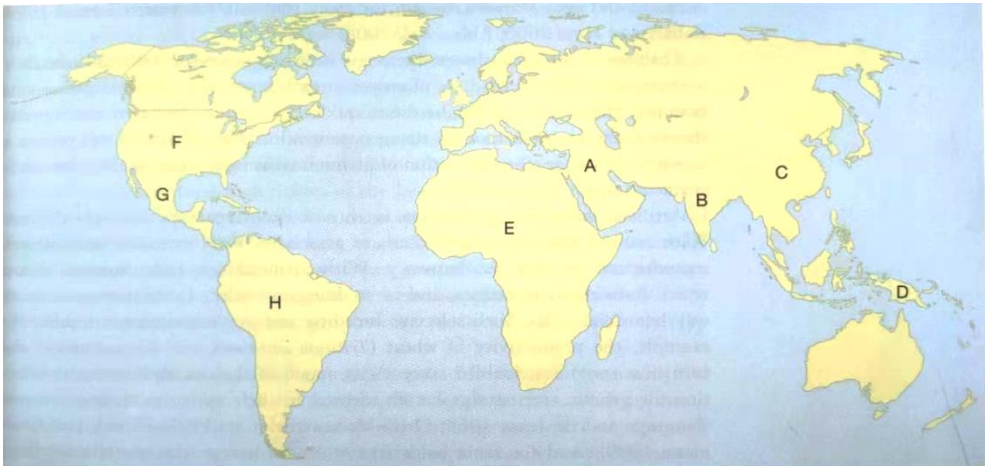

Figure 1: A map showing location where at least one animal domestication event is thought to have occurred. "A" is Southwest Asia, "B" is South Asia, "C" is East Asia, "D" is New Guinea, "E" is East Africa & South Arabia, "F" is North America, "G" is MesoAmerica, "H" is South America. No animal was domesticated in B and F in this map, only plants were domesticated there (Bergstrom and Dugatkin 2016).

Table 1: The time frame and geography of domestication for key vertebrate domestic species (MacHugh et al. 2017)

Common name	Scientific name	Approximate time frame for domestication (years before present)	Geographical location
Dog	*Canis familiaris*	15,000	Eurasia
Sheep	*Ovis aries*	11,000	Southwest Asia
Goat	*Capra bircus*	10,500	Southwest Asia
Humpless cattle (taurine)	*Bos taurus*	10,300	Southwest Asia
Pig	*Sus scrofa*	10,300	Southwest Asia
Cat	*Felis catus*	9,500	Southwest Asia
Humped cattle (zebu)	*Bos indicus*	8,000	South Asia
Water buffalo	*Bubalus bubalis*	4,500	South Asia
Pig	*Sus scrofa*	8,000	East/Southeast Asia
Chicken	*Gallus gallus*	4,000	East/Southeast Asia
Duck	*Anas platyrbyncbos*	1,000	East/Southeast Asia
Horse	*Equus caballus*	5,500	Central Asia
Bactrian camel	*Camelus bactrianus*	4,500	Central Asia
Dromedary camel	*Camelus dromedarius*	3,000	Arabian Peninsula
Donkey	*Equus asinus*	5,500	North Africa
Llama	*Lama glama*	6,000	South America
Alpaca	*Vicugna pacos*	5,000	South America

Table 2: The period and location where the animals were domesticated (Bergstrom and Dugatkin 2016).

Species of Domestication	Probable Period of domestication	Geographic regions in the map
Sheep	8000-9800	A. Southwest Asia
Goat	8000-9800	A. Southwest Asia
Pig	9000-10200	A. Southwest Asia
Cattle (taurine)	8000-10200	A. Southwest Asia
Cat	4000	A. Southwest Asia
Cattle (zebu)	6300-8000	B. South Asia
Water buffalo	4400	B. South Asia
Pig	6000-8400	C. East Asia
Silkworm	5200	C. East Asia
Yak	4200	C. East Asia
Horse	4000-5400	C. East Asia
Bactrian camel	4400	C. East Asia
Duck	1000	C. East Asia
Chicken	4000	C. East Asia
Cattle (taurine)	6400-7800	E. East Africa & South Arabia
Donkey	3400-5500	E. East Africa & South Arabia
Dromedary camel	3000	E. East Africa & South Arabia
Guinea fowl	1400	E. East Africa & South Arabia
Turkey	2000	G. MesoAmerica
Llama	4000-6000	H. South America
Alpaca	3000-5000	H. South America
Guinea pig	4000-5000	H. South America
Muscovy duck	2000-3900	H. South America

know about their target populations and habitats. Leopold (1933) described the historical development of wildlife management in the United States and elsewhere in the following sequence: i) restriction of harvest, ii) control of competing predators, iii) establishment of refuges, iv) restocking, game farming, and transplanting, and v) habitat management; Bailey (1984) added vi) informing and communicating with the public and other land users (Bailey 1984).

Wildlife managers pay attention to manage habitats, including plants and invertebrates that could serve as foods or disease vectors for vertebrates. However, the aims of most wildlife management programs are to control the abundance of distribution and population size of vertebrates. It becomes more difficult to manage all the wildlife on the earth now-a-days because so many species had been captive in the zoos or wildlife farms, which caused so serious contagious diseases in the world, such as COVID-19.

Ecological and Economic Carrying Capacity

Ecological Carrying Capacity (ECC1) is one of the most important and common terms in wildlife management. The ECC1 can be regarded abstractly as the K in the logistic equation. In fact, it is set to be the limit of natural resources for a population of wildlife in a particular environment (Fryxell et al. 2014). It is an equilibrium point that a wildlife population tends to through density-dependent effects because of shortage of food supply, habitat (e.g. territoriality), cover, or other resources. If the wild environment changes, it would cause the population to deflect from equilibrium and produce fluctuations about the equilibrium. The environment change for a long time would affect resources, which in turn changes K. In other words, the population changes would be followed by the environmental trend. Predators, parasites, or diseases can regulate the size of population and form other equilibria. In order to distinguish the equilibrium produced by limited resources, predation, and by a combination of the two, whether resources limitation or predators or both affect birth rate and death rate, should be noted (Bailey 1984).

Economic carrying capacity (ECC2) is the maximum sustained yield of wildlife population. It should be known that the population level is lower than the ECC1. For a population growing logistically, its level is half of K (Caughley 1976; Fryxell et al. 2014).

Birth rates are inputs to the population (Fryxell et al. 2014).

Death rates are losses to the population (Fryxell et al. 2014).

Wildlife management could be defined as the management of wild population in the ecological system; however, it may be limited for some researchers. Some scientists believe that many problems for wildlife management are related with humans. Therefore, popularization, education, park management, economics, law enforcement, and land evaluation are legal aspects of wildlife management, and they should be included in its definition (Fryxell et al. 2014).

Wildlife management means looking after a population. A population is a group of individuals belonging to the same species. When the population size becomes too small and near to extinct, conservation becomes urgent. In this condition, wildlife managers pay more attention to restoration activities. Wildlife management may be either controlled or supervised. A wildlife population could be managed in one of the following four ways: i) increase it; ii) decrease it; iii) harvest it for a sustainable yield; and iv) leave it as such but supervise it. Moreover, three decisions are needed: i) desired management goal; ii) appropriate management option; and iii) best action (Bailey 1984; Fryxell et al. 2014).

Wildlife Conservation and wildlife management

The aim of the wildlife conservation is to keep the wildlife surviving and reduce the trend of its extinction. Wildlife conservation depends on the kind of measures of wildlife management, such as habitat management (renovation, recover, rebuild etc.) and population management (number, sex ratio, age structure, pest control etc.). The wildlife management is the coexistence of humans and wildlife, or coexistence of humans and ecological system or species, including people using the wildlife resources efficiently, binding the utilizing and population optimization, preventing contagious diseases. Therefore, there are some common and overlapped points in both wildlife management and wildlife conservation, while difference is there at the same time.

The Convention on International Trade on Endangered Species of Wild Fauna and Flora (CITES) is a multifaceted deal, which intends to standardize the international wildlife trade and protect the threatened wildlife. The convention became effective on July 1, 1975 (Borsky et al. 2020). Now-a-days, the agreement has been approved by 183 countries. To some extent, CITES provides security to about 5,800 animals and 30,000 plants; these are classified into three Appendices, based on the consequences of the wildlife' deal on their long-term sustainability. Appendix I consists of around 1,000 species, which are endangered with annihilation. The business for these species is allowed under special conditions. Appendix II includes around 34,500 species, which are not endangered with extinction if trade is not effectively monitored. Appendix III includes 200 species that are already protected by some countries, but the cooperation is needed from other countries to avoid the unsustainable exploitation (Borsky et al. 2020). For these species, group could be adjusted by their abundance and changed in different countries or even in the same country from year-to-year, according to the population size. Every trade flow should be documented for the specific species in the CITES lists or from a signatory country. CITES plays an important role to forbid illegal international trade. Moreover, Convention on Wetlands of Importance Especially as Waterfowl Habitat pays more attention to protect the

waterfowls in the wetlands, which was implemented on December 21, 1975. Convention of Migratory Species or Bonn Convention could help to protect migratory wildlife on the land, in the ocean and sky, which was implemented on December 1, 1983. Convention on Biological Diversity could help to protect wild animals and plants, which was implemented on December 29, 1993. The above four conventions emphasize the international agreement to protect wildlife. Moreover, there are legal systems and acts to limit the citizens' behavior to manage and protect the wildlife and their habitats in the US and China, including Endangered Species Act (Noss et al. 1997; Malcom and Li 2015), Fish and Wildlife Coordination Act (Miller et al. 2011) in the US, and Wildlife Protection Act (2018) and Fisheries Act (2013) in China.

Louis Bernatchez emphasized on the important contributions to harvest management, stocking programs, definition of conservation units, recovery of threatened species, management of invasive species and forensic applications. In the fields of wildlife management, conservation and forensics (Figure 2), the Quebec government wildlife department greatly benefits from the maturity of genetics and genomics (Bourret et al. 2020).

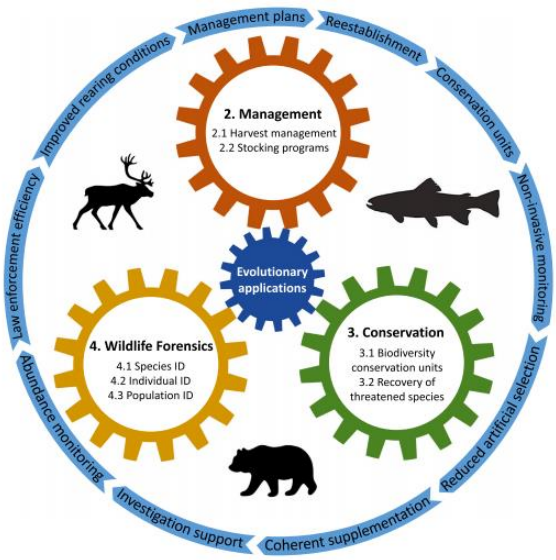

Figure 2: The Schematic themes (sections and subsections) showing the implementation outcomes set in motion by the integration of evolutionary applications (Bourret et al. 2020).

Zoonotic diseases

A zoonotic disease can be transmitted from vertebrate animals to humans or from humans to vertebrate animals in the nature (Rahman et al. 2020; WHO 2020a). The "Asia Pacific strategy for emerging diseases 2010" reported that about 61% of the existing human infections are zoonotic, and more than 70% came from wildlife, resulting from 1400 pathogens (Cleaveland et al. 2007; WHO 2020b). According to an estimate, there are between 650,000 and 840,000 known zoonotic pathogens that could have crossed the species barrier but have not yet done so (Perrings et al. 2018). Some major zoonotic diseases, such as Ebola, tuberculosis, bird flu and COVID-19 have their origins in wildlife (Borsky et al. 2020). Different zoonotic diseases are caused by various pathogens, including bacteria, viruses, fungi, protozoa, parasites, and other pathogens. The emergence, distribution, and patterns of zoonotic diseases are greatly influenced by biological and abiotic factors. COVID-19 is a new zoonotic disease, and its pathogen seems to have been originated from bats. At present, the main issue is to effectively control the zoonosis. The implementation of One Health measures is highly recommended for this purpose (Rahman et al. 2020). Close contact between humans and animals is needed for the spread of some wildlife-origin infectious diseases, such as HIV, SARS, and COVID-19 (Wolfe et al. 2007; Borsky et al. 2020).

Wildlife values

In "Principles of wildlife management", the author described the following wildlife values (Bailey 1984):

Commercial value: The capitalized value of the income derived from selling or trading wild animals or their products (Bailey 1984). Some wildlife can be legally bred for consumption in wildlife farming like agricultural animals, such as tigers for bones, bears for bile, porcupines for meat, birds for pets, turtles and snakes for both food and pets, crocodiles for skin, frogs and shrimp for food etc. (Rizzolo 2020).

Biological value: It is the contribution of wild animals to productive ecosystem. Wildlife is a part of the complex biotic "machinery" of ecosystems that we rely on for food, water, fertilizer, and aesthetic and recreational values (Bailey 1984).

Recreational value: People derive benefits of pleasure, adventure, and enhanced physical and mental health from outdoor activities involving the pursuit or sometimes accidental enjoyment of wildlife (Bailey 1984).

Scientific, philosophical, and educational value: The scientific value of wildlife is the use of wild populations as experimental material for scientific study. Ecologists, ethologists, physiologists, pathologists, demographers, sociologists, and anthropologists have conducted studies on wild animals to extend their knowledge. The educational value of wildlife is realized from the use of wildlife examples in schools, at nature centers and park exhibits to enhance people's understanding of their environment (Bailey 1984).

Aesthetic value: The use of wildlife and their habitats as objects of beauty or historical significance, and as they become part of literature, poetry, art, and music, are the most personal and variously conceived wildlife values (Bailey 1984).

Social value: People live in the community and wildlife can help us to get more benefits, not only for ourselves, but also for our neighbors (Bailey 1984).

Negative value: The negative values of wildlife include the costs of wildlife damages to crops and other property and the costs of controlling these damages. Where these values can be expressed in cash, they should be subtracted from the total commercial and recreational value of the wildlife resources (Bailey 1984).

The total value of a wildlife resource is the sum of all the above positive values, minus the negative values. However, since the values are not commensurable (not measured in common units) and some are not even quantifiable, we cannot produce a single number to represent the total value of wildlife. This makes it difficult to compare the value of a new reservoir or strip mine with the value of the wildlife habitat that would be destroyed in developing these other resources. Since wildlife is a public owned resource, what total compensation is due the public when a mine is developed on public land and destroys a wildlife resource? One way to answer this question is to consider the total value of a wildlife resource as the cost necessary to replace it. Colorado has developed this method for calculating the costs of mitigating loss of wildlife habitat (Norman et al. 1975). Replacement costs include those for purchasing and developing new habitat and for improving existing habitat to increase its productivity (Bailey 1984). According to the survey in the surrounding areas of the Changqing National Nature Reserve in China (Hou et al. 2020), the cultural value of wildlife is significantly higher than that of the infrastructure and other natural landscape features.

Wildlife strike risk

The habitats for wildlife and humans are overlapped with the exploration of lands. Therefore, some animals would be hurt or killed by the vehicles, trains and ships, even airplanes. In the street, the dead body of skunk (*Mephitis mephitis*) and white-tailed deer were found in Washington State, and coyote (*Proteles cristatus*) was found on the street in Oregon State, USA in 2020. Some homeless dogs were killed on the streets in China in 2021. These animals were killed always by the cars and vehicles. Some marine wildlife even could be hurt by ships (Sèbe et al. 2020). In the airport, the birds are so often hit by the airplane in the world (Hu et al. 2020). How to reduce the risk and take some measures to protect the safety of humans and wildlife? It is a big problem for the scientists and governments. In recent years, traffic caused by bird strike events has increased along with the number of aircraft taking off and landing increased continuously. According to the statistics in "Bird Strike Aircraft Information Analysis Report" from the Civil Aviation Administration of China (CAAC), bird strike accidents increased from 326 in 2007 to 4618 in 2016. The number of bird strikes in the US has increased from 1851 in 1990 to 13,668 in 2014, according to the data from the Federal Aviation Agency (FAA), USA (Lopez-Lago et al. 2017). The increasing bird strikes can cause aircraft accidents, which would bring the huge security risks and economic losses (Allan 2000). Bird strikes became an "A" aviation disaster in the International Federation of Aviation (Hu et al. 2020).

COVID-19, Global Climate Change and Pollution: Threatening Wildlife Management and the Public Health

COVID-19 has challenged the wildlife management and public health

The wildlife management becomes more and more popular as a research area for scientists in recent years, especially during COVID-19 epidemic time. COVID-19, coronavirus disease 2019, was found for the first time in China in December 2019, but in a short time, it became a serious infectious disease threatening all the people on the earth. There were over 185 million confirmed cases and over 4 million dead cases till July 9, 2021 (UTC/GMT+08:00) (Chinanews 2021). Human are suffering from the big disaster, and our life has totally changed after the new virus came to the world in December 2019. SARS-CoV-2, the pathogen of COVID-19, is the seventh coronavirus to infect humans, and other six coronaviruses included 229E-CoV, NL63-CoV, OC43-CoV, HKU1-CoV, SARS-CoV, and MERS-CoV (Hedman et al. 2021). At the same time, a large number of captive wildlife were killed for preventing the spread of COVID-19; this includes the domestic mink, which is the only known animal that can transmit SARS-CoV-2 to other minks and humans (Hedman et al. 2021). Denmark ordered to kill 17 million captive minks and UK ordered to kill all the minks to prevent COVID-19 transmission on November 2020, but the policy was opposed by some officials and businessmen (Shanxi Evening New 2020). The wildlife farming industry has been dramatically developed in the past decades in China (Anonymous 2017). According to "Report on the sustainable development strategy of wildlife farming industry in China", the annual output value was 133 billion RMB (about 20 billion dollars) for wildlife farming in 2016. International trade of wildlife is one of the important factors for zoonotic disease risk, as the zoonotic pathogens are traded as well (Borsky et al. 2020). The worst thing is that some captive animals were killed or burned to cut the spread of COVID-19, which caused huge economic losses for some businessmen and wildlife farming met the biggest obstruction during COVID-19 pandemic. All the governments in the world have already taken some strategies to recover the economy and people's daily life, but it is not so easy to achieve this. The public health has been badely challenged due to COVID-19 pandemic. There has been always new variant of coronavirus that can appear anytime and anywhere in the world. The scientists in Harvard University hypothesized that COVID-19 would probably end in 2025, or it would coexist with humans forever, like flu (Liu 2021). Therefore, the wildlife management should be paid more attention than before in the current time. How to keep the wildlife and humans to live in harmony on the earth is the most important issue of the time.

Another disaster for public health is global climate change

The melt ponds were found on the Southern pole (Figure 3) and scientists recorded the highest temperature up to

20.75°C on February 9, 2020 (Anonymous 2020a). The researchers also found 2 million methane emission spots on the Northern pole on February 14, 2020 (New observations on Cosmology, February 16, 2020; Anonymous 2020b). The global warming would cause glacier melting and about more than 30% polar bears could not survive in the next 35-40 years, as shown in the report from International Union for Conservation of Nature (IUCN). Red snow appeared on the Southern pole on February 27, 2020 (Anonymous 2020c) and green snow was found there on May, 2020 (Woodyatt 2020). Glacial algae could be found on all continents, and most algae belong to Chlamydomonadales (Chlorophyta) and Zygnematales (Streptophyta). Other algal groups include euglenoids, cryptomonads, chrysophytes, dinoflagellates, and cyanobacteria. They can live under extreme conditions near 0°C, high or low irradiance levels, acidic pH, low conductivity, and dryness after snow melts. These algae change the snow color to red, green, golden-brown, or purple-grey, and they are part of communities that include other bacteria, viruses, fungi, eukaryotes, and archaea.

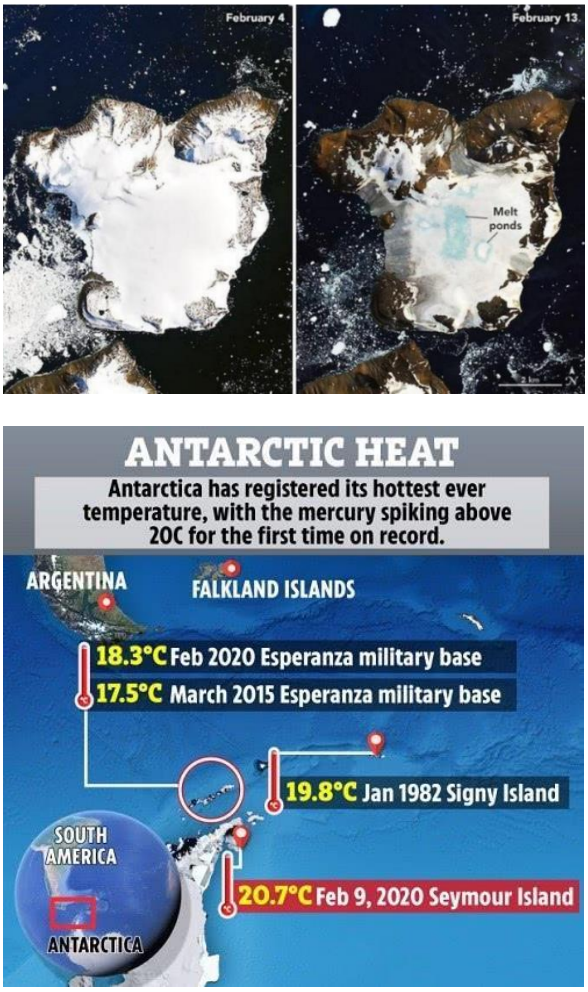

Figure 3: The southern pole was melting on February 2020, and 20.7°C hit the record (Anonymous 2020a).

They are one of the important components of the global biosphere and carbon and water cycles. Life cycles in the Chlamydomonas, Chloromonas and Chlainomonas include migration of flagellates in liquid water and formation of resistant cysts, many of which were classified as other algae (Figure 4). Glacial algae reduce albedo, accelerate the melting of snowpeaks and glaciers, and can be used to monitor climate change. Some selected strains of the above algae have potential for producing food or fuel products (Hoham and Remias 2020).

Pollution is another big problem for wildlife

The water system is polluted most seriously in the globe. The river, lake and ocean can be polluted by different kinds of algae, which can also cause death of some wildlife in the water, resultantly, the water surface looks like grassland with malodorous smelling. In China, pollution caused the harmful effects for some wildlife, especially for some waterfowls on December 2020. The reason could be the algae in the water because not many terrestrial birds died at that time, and the temperature dramatically dropped. Some local birders found the egret, heron and colymbiformes died on the frozen surface in the river or lake in Weifang, a small city in Northeast of China. In the local botanic garden, the dead fish were seen floating on the water surface after melting of the frozen water. There should be some waterfowls swimming in the river in the city, but the pollution may drive them far away, only some terrestrial birds could have been found in the trees.

Harmful algal bloom events can be caused by rapid growth of photosynthesizing organisms in natural bodies of fresh and salt water (Roberts et al. 2020). These events can be intensified by nutrient pollutants (e.g., phosphorus) and warming waters or other climate changes (Wells et al. 2020), which have a negative effect on health of humans, animals and the environment (Backer et al. 2015; Adams et al. 2018). The public health concerns caused by harmful algal bloom events are centered on a subset of phytoplankton: diatoms, dinoflagellates, and cyanobacteria (or blue-green algae) in the US. One Health Harmful Algal Bloom System (OHHABS) was launched by Centers for Disease Control and Prevention (CDC) to inform efforts to prevent human and animal illnesses associated with harmful algal bloom in 2016 in the US. A total of 421 harmful algal bloom events, 389 cases of human illness, and 413 cases of animal illness were reported in 18 states during 2016-2018. Gastro-intestinal diseases or generalized illness symptoms were the most frequently reported (>40% in human cases and >50% in animal cases). However, other signs were also reported. The surveillance data from harmful algal bloom events could have been used to control and prevent the harmful algal bloom–related diseases (Roberts et al. 2020).

Algal bloom events have become a serious water pollution problem in freshwater ecosystems in the globe because harmful algae can gradually degenerate the self-purification function of water and decrease the water quality (Paerl et al. 2011; Šulčius et al. 2017). In the past several decades, many scientists have studied the environmental factors of algal bloom outbreaks and

decline (Paerl et al. 2011). High temperature, adequate light intensity and excessive exogenous nitrogen (N) and phosphorous (P) have been regarded as the three major abiotic factors (Metson et al. 2017).

Moreover, aquatic microorganisms play an important role in regulating water quality and composition of other organisms (Shao et al. 2014). The characterization of bacterial communities related with algal blooms in eutrophic lakes from China (Su et al. 2017) and the US (Berry et al. 2017), investigated by the 16S rRNA gene sequence method, have shown that algal blooms are formed by a biological disturbance in the bacterial communities of freshwater lake. In freshwater ecosystem, organic carbon, nitrogen and phosphorus cycling is greatly influenced by the microbial communities related with algal blooms (Zhang et al. 2018).

Furthermore, the used masks in the ocean killed and hurt so many wild animals during COVID-19 pandemic, which is the worst thing to the wild environment. During the coronavirus pandemic, more and more disposable masks could be found anywhere, on the lands or in the water where people could arrive. According to a new report, an estimated 129 billion face masks and 65 billion plastic gloves were used every month for preventing the spread of COVID-19 on the globe (Sophie 2020).

Conflict Between Human and Wildlife

The conflict between human and wildlife usually occurs when the habitats for humans and animals overlap, and wild animals are increasingly adapting to urbanizing environments (Hadidian 2015). Now-a-days, the problem is more serious than ever before because of the explosive growth of human population. According to an American official site, the following ways could be used to prevent human/wildlife conflict: no food for wildlife, secure storage for garbage, pets feeding in the house not outdoors to avoid attracting unwanted animals, fencing in the garden and preparation for learning about how to treat animals in the wild environment (McCarthy 2015).

The following cases will show seriousness of the conflict between human and wildlife in recent years.

Case I: On May 15, 2020, a 110-pound male cougar was spotted by infrared camera in the backyard of a house near Mountain View Park in Ellensburg, Washington, USA and this big cat was killed by police in order to protect local citizens (Figure 5). For Washington Department of Fish and Wildlife agents, it was difficult to decide how to deal with the case. The officer explained that it was legal to take lethal measures for the citizens if they could demonstrate their personal safety and pets or livestock were at risk in front of some wild animals (Holappa 2020).

Case II: Kuzya, a Siberian tiger (*Panthera tigris altaica*/Putin's Tiger in Chinese) from Russia, was inspected in a Chinese reserve on October 9, 2014, according to GPS satellite data opened by a Russian scientist (Figure 6). Some Chinese researchers were

Figure 4: The microalgae (*Chlamydomonas nivalis*) caused the redlike snow on Southern pole on February 2020 (Anonymous 2021).

Figure 5: A 110-pound male cougar was killed because it was near a homeowner's house, near Mountain View Park in Ellensburg, WA, USA (Holappa 2020).

requested by government to inspect the beast anytime, anywhere to protect local citizens and domestic animals near the natural reserve after the big cat entered Chinese land at that time. The Russian scientists contacted some Chinese organizations for protecting animals and asked them to treat the tiger better, keep it with no chicken or other meat, and avoid degrading its wild viability. Then Chinese local government began to launch a monitoring, early warning and protection mechanism to protect it. In the next two months, Chinese government paid more attention to this big cat because there were some trackers in Taipinggou Natural Reserve in Luobei county. According to the Director of the reserve, people felt excited about the finding of the first Siberian tiger there. But it was a huge challenge to China to protect the tiger back to Russia. In one month, the tiger hunted wild boars and deer in the reserve. Dr. Jiang Guangshun, a professor in Northeast Forestry University and the Executive Deputy Chief of the Field Research Center in the national forestry administration, said the Russian researchers had updated the new findings with the center. Moreover, the tiger was trained, especially before being released, and kept away from people. The wild boars and rabbits, as its food, could be easily found in the area where it was staying. Hair, feces and tracks left by the big cat were discovered in the areas where the beast was suspected to have travelled in the forestry area of the Lesser Xing'an Mountain in the northeastern province of Heilongjiang. Eventually, the tiger went back to Russia from Heilongjiang in China on December 7, 2014. Dr. Jiang Guangshun told the students in the class about Putin's Tiger in Northeast Forestry University in 2015. Chinese State Council required the specialists to report the activity of Putin's Tiger every day. There was a Chinese researcher in Russia at that time to transfer the data for the tiger from Russia to China and to make sure that both the tiger and local community were safe. The challenge could be regarded as a chance to evaluate the effectiveness of wildlife management in China. The good thing was that no one was hurt by the big animal, only some wildlife had been hunted, such as Chinese wild boars, roe deer (*Capreolus pygargus*), Northeastern hare (*Lepus mandshuricus*) etc. One Russian scientist said Kuzya found a good natural environment and sufficient food in the reserve. Now there are more than 500 wild Siberian tigers in the world. Chinese and Russian governments have agreed to save the tigers from extinction (Zhou 2014).

Case III: Fifteen Asian elephants left their habitat in Mengyangzi Nature reserve in Xishuangbanna, Dai autonomous prefecture, Yunnan province in April 2021 (Figure 7). They were found directed to the north of the province, just near the capital city Kunming about 120 km (Figure 8). Research workers tried to guide them to protect local residents and elephants themselves. Moreover, the elephants left their habitat last December to Mojiang county, and they traveled tens of Kilometers from Mojiang to Yuanjiang county during this April (Chinadaily 2021). The human-elephant conflict is the prominent problem in China. According to a study for human-elephant conflicts in 2011 to 2018 and questionnaire survey with 217 villagers, the number of elephants increased in the past 40 years, from 101 in 1976 to about 184-205 in 2016, and near 300 in 2021 (Su et al. 2020; Nangongxiaopang 2021). The main reason was the efficient work of Xishuangbanna nature reserve and improved awareness among local residents. However, the habitat loss and fragmentation for elephants may be the main reason to force them to leave the original habitat. The economic loss and threatens safety for local residents from elephants are so urgent that the efficient protection and management of Asian elephants are needed now-a-days (Su et al. 2020).

Figure 6: The tiger, named Kuzya, was one of three rare wild cats that Putin helped release into Russia's remote Amur region in eastern Siberia in May (Anonymous 2014a).

Figure 7: The Asian elephants are sleeping on the land near Kunming, Yunnan province in June 2021 (Shangfangwen 2021).

Figure 8: The Asian elephants are walking in the land near Kunming, Yunnan province in June 2021 (Shangfangwen 2021).

Wildlife Observation Practice in The United State

The free terrestrial wildlife in the United State - Observation Record

Why could the wildlife have chance to get more freedom in the US? According to the observation in Ellensburg, Washington and Redmond, Oregon during October 16, 2019 and November 7, 2020, the wild animals can easily move from one spot to others because there are not many fences and walls. For example, the buildings in Central Washington University (CWU) and houses of local citizens in Ellensburg are standing there, only lawns are regarded as boundary, with almost no barrier for the wildlife in the natural environment. Moreover, human interference is rare in the local parks and neighborhood. And the air quality is always very clean during the whole year, except for the wildfire time in 2020.

The Mallards were found in the cricks near CWU campus in the whole year. They could prey, swim and rest in the small rivers and fly to the near lakes or rivers to get food during winter when the local water surface was frozen. Even they could eat some ice and nuts under the trees and on the lawn in winter in front of Science Building in CWU. The California Quails could hide themselves in the bushes and take their several chicks to find fresh and tender grass and lie on the ground in the sunny days in summer. Mallards, California Quails and Doves could be found walking across the streets when the drivers would stop to wait for them first. Sometimes Mallards and doves stayed in the middle of streets, not hurry to go, then some drivers probably started engine and the birds would fly away immediately. There were about twenty crowns croaked in the neighbor's yard in a 2020 summer evening, an owl standing on the pine tree. Then the noise attracted an eagle and a crown was prayed by the eagle. At last, other crowns flew away from the yard. Robin was found on the lawn to dig earthworms and it was aggressive. It could drive other small birds away from the lawn, except for magpie. Magpie could still stay on the lawn, while robin showed aggressiveness, because magpie could dig earthworms and it is bigger than robin. Around 10 wild turkeys were found near a forest in Kittitas county, WA. Hummingbirds were attracted to get food by some feeders near a mountain in Redmond, Oregon. It was so small like a black spot when birding location was a little far near the hill, and it flew up and down like a helicopter in Ellensburg, WA. A Wilson's snipe was found after rain near the grassland for feeding cattle, some temporary cricks were also there. Bald eagle could be found in the sky near the Yakima River and in Irene Rinehart Riverfront Park. Another eagle stood under a pine tree on the snowy lawn in front of the library of CWU in 2019 winter, frightening a crown away. Swallow could be found in a grassland in summer. Woodpecker was always in the park or on the big tree. Blue jay was spotted on the lawn and in the mountain. Red-winged blackbird could be found in the trees near a trail. Falcon could be found in the suburb. Several seagulls were found in the sky near Columbia river in summer. The Canada geese could live the whole year in Carey Lake in Irene Rinehart Riverfront Park, taking the branches from beavers' cutting to build nests. They could migrate in summer, west to east in the morning and east to west in the evening located by the sun from July to September. Moreover, so many small birds could not be recognized one by one. All the birds live in the peaceful and quiet environment in a small city, with no more than 20,000 people there.

The wild mammals are not easy to be found in the natural environment. Only white-tailed deer appeared often in the golf course turf in Ellensburg, WA. One squirrel was found in the turfgrass in Redmond, Oregon. The beavers left imprints by strong teeth on the tree trunks and dragged them and small branches to build dams to be used as home and get food for families in Carey Lake, Irene Rinehart Riverfront Park. The skunk's dead body was found on the street in summer, dying of roadkill. Both coyote and white-tailed deer were killed by the vehicles. The chipmunk was found on the top of the local mountain. The rabbit and hare could be seen near a trail and in a small park. In winter, there were some imprints on the snowy lawn in the backyard and in Irene Rinehart Riverfront Park. The foot imprints of weasel (*Mustela sibirica*), raccoon (*Procyon lotor*), American black bear, deer, rabbit and hare, wild cats could also be seen. The cave's imprints of rodents could be found on the lawn in the backyard and park. They could be moles.

The reptiles and amphibians were not easily observed and recorded. But one small snake appeared on the trail after rain in summer. The voice of some frogs could be heard in Irene Rinehart Riverfront Park or in the ponds in Ellensburg, and some frogs could be heard in the pond in Redmond, Oregon.

Why the wildlife could be so free in the USA? Why could they keep the natural environment so well? How can other countries get some inspire and wisdom from the wildlife management in the USA? All this is due to the fact that the constitution and acts are very strong and strict in USA (Treves et al. 2017). There are over 170 Federal laws that regulate environmental activities which may affect wildlife, such as Lacey Act of 1900, Migratory Bird Hunting Stamp Tax Act of 1934, Pittman-Robertson Act of 1937, Fish and Wildlife Coordination Act of 1958, National Environmental Policy Act of 1969 (Fairbrother 2009) and Endangered Species Act of 1973 (Carroll et al. 2010) etc. The wildlife is protected by the laws and principles. Even in the hunting season, citizens can hunt only some wild animals if they have legal hunting licenses, but there are strict rules for hunting (Messinger et al. 2019). All the people in USA have consciousness to protect the wildlife in their mind, which is necessary in the world to keep our natural resources - wildlife and helpful to decrease the trend of extinction for some endangered species (Treves et al. 2017). The suggestion could be developing the acts and popularizing laws and principles among the communities in the underdeveloped and developing countries, and all the governments need to cooperate in the human's common assets - wildlife.

An observation record on Canada geese in the United State

The Canada goose (*Branta canadensis*) is the largest wild goose in the world, with the subspecies "giant Canada goose" having specimens weighing over 9 kg. However, most Canada geese weigh between 2.3 and 6.4 kg (5 and 14 pounds), with females weighing slightly less than males. Canada goose measures from 76 cm to 114 cm in length and has a wingspan between 127 cm and 190 cm. The Canada goose can be distinguished from other species of geese by its distinguished black neck and head, by a striking white "chinstrap" on the neck, and by a lightly colored breast and a brown back (Figure 9). Like other geese, they have an elongated neck, as well as webbed feet, and both these adaptations make them quite agile in the water when they are going after food.

The observational spot on Canada geese was near Central Washington University and Yakima River in Ellensburg, Washington State in the USA. Observations were made during 18 mornings and 12 evenings from July 27 to September 18. The date, time, weather, and all observations were recorded to account for differences in environmental conditions. Data collected included: the number of geese present and the flock activities and behaviors. OPPO R17 application was used to take photographs and video recordings of the geese were taken to document the observational data.

In Ellensburg, the Canada geese were found throughout the year according to the map of Canada goose distribution (Figure 10). There are more than 70 observers' data of Canada geese in Ellensburg Valley from September 1998 to April 2020 on the current eBird website (Table 3). The most observation spots are near the I-90 highway and Yakima River (Figure 11). In this report, the records for Canada geese were found in the Carey Lake at Irene Rinehart Riverfront Park near Yakima River in winter (Figure 12 and 13), while they were found in sky on Abel place during summer (Figure 14) and on the grassland near John Wayne Trail in autumn (Figure 15). The focus was the movement behavior from July 27 to September 18 in this report.

Figure 9: The superficial character of Canada goose. https://forum.americanexpedition.us/canada-goose-information-facts-photos-and-artwork

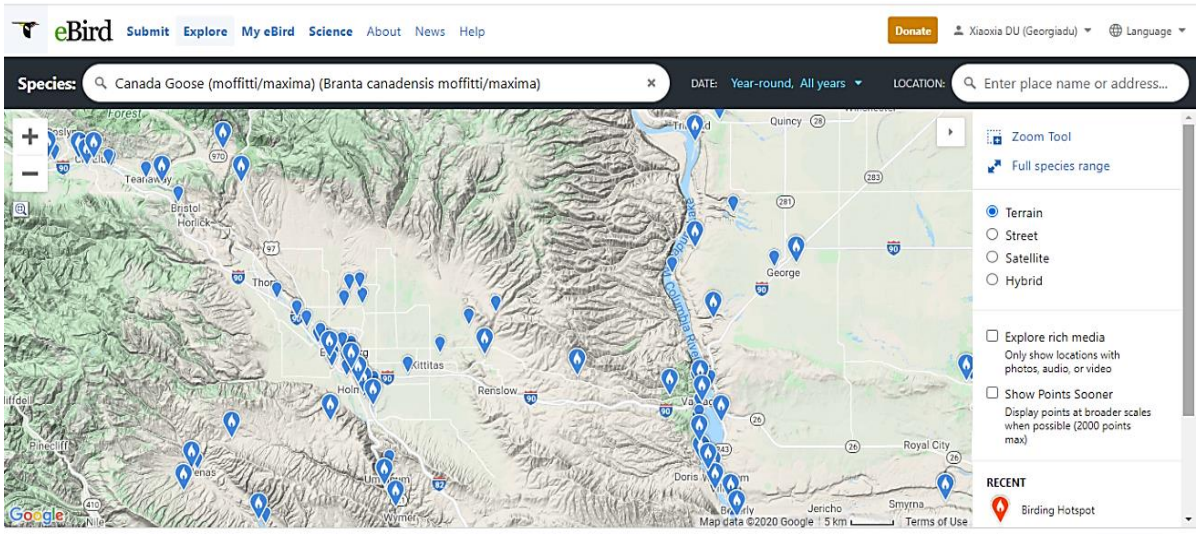

Figure 10: Spots of Canada geese in Ellensburg Valley on the current eBird website from September 1998 to April 2020 (https://ebird.org/map/cangoo4?neg=true&env.minX=&env.minY=&env.maxX=&env.maxY=&zh=false&gp=false&ev=Z&mr=1-12&bmo=1&emo=12&yr=all&byr=1900&eyr=2020).

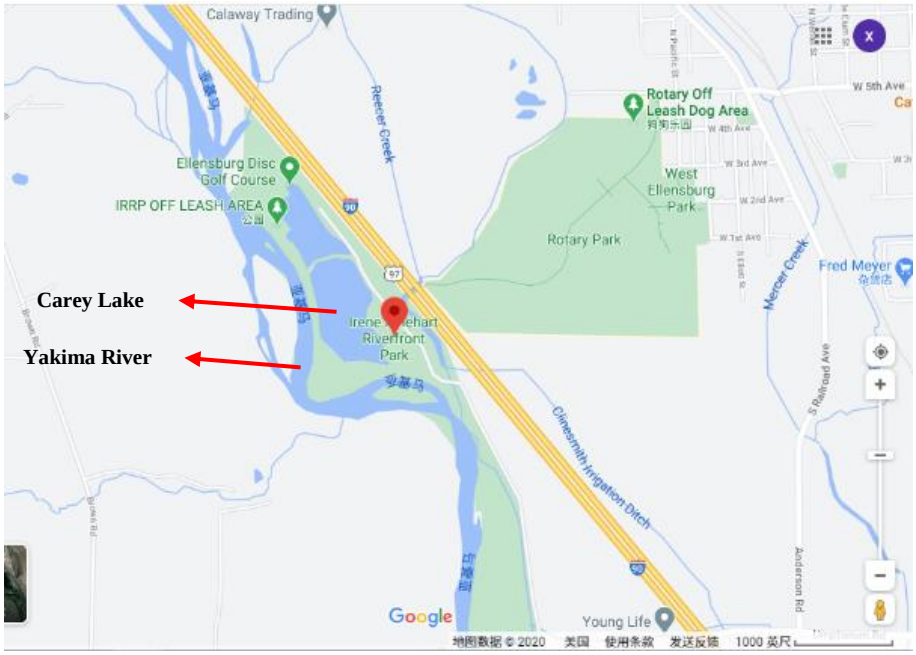

Figure 11: Carey Lake at Irene Rinehart Riverfront Park near Yakima River in Washington State in USA in Google map.

Figure 12: Canada geese are in Carey Lake at Irene Rinehart Riverfront Park in winter. Date/time: 15:09:05, Dec 8,2020 Location: Carey Lake at Irene Rinehart Riverfront Park, Ellensburg.

Figure 13: Canada geese are in Carey Lake at Irene Rinehart Riverfront Park in winter. Date/time: 15:09:01, Dec 8,2020 Location: Carey Lake at Irene Rinehart Riverfront Park, Ellensburg.

Figure 14: Canada geese in flock in V-shaped in summer. Date/time: 08:10:30 Aug 19, 2020. Location: Abel Place, Ellensburg.

Figure 15: Canada geese on the grassland in fall. Date/time: 18:26:07 Oct 4, 2020. Location: John Wayne Trail, Ellensburg.

The Canada geese showed a relatively regular movement schedules in the morning and evening during summer and autumn in 2020. The Canada geese were observed from July 27, 2020 to September 18, 2020 in Ellensburg. They didn't show the regular movement schedule after September 18, 2020 (Table 4).

The Canada geese can fly in the morning and in the evening during the observation time. But they can show different kinds of groups and different characters during the movement. In the morning, they began to group and fly from the Carey Lake (west) to sunrise location (east), and they migrated from east to west in the evening. The population size for each flock varied from 2 to 39, occasionally single goose, but most time it was around 10 to 30. In the morning, the movement time lasts longer than evening from the beginning to the end. The movement time was about 7:50 am in the morning, while 5:30 pm in the evening, occasionally they were found in the sky on other times, such as 6:00 am and 3:00 pm (Table 5).

Morning movement behavior

Most of the time, the Canada geese moved from west to east, but sometimes toward north in the morning from July 27, 2020 to September 18, 2020. Normally, their staring time and ending time could last 5-43 minutes in the morning. The movement time was earlier on 7:50 am (August 3, August 4, 2020, when highest temperature was 32-33°C) in the hot days, and later until 9:36 am (September 18, 2020, when highest temperature was near 24°C) in the cool days. At the beginning of grouping, the shape of their flocks looked like a straight line (August 18, 2020), and sometimes they changed the shape gradually, but sometimes they kept the straight line for several minutes, and then changed the formation. During this formation, each goose found its own position in the flock and cooperated with other individuals to go forward. The largest population size was about 800 on August 19, 2020, about 600 on August 17, 2020 and 560 on August 18, 2020 (The highest temperature was higher than other days, up to 34-38°C). The mountain fire made pollution in Ellensburg from September 15, 2020 to September 18, 2020. The formation of folks was different from other days in the smoking condition (Figure 16), more geese were together, and sometimes shaped like double "V" shaped (Figure 17). The smallest population size was about 20-30 on August 3, 2020 and August 11, 2020 (The highest temperature was near 32°C.

Evening movement behavior

The Canada geese moved from east to west in the evening from July 27, 2020 to September 18, 2020. The movement time was about 5:30 pm on the hot days on August 8, 2020 and August 31, 2020 (The highest temperature was up to 30°C), and about 7:00 pm on the cool day on August 12, 2020 (The highest temperature was near 26°C). But the geese movement schedule was much earlier than other days on August 28, 2020 because the lowest temperature dropped to 9°C, and lower than the lowest temperature, higher than 11°C on other days. There was a special day on August 13, 2020, when the lowest temperature was near 6°C, the movement time didn't change too much, but the population size was about 270. Therefore, the population size in the evening was bigger when the temperature was lower on August 28, 2020, while it was smaller when the temperature was higher on August 8, 2020.

Figure 16: Some flocks of Canada geese together to go forward in smoking condition in the morning. Date/time: 09:36:39 Sep 18, 2020. Location: Abel Place, Ellensburg.

Figure 17: The Canada geese in folk shaped like double "V" in smoking condition in the afternoon. Date/time: 15:39:22 Sep 15, 2020. Location: Abel Place, Ellensburg.

It was not easy to identify whether their movement was short or long according to observation. This was because the movement time was in summer, and it was different from autumn and spring migration. Some researchers found that Canada geese had movement in summer. The short-distance movement of Canada geese migration can be seen from September to the beginning of November (Landys et al. 2004). The late summer movements by giant Canada geese were related with the September hunting season (Dieter et al. 2010). The movement schedule of Canada geese can change with the temperature, so there is possible correlation between movement in summer and temperature. The data was

insufficient to analyze statistically, but some data showed the regular patterns.

The sun and star may help Canada goose to locate the movement

The sun may help Canada geese to locate the movement direction. This is because their movement went toward sunrise direction from west to east in the morning, and toward sunset direction from east to west in the evening. There was a famous experiment published in New York Times on September 28, 1993, titled "Migrating birds set compasses by sunlight and stars". Dr. Kenneth P. Able and Mary A. Able, both in the State University of New York at Albany, showed evidence that Savannah sparrows, migrating between the Northeast and the Deep South or Mexico, could see polarization pattern in the daylight, but also use the orientation patterns as a navigation aid for calibrating their magnetic directional sense. The birds were not influenced by the position of the sun, but by the polarization direction of sunlight scattered by the atmosphere. Migratory birds could use a magnetic compass in their eyes for navigation. Its basic sensory mechanisms have long remained elusive, but now researchers have revealed exactly where in the eye, the birds' control center for navigation is situated (Anonymous 2018).

The movement behavior of Canada goose may find suitable habitats in summer in Ellensburg, WA, United States

The observation for the moment behavior of Canada goose was observed in summer and autumn in Ellensburg, and published in a paper, titled "Canada Goose-Early Summer Migration" (Martin and Foote-Martin 2018). The first movement of molt migrants of Canada geese was in the beginning of June, along with other migrants, and continued for next two weeks or so in Wisconsin. The non-breeders started to make flock and inviting others to prepare for their northern flight, where they could replenish feathers. At that time, nearly all the young ones had hatched and it was most likely to allow failed breeders to join the flocks. The only clutches that were still to hatch in the southern United States were making re-nesting efforts and some of the early hatched young ones were nearly half grown-up. When geese are traveling, they mostly flew a few hundred feet high, but the flocks present at higher altitude and toward north were a sign of the molt migration. Michigan's Waterfowl Biologists discovered that the geese migrated over 600 miles to the place around James Bay. They also discovered that most non-breeding geese in the countryside migrated, whilst a quarter of their "park" geese took part in the migration.

The geese were "giant" subspecies (*Brania Canadensis maxima*). In the 1800's, the giant goose was hunted all the year, and eggs and young birds were also taken for food. By the 1900s, there were almost certainly no nesting giant Canada geese in Wisconsin. In spite of this, they could be found in game breeders' hands and the breeders supplied the source for the DNR reintroductions in the 1960s and 1970s. Mark assisted the pinioned adults at Crex Meadows State Wildlife Area in 1970 from their winter-pens to summer-pens so that their offspring would fly out of the summer-pens to build a population. Canada geese nested during 1996 for the first time at Goose Pond. The Waterfowl Biologists in Wisconsin Department Natural Resources (WIDNR) anticipated in 2017 that the giant Canada goose population could be 158,000 (Martin and Foote-Martin 2018). Until reaching the age of two years, Canada geese would not start nesting. There must be a thrilling time for a-year-old birds led by failed breeders to north for a summer vacation in the land of the polar bears.

The movement behavior of Canada goose may have special function in summer in Ellensburg, WA, USA. It is possible that Canada goose moved to the north or east direction in summer hot days in order to be away from the hot weather, searching for suitable habitats. It is evident that the movement population of Canada goose in cool days was less than that in hot days. But the data is not enough to analyze the relationship between temperature and their schedules in the morning and in the evening. Further observation could be conducted to find the rule of migration in summer.

The summer movement may help Canada goose from being hunted

The late summer movements by giant Canada geese were related with the September hunting season (Dieter et al. 2010). The population size is growing substantially in recent years, a September hunting season was implemented in more than 40 states in USA to control the number of Canada goose, which may change their movement behavior. Sometimes there will have a conflict to get benefits between wildlife and humans. It is not easy to manage wildlife without human's interference.

For example, the population size for Canada goose declined by early 20th century, and almost extincted in the 1950s (Hanson 1997), but Northern Prairie Wildlife Research Center and center's Canada goose production and restoration program protected the species between 1964 and 1981. Then 64 pens with 64 breeding pairs of screened, high-quality birds were used to conduct the project, and the population size of Canada geese was raised to more than 60,000 in North Dakota (Anonymous 2013; Figure 18). Their number recovered in most ranges with improved game laws and habitat recreation and preservation programs. But later, their populations in some places grew substantially. Therefore, many people regarded them as pests for their droppings, noise, and confrontational behavior. This problem was partially due to lack of natural predators and an abundance of safe food sources in man-made bodies of water (Anonymous 2015), such as golf courses, public parks and beaches, or planned communities. Canada geese were frequently a year-around feature of urban environments due to the partly interbreeding of various migratory subspecies with the

Table 3: Canada goose records on Ellensburg valley in eBird website from September 1998 to April 2020 (Anonymous 2020d)

No.	Numbers	Date	Address	Observer
1	5	Sep 1, 1998	Vantage Area, Kittitas County	Steven Mlodinow
2	10	May 20, 2010	Woodhouse Loop	Caleb Davidson
3	2	Sep 18, 2010	Vantage Area, Kittitas County	Steven Mlodinow
4	25	Feb 2, 2013	Irene Rinehart Riverfront Park	KAS trips, Eric Heisey
5	2	Mar 2, 2013	Ellensburg	Eric Heisey
6	6	May 31, 2013	Kittitas County - Parke Creek Road	Bradley Waggoner
7	21	Nov 29, 2013	KitC-I-90	Jon Isacoff
8	5	Jan 26, 2014	Irene Rinehart Riverfront Park	Shep Thorp
9	4	Jun 1, 2014	Bettas Rd	Bradley Waggoner
10	60	Sep 6, 2014	Ellensburg - I-90 corridor	Michael Shepard
11	30	Nov 16, 2014	Bar 14 Pond	Jeanelle Richardson
12	55	Nov 26, 2014	KitC-Ellensberg	Jon Isacoff
13	22	Mar 24, 2015	Vantage Hwy at Parke Creek	Pamela Myers, John Grettenberger, Teri Martine, Jon. Anderson
14	15	Mar 29, 2015	Kittitas Valley--Tjossem Pond	Andy Stepniewski, Eric Heisey
15	60	Mar 29, 2015	Kittitas Valley--Tjossem Pond	Jon. Anderson, Teri Martine, John Grettenberger, Pamela Myers
16	2	May 15, 2015	KitC-Ellensberg	Jon Isacoff
17	5	Apr 9, 2016	Irene Rinehart Waterfront Park	Carol Smith, Faye Hands, Diane Yorgason-Quinn, Laurel Parshall
18	6	Apr 30, 2016	Kittitas Valley--Tjossem Pond	Barbara Petersen, Marissa Benavente, Jim Flynn
19	9	May 1,2016	Kittitas Valley--Tjossem Pond	Eric Heisey
20	15	May 26, 2016	Kittitas County - Parke Creek Road	Bradley Waggoner
21	2	May 26, 2016	Kittitas Valley--Tjossem Pond	Bradley Waggoner
22	8	May 26, 2016	Bar 14 Pond	Bradley Waggoner
23	36	Sep 1, 2016	Ellensburg - general location	Michael Shepard
24	175	Nov 12, 2016	sorenson pond	steve giles
25	7	Nov 13, 2016	Tipton rd	steve giles
26	25	Nov 13, 2016	hungry junction rd kittitas co	steve giles
27	290	Dec 3, 2016	sorenson pond	steve giles
28	33	Dec 3, 2016	Bar 14 Pond	steve giles
29	35	Apr 2, 2017	Kittitas Valley--Tjossem Pond	Adrian Lee
30	3	Apr 12, 2017	Ellensburg -- EconoLodge	Craig Tumer
31	7	May 28, 2017	Parke Creek Road	Guy McWethy, Carol Riddell
32	93	Oct 23, 2017	Kittitas Valley--Tjossem Pond	Carol Riddell
33	150	Nov 16, 2017	Number 6 Gravel Ponds	Carol Riddell
34	100	Nov 16, 2017	Bar 14 Pond	Carol Riddell
35	115	Jan 12, 2018	I-90 at Umptanum Road	Jon. Anderson
36	25	Jan 12, 2018	Yakima River ponds I-90	Jon Anderson
37	42	Mar 10, 2018	1304 S Canyon Rd, Ellensburg	Sheryl Bayles
38	2	Apr 5, 2018	1138–1188 Tipton Rd, Ellensburg (47.0586-120.5221)	Sheryl Bayles
39	15	Apr 22, 2018	Bar 14 Pond	Jaimo Goff, Eric Heisey
40	30	May 14, 2018	Number 6 Gravel Ponds	Walter Szeliga
41	55	Oct 21 ,2018	Number 6 Gravel Ponds	Carol Riddell
42	150	Oct 21, 2018	Kittitas Valley--Tjossem Pond	Carol Riddell
43	6	Jan 26,2019	4761 Number 6 Road, Ellensburg, Washington, US (46.957, -120.501)	Terry Carkner
44	9	Feb 2, 2019	Irene Rhinehart Riverfront Park	Terry Carkner
45	4	Mar 29, 2019	roadside ponds	Joshua Glant
46	8	Apr 12, 2019	Woodhouse Loop	Dan Waggoner
47	16	Apr 12, 2019	Kittitas Valley-Tjossem Pond	Dan Waggoner
48	7	Apr 15, 2019	Kittitas Valley-Tjossem Pond	David Poortinga
49	12	May 17, 2019	Woodhouse Loop	Bradley Waggoner
50	32	May 17, 2019	Kittitas County - NE Ellensburg pastureland	Bradley Waggoner
51	6	May 17, 2019	Kittitas--Parke Creek Road	Bradley Waggoner
52	2	May 17, 2019	Kittitas I5 Ponds	Bradley Waggoner
53	2	May 25, 2019	Ellensburg region	Adrian Lee
54	45	Jul 25, 2019	I-90 Kittitas Co	Adrian Lee
55	10	Jul 26, 2019	I-90 Kittitas Co	Adrian Lee
56	3	Apr 12, 2020	EconoLodge	Craig Tumer

Table 4: The movement of Canada geese during the summer and autumn in the morning

Date	Time		Temperature (°C)		Population size
	Beginning	Ending	Lowest	Highest	
2020.07.27	8:00	8:01	11.67	36.67	28
2020.07.30	8:41	8:42	18.89	37.78	3
2020.08.03	7:50	8:00	13.89	32.22	32
2020.08.04	7:50	8:20	15	32.78	64
2020.08.10	7:53	8:06	11.11	34.44	107
2020.08.11	8:06	8:26	13.89	32.78	32
2020.08.13	7:53	8:40	5.56	27.78	170
2020.08.14	8:31	8:32	8.89	31.11	3
2020.08.17	7:57	8:23	16.67	38.89	591
2020.08.18	7:55	8:38	14.44	37.78	561
2020.08.19	7:55	8:25	11.67	34.44	798
2020.08.20	8:22	8:55	21.11	31.67	123
2020.08.21	8:47	8:50	18.33	30.56	202
2020.08.25	8:38	8:45	15	31.11	54
2020.09.03	8:54	8:59	11.67	33.89	135
2020.09.04	8:59	9:08	13.89	35	84
2020.09.08	8:14	8:15	3.89	23.33	21
2020.09.18	9:36	9:37	9.44	24.44	104
2020.10.07	8:13	8:14	12.22	26.11	18

Table 5: The movement of Canada geese during the summer and autumn in the evening

Date	Time		Temperature (°C)		Population size
	Beginning	Ending	Lowest	Highest	
2020.08.08	17:36	18:09	16.11	30	84
2020.08.10	18:58	19:02	11.11	34.44	62
2020.08.11	18:04	18:05	13.89	32.78	54
2020.08.12	17:53	18:19	16.11	26.67	123
2020.08.13	18:04	18:31	5.56	27.78	269
2020.08.18	18:42	18:43	14.44	37.78	19
2020.08.28	15:44	15:50	9.44	31.11	418
2020.08.31	17:36	17:46	16.67	28.33	238
2020.09.01	18:04	18:05	11.67	33.33	46
2020.09.02	18:07	18:08	13.89	35	206
2020.09.15	15:39	15:40	11.67	25	59
2020.10.03	15:52	15:53	7.78	27.22	50

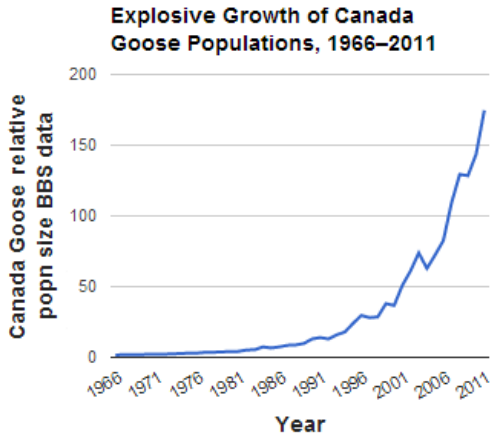

Figure 18: The population size of Canada goose was dramatically increased during 1966 and 2011 (Leonard 2013).

introduced nonmigratory giant subspecies (Anonymous 2014b). The geese were considered protected, though there is a hunting season from September 1 to 15 in the USA, with a daily bag limit of five (Hamrick 2015). The Ohio Department of Natural Resources recommends a number of non-lethal scare and hazing tactics for nuisance geese, but if the methods could not work, they may destroy nests from March 11 through August 31, or conduct a goose roundup or shoot geese.

Suggestions

The Canada goose is a kind of giant wild bird in North America. It will be helpful to keep the bird population size stable if the natural predators can be protected and natural biological web would work efficiently. The nature itself can keep the ecological substance and energy balanced, and it will help human to pay more attention to develop substantially for future generations.

Current Situation of Wildlife Management in China

There is a vast territory, complex terrain and climate, high ecological diversity, and rich wildlife resources in China (Ren and Zhaohui 2021). In the ancient China, a relatively perfect foundation had been laid on wildlife management in the Western Zhou Dynasty (about the 11th century BC to 770 BC), such as the protection of female and young birds and mammals, the establishment of gardens and so on (Chen et al. 1985). After new China was founded, laws, regulations and provisions on wildlife management were

successively publicized. On November 8, 1988, the Wildlife Protection Act was passed in the fourth meeting of the Standing Committee of the Seventh National People's Congress, which was enforced on March 1, 1989. The attributes and utilization of wildlife were considered in this act (Zhang et al. 2020). With increasing necessities and urgency of wildlife protection, the conflict between the pursuit of immediate profits and the requirements of ecological protection became more and more intense. Therefore, the Wildlife Protection Act was revised in 2018 (The Wildlife Protection Act in China 2020), and the principles of "protection priority, standardized utilization and strict supervision" were emphasized.

Since 21st century, the outbreak of large-scale epidemic diseases, including SARS in 2003, MERS in 2015, Ebola in 2019, and COVID-19 from 2019 to now, the close relationship between the improper use of wild animals and the risk of public health and safety became more serious than before (Zhang and Zhigang 2021; Stawicki et al. 2020). The Decision on Comprehensively Banning Illegal Wildlife Trading, Getting Rid of the Negative Habit of Eating Wild Animals Indiscriminately, and Effectively Protecting People's Health and Safety was passed in the 16th session of the Standing Committee of the 13th National People's Congress on February 24, 2020 (Standing Committee of the National People's Congress 2020). "Illegal hunting, killing, trading and eating of wild animals should be severely punished" was clearly pointed out in the Government Work Report in May 2020. The clear tasks were put forward in these strategies to improve the management of ecological environment and wildlife protection, which fully reflects the attached importance of Chinese government on the rational utility of wildlife resources.

The utilization of wildlife resources was managed in the mode of classification

At present, scientific research, public exhibition, medicinal and edible usages are the four main legal ways of wildlife resources in Chinese legation system (Yu and Siying 2020a). The wild animals are managed into different classifications according to different objects and ways of utilization (Zhong 2008).

The wild animals could be divided into two categories in “the Wildlife Protection Act”: key protected wild animals and terrestrial wild animals with important ecological, scientific and social values (Three Values Animals). Among them, the key protected wild animals are divided into national key protection level and local key protection level (Anonymous 2020e).

The utility of national key protected wildlife is limited to scientific research, public exhibition and other public purposes and special uses such as medicine. Whether the wild or artificial population would be used, it must be approved by the relevant department in-charge of wildlife protection, and the corresponding special identification and quarantine inspection should be conducted (Anonymous 2020e). The artificial population with mature and stable artificial breeding technology and listed in the list of national key protected wild animals for artificial breeding could be sold and utilized with Artificial Breeding License, special identification and corresponding quarantine certificate. However, since February 24, 2020, the Standing Committee of the National People's Congress issued the Decision on Comprehensively Banning Illegal Wildlife Trading, Getting Rid of the Negative Habit of Eating Wild Animals Indiscriminately, and Effectively Protecting People's Health and Safety, the wild animals in the list of artificially bred National Key Protected Wild Animals are not edible.

For the utility of Local Key Protected Wildlife and Three Values Animals, the artificial breeding population should be applied in scientific research, public display and medical use, and it can be used with the legal certificate of hunting, and import and export quarantine certificate (Anonymous 2020e). Moreover, the relevant laws and regulations of drug administration should be obeyed if the wildlife would be in medical usage. The restrictions on the use of non-rare and non-Three Values Animals are not strict like key protected wildlife because they are not within the protection scope of the Wildlife Protection Act (Anonymous 2020e).

This scoping for the wildlife based on rarity and value essentially reflects the thoughts of "key protection" rather than "comprehensive protection".

License system, special identification system and quarantine system are adopted in the management measures of wildlife utilization

Article 33rd in the Wildlife Protection Act in China (Anonymous 2020e), the special hunting license or artificial breeding certificate, copy of them or special mark, and quarantine certificate should be prepared if a national key protected wildlife and its products from wild environment or artificial population would be transported, carried or delivered across the county. The marking technical services, management and identification agencies were provided in the Notice on the Enterprises Produced Some Wildlife Products Should Be Cleaned up and Rectified and Carried out the Pilot Marking was issued by the State Forestry Administration and the State Administration of Industry and Commerce in 2003. The regular inspection system was set in Measures for the Administration of Domestication and Breeding Licenses of Wildlife under Special State Protection. The Measures for the Administration of Domestication and Breeding Licenses for National Key Protected Wildlife under Special State Protection stipulates a regular inspection system.

The wildlife management system is dominated by Forestry departments, supplemented by other relevant administrative agencies and non-governmental organizations

National Forestry and Grassland Administration is responsible for terrestrial wildlife, and Bureau of Fisheries

(in Ministry of Agriculture and Rural Affairs) is responsible for aquatic wildlife (Anonymous 2020e), and other relevant departments are responsible to supervise and manage the related issues in the process of wildlife utilization according to the division of responsibilities in Chinese current wildlife management system. In practice, wildlife management mainly involves the departments of ecological environment, market supervision, agriculture, health, police and customs. Specifically, National Forestry and Grassland Administration and Bureau of Fisheries are mainly responsible for formulating and updating the List of National Key Protected Wildlife and the List of Three Values Animals, and also responsible for the approval of utilization of National Protected Wildlife and the issuance of special identification, the License for Artificial Breeding of Wildlife and the issuance of special identification, etc. The Department of Ecology and Environment is mainly responsible for the work related to biodiversity protection and the supervision of ecological protection red line and nature reserves. The Department of Market Supervision is responsible for supervising the business, utilization and advertising of wild animals. In addition to protecting aquatic wildlife, Ministry of Agriculture and Rural Affairs also manage alien species. National Health Commission focuses on the epidemic work and drug management of wild animals. The Ministry of Public Security is mainly responsible for the law enforcement of wildlife crimes (Zhang et al. 2020). General Administration of Customs is responsible for the control of the inspection and quarantine of entry-exit wild animals and plants and other transnational use of wild animals. There are overlapping responsibilities among different departments, with some departments are responsible for both management and supervision, which affects the effective implementation of the Wildlife Protection Act to a great extent (Yu et al. 2020b).

Conclusion

The chapter includes the following five parts. Some important scientific definitions and explanations were listed in wildlife management, such as “wildlife” and “wildlife management” etc. in the first part. The implication of “wildlife” could be changed with the time going. COVID-19, global climate change and pollution are threatening wildlife management and public health, which were described in the second part. COVID-19 pandemic caused big disaster for humans in recent years, and the glacier melting in the southern pole and colorful algae show the global consistent warming. Water pollution and algal bloom events are warning humans, so harsh environments are faced with now. It is urged to improve the efficiency of wildlife management. Three cases on conflict between humans and wildlife in the USA and China are in the third part. Both humans and wildlife need to survive in the world, so it is necessary to control the population size of wildlife in the nature by different strategies. The wildlife observation practice in the USA is in the fourth part. The free terrestrial wildlife in the backyard, campus and suburb showed the efficient wildlife management in the USA. The Canada geese were observed in Ellensburg, Washington throughout the year, and they may locate the flying direction by sun and stars, and summer movement may help save Canada goose from being hunted in the hunting season. The current situation of wildlife management in China is in the fifth Part. More and more strict policies and acts have been implemented to improve the level of wildlife management in China to protect wildlife.

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WILDLIFE DISEASES OF TROPICS AND SUB TROPICS

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INTRODUCTION

Monitoring the wildlife diseases is of vital importance in safeguarding the Public health and the health of livestock (Medley et al. 2021; Nyarko et al. 2021; Chowdhury et al. 2021; Nnko et al. 2021; Ong et al. 2021). Environmental changes and the human activities have resulted in the modification of the pathogens to infect many species rather than to infect a particular specie (Hedman et al. 2021, Ellwanger et al. 2021; Peterson et al. 2021; Turkson 2021). To achieve this goal of infecting many species, the pathogens have also modified their genetic makeup that favors them to dodge the immune system of the diversified host species (Medley et al. 2021; Nyarko et al. 2021). New species that become the victim of these pathogens are not limited to the wildlife species, but the livestock and even the humans too are included in that list (Ellwanger et al. 2021; Peterson et al. 2021; Turkson 2021). In this chapter, the neglected diseases of the tropics and subtropics have been discussed. The main emphasis is on the diseases that infect the wildlife and have the veterinary importance too. We divided These diseases have been divided in two main categories, the infectious and non-infectious diseases. Infectious diseases include Caseous Lymphadenintis, Tuberculosis (Wildlife), Mycobacteriosis, Brucellosis, Rabies, Anthrax, Vesicular Stomatitis (Sore Mouth, Indiana Fever), Paratuberculosis, Peste des Petits Ruminants, Stomach Fluke Disease (Intestinal Amphistomosis), Fasciolosis (Liver Fluke Disease), Tick-Borne Fever, Anaplasmosis, Equine Granulocytic Anaplasmosis, Babesiosis, Malaria, Filariasis, and Winter Dysentery of Cattle. Non-infectious diseases of tropics and subtropics, having wildlife and veterinary importance, are the Myocardial Disease and Cardiomyopathy, and Acute Carbohydrate Engorgement of Ruminants (Quinn et al. 2011; Constable et al. 2016). There are many other diseases of wildlife, such as Trichostrongylus infestation etc., which are not discussed in this chapter, as to discuss each and every disease of wildlife is far beyond the scope of this chapter (Lateef et al. 2021). There is a need of the time that international organizations should implement the rules that will not let the infected animals and humans to travel to the disease free regions of the world. Despite the fact that a pathogen can infect more than one species, it is still possible to curb the pathogen and there are many examples of controlling the pathogens having diversified hosts in a region. One such example is the Brucellosis (Simpson et al. 2021; Khan et al. 2021). There is approximately no mammalian species that may have resistance against Brucellosis to an extent that it may not be infected from that disease. Even, along with land mammals, marine mammals are also infected with Brucellosis (Turkson 2021; Simpson et al. 2021; Khan et al. 2021). Despite that host diversification of Brucellosis, there are many countries in the globe which are free from brucellosis. Examples of such countries are Australia, New Zealand, Japan, and many regions of the North America and Europe (Turkson 2021; Simpson et al. 2021). These countries have achieved that milestone by the strict check on the entry of these pathogens through humans, animals, agricultural products and the meat etc. Rest of the world should have to learn from the strategies of these countries to declare their regions as the potent pathogen free regions (Simpson et al. 2021).

Infectious Diseases

Caseous-lymphadenitis

This disease is caused by *Corynebacterium pseudotuberculosis*. It is a Gram positive, pleomorphic, fastidious, non-motile, catalase positive, oxidase negative, and facultative anaerobe. It is commensal on mucus membrane and can survive for months in the environment (Quinn et al. 2011; Constable et al. 2016; Bezerra et al. 2021). The animals that are more susceptible to this disease are sheep, goats, horses and camels. The wildlife ruminants are reported to be infected with this disease with increase in age (Kimberling 1988). The disease is distributed worldwide and is characterized by suppurative necrotizing inflammation of parotid, submandibular, popliteal, pre-crural, and pre-scapular lymph nodes (Kimberling 1988; Zaitoun and Ali 1999). Economic losses caused through this disease include reduced milk and wool production (Paton et al. 1994), weight loss (Davis 1996), carcass condemnation (Kimberling 1988) and restricted trade. *C. pseudotuberculosis* also affects horses, cattle, camels and wild ruminants in some countries. Rarely, humans infected sheep on farms or in slaughter plants may develop regional lymphadenitis. Ulcerative

lymphangitis is the term classically used to describe cuticular manifestations of *C. pseudotuberculosis*, although it may also be less commonly caused by other bacteria (Quinn et al. 2011; Constable et al. 2016).
Laboratory test that can aid in the diagnosis of this disease is the ELISA. Post-mortem findings of diagnostic importance in this disease include abscesses in lymph nodes and internal organs. Confirmatory diagnosis associated with this disease is based on bacterial culture and PCR against proline iminopeptidase (PIP) gene present in *Corynebacterium pseudotuberculosis* bacterium. This disease is treated by surgical intervention for superficial abscesses (Connor et al. 2007; Fontaine and Baird 2008; Baird and Malone 2010; Voigt et al. 2012).

Tuberculosis (Wildlife)

Wildlife tuberculosis (TB) is one of the neglected zoonotic diseases and its importance is more in the regions with more diverse mammal species, as the South Africa and South Asia (Pakistan, Nepal, India, Bangladesh and Sri Lanka). Many animal species form these regions have been eliminated from this world by the harsh realities of life, as the poor immunity, lack of adaptability with the environmental conditions and the disease outbreaks (Clarke et al. 2021; Devi et al. 2021; Thomas et al. 2021). The animals that are on the verge to extinction in South Asian countries include elephants, rhinoceros, and Bengal tigers. More than one-third infected cases of human TB, as compared to the rest of the world, are reported in South Asia. *M. orygis* has been reported to infect the rhinoceros (Helke et al. 2006; Burrill et al. 2007; Hunter et al. 2018).

Mycobacteriosis

This disease is caused by *Mycobacterium avium*–intracellular complex. Infection is resulted by ingestion. High concentrations of the organism are reported in animal bedding (Quinn et al. 2011; Constable et al. 2016; Didkowska et al. 2020; Barroso et al. 2020; Steinparzer et al. 2020). Wild birds are a source of classic avian tuberculosis. Clinical signs associated with this disease are sub-clinically draining lymph nodes of the alimentary tract (Prince et al. 1989; Biet et al. 2005; Anusz 2021). Laboratory tests that can aid in the diagnosis of this disease are Tuberculin test and PCR. Post-mortem findings of diagnostic importance in this disease include micro-granulomas (Prince et al. 1989; Biet et al. 2005; Jarzembowski and Young 2008; Alvarez et al. 2011).

Brucellosis

This disease is caused by *Brucella species,* and is most prevalent in developing countries (Quinn et al. 2011; Godfroid et al. 2011; Scholz and Vergnaud 2013; Revez et al. 2014; Constable et al. 2016). Laboratory tests that can aid in the diagnosis of this disease are serological test, which include SAT, RBPT, CFT and ELISA. Another screening test is the Milk ring test. Lesions in animals with this disease include necrotizing placentitis and inflammatory changes in the fetus. Confirmatory diagnosis associated with this disease is culture of organisms from the fetus (Quinn et al. 2011; Constable et al. 2016). This disease has no satisfactory treatment to date. Control measures for this disease include reducing reservoirs of infection, quarantine, and depopulation. Vaccination is also practiced (Godfroid et al. 2011; Scholz and Vergnaud 2013; Revez et al. 2014). Recently, different species of Brucella have been isolated from different domestic and wildlife mammalian species that has sum up the total number of known Brucella species to 10. Brucella has been isolated from different mammals including humans, cattle, buffaloes, sheep, goats, pigs, reindeer, caribou, cetaceans, pinnipeds, foxes, marine mammals etc. (Quinn et al. 2011; Constable et al. 2016). Despite the fact that there have been many advancements in the understanding of the pathogenesis of Brucella species, but till date there is no corroborative study regarding the exact mechanism by which these bacteria hijack the intracellular immunogenic machinery to reside within the cell. There is also a conflicting advocacy in the mechanism by which Brucella colonises the pregnant uterus. Vaccines have been developed with a limited specie specification as S19, RB51 for cattle and Rev 1 for small ruminants, with no vaccine for humans, wildlife and pigs (Godfroid et al. 2011; Scholz and Vergnaud 2013; Revez et al. 2014). So, there is the urge to develop an ideal vaccine with optimal potency with the advancement of the biotechnology. Different countries have different strategies to control the disease, such as to vaccinate or test and slaughter the infected ones (Farhab 2020). The protective effect of vaccine in developing countries is masked by the poor management and use of this organism for bioterrorism. Its zoonotic importance emphasizes to control it with the "One Health" approach (Scurlock and Edwards 2010; Godfroid et al. 2011; White et al. 2011; McDermott et al. 2013; Scholz and Vergnaud 2013; Revez et al. 2014; Mathew et al. 2015).

Rabies

Rabies is caused by Lyssavirus of family Rhabdoviridae. The animals that are more susceptible to this virus are all farm animals worldwide. It is mainly transmitted by bites of rabid animals (Quinn et al. 2011; Constable et al. 2016). Different animals serve as vectors, like foxes, skunks, raccoons, mongoose, and vampire bats. Paralytic form of the disease in cattle is characterized be clinical signs as bizarre mental behavior, and death within seven days (Shankar 2009; Reddy et al. 2014; Hampson et al. 2015). Symptoms of the furious form in cattle include hypersensitive, then paralysis and death. Sheep presents sexual excitement, attacking, and then paralysis (Quinn et al. 2011; Constable et al. 2016). There is no antemortem test that can aid in the diagnosis of this disease. Characteristic lesion associated with this disease is non-suppurative encephalomyelitis.
Confirmatory diagnosis associated with this disease is FAT of brain, with observation of Negri bodies in histological smears (Quinn et al. 2011; Constable et al. 2016). This disease has no treatment. All rabid cases are fatal. This

disease can be controlled by prevention of exposure to infected animals, vaccination, quarantine, and biosecurity measures (Shankar 2009; Banyard et al. 2010; Den et al. 2012; Reddy et al. 2014; Hampson et al. 2015; Papaneri et al. 2015; Boyong et al. 2018).

Anthrax

Anthrax is caused by *Bacillus anthracis*. This disease has global occurrence (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease may be acute or per-acute. Laboratory tests that can aid in the diagnosis of this disease are not performed (Jernigan et al. 2001; Rashid et al. 2020). Demonstration of the causative organisms is the confirmatory test. Postmortem finding of diagnostic importance in this disease is no splenomegaly. Diagnosis this disease is based on the identification of organisms, culture, Ascoli test, and PCR (Quinn et al. 2011; Constable et al. 2016). This disease is treated by procaine penicillin and Anthrax hyperimmune serum (Williams et al. 1992; Jernigan et al. 2001; Hugh-Jones and Blackburn 2009; Fasanella et al. 2010; Rashid et al. 2020).

Winter Dysentery of Cattle

This disease is caused by Bovine coronavirus. It is most prevalent in Northern climates. Animals susceptible to this disease are the adult lactating dairy cows (Quinn et al. 2011; Constable et al. 2016). This is transmitted by feco-oral route, affects intestinal and respiratory tracts and shows high morbidity rates. The BCoV is also on the diarrheic adult wild ruminants (sambar, waterbuck, and deer). A coronavirus has been isolated from wild ruminants (Natsuaki et al. 2007; Boileau and Kapil 2010). Clinical signs associated with this disease are sudden onset of diarrhea, sometimes coughing, mild fever, decreased milk production and inappetence. Infected animal recover in a few days. Laboratory tests that can aid in the diagnosis of this disease are not established or are for research purposes only (Quinn et al. 2011; Constable et al. 2016). Lesions include crypt atrophy and enterocolitis. Confirmatory diagnosis of this disease is based on the isolation of virus in feces, serology and PCR (Natsuaki et al. 2007; Boileau and Kapil 2010).

Vesicular Stomatitis

Vesicular stomatitis is caused by Vesicular stomatitis virus. This virus is not as stable as the Foot and Mouth Disease virus and can easily be killed by the routine disinfection procedures. It can also be killed by direct sunlight, but in the absence of light and in darkness, it can survive for longer duration (Rozo-Lopez et al. 2018). This virus can spread rapidly, but only can present the clinical signs in the immunocompromised individuals, while healthy adult animals usually can cope with this virus. The animals that are more susceptible to this disease are equines (horses and donkeys), cattle, wild birds, canines, mice, humans and pigs (de Souza et al. 2018; Martella et al. 2020). Camels and small ruminants are also infected with this disease, but to very less extent and without clinical signs. Once, this vesiculovirus enters the ruminants, it can't continue its life cycle of reinfecting the equines, so, ruminants are considered as the dead host for this vasiculovirus (Quinn et al. 2011; Constable et al. 2016). This disease affects adults, has seasonal occurrence, is vector-borne, and is OIE List A disease. It usually has a low morbidity rate that does not exceeds 10%, but there are also some reports of morbidity rate of up to 80% (Patterson et al. 2017; Peek et al. 2018). The primary hosts, such as the equines, usually don't die of disease, but the other hosts, like the large ruminants, may show mortalities, with percentage not exceeding 15 percent. The main factor that has led to study and concentrate on this disease is the fact that it closely resembles with the FMD (Fowler et al. 2016). It is usually transmitted through insect vector, but can also be transmitted by the direct contact (Rozo-Lopez et al. 2018). It is differentially diagnosed with foot-and-mouth disease (Fowler et al. 2016). Clinical signs associated with this disease are vesicular lesions. Confirmatory diagnosis associated with this disease is virus isolation, ELISA, CFT, and PCR (Quinn et al. 2011; Constable et al. 2016). This disease has no specific treatment and is only managed through supportive treatment. It is controlled by quarantine and movement control (OIE 2021).

Peste Des Petits Ruminants

This disease is caused by Peste des Petits Ruminants virus. This is the contagious disease of small ruminants, and is mostly prevalent in Africa, the Middle East, and Asia (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease are fever, oculo-nasal discharge, stomatitis, diarrhea, and respiratory distress. Laboratory tests that can aid in the diagnosis of Peste des Petits Ruminants are marked leukopenia and hemoconcentration. Confirmatory diagnosis associated with this disease is through VNT, immunohistochemistry, and PCR. This disease is differentially diagnosed with Rinderpest, Contagious ecthyma, Bacterial pneumonias, and Coccidiosis. There is no specific treatment for this disease, but symptomatic treatment and use of hyperimmune serum are usually advised. It is controlled through segregation of the new stock, vaccination and possible eradication, as has been done for rinderpest (Quinn et al. 2011; Constable et al. 2016).

Stomach Fluke Disease (Intestinal Amphistomosis)

The stomach fluke disease is caused by *Paramphistomum cervi* and related flukes. Infection is caused by ingestion of contaminated feed (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease are severe enteritis, with fetid diarrhea. Laboratory tests that can aid in its diagnosis are hypoalbuminemia and observation of flukes in feces (Toledo et al. 2006; Millar et al. 2012; Mason et al. 2012). Main lesion reported in this disease is thickened duodenal mucosa. Confirmatory diagnosis of the stomach fluke disease is through demonstration of flukes in feces

Table 1: Etiology and microbiology of the diseases of wildlife

Disease	Aetiology	Definitive host	Reported wildlife animals	Reference
INFECTIOUS DISEASES				
Caseous Lymphadenitis	*Corynebacterium pseudotuberculosis*	Sheep, horses, camels and goats	Wild ruminants	Connor et al. (2007)
Tuberculosis	Mycobacterium species	humans and animals	Europeans Badger, Brush tailed possum, White tailed deer, Wild boar, African buffalo, Lechwe, Seal, Sia lion, Voles, Antelopes, deer, Antelope, Meerkats, Rock hyraxes, Chimpanzee,	Thapa et al. (2017)
Mycobacteriosis	*Mycobacterium avium*	Bovids	baboons, Colobus monkeys, macaques, gibbons, Patas lemurs, bears, leopards, lions, tigers, lynx, bobcats, hyenas, foxes, llamas, alpacas, antelope, giraffes, wildebeests, impalas, yaks, deer, muntjac, rhinoceroses, voles, moles, mice, rats, squirrels, ferrets, and marine mammals	Schrenzel (2012)
Brucellosis	Brucella species	Ruminants and many more	Bears, Bison, Caribou, Deer, Elk, Ferrets, Foxes, Rodents, Wolves	Coelho et al. (2015)
Rabies	Lyssavirus of Rhabdoviridae	Mammals	dogs, foxes, raccoon dogs, raccoons, mongooses and skunks	Fooks et al. (2017)
Anthrax	Bacillus anthracis	Mammals	Amphibians, birds, carnivores, mammals, ungulates, elephants, carnivores, primates, struthioniformes, falconiformes	Hugh-Jones and De Vos (2002)
Winter Dysentery of Cattle	Bovine coronavirus	Cattle	sambar deer, waterbuck, and deer	Boileau and Kapil (2010)
Vesicular Stomatitis	*Vesiculovirus* of Rhabdoviridae	cattle, horses and pigs	Elk, mule deer, pronghorn	Webb et al. (1987)
Paratuberculosis	*M. avium subsp. paratuberculosis*	cattle, small ruminants and camelids	Deer, cervids, bovids, rabbit, carnivores, mouse, mouflon, kangaroos, and tammar wallabies	Carta et al. (2013)
PPR	Peste des petits ruminants virus, a morbillivirus	goats and sheep	Sumbar deer, mouflon sheep, chinkara deer, Hog deer, Urial, blackbuck deer, nilgai	Abubakar et al. (2016)
Stomach Fluke Disease	Flukes related with *Paramphistomum*	Cattle, sheep, goat, buffalo.	Reindeer, roe deer, moose, antelope, wild ruminants and caribou	Taylor et al. (2016)
Fasciolosis	*Fasciola hepatica* and *Fasciola gigantica*	Sheep, cattle, goat, horse, camel & human	Deer and other mammals	Taylor et al. (2016)
Tick-Borne Fever	*Anaplasma phagocytophilum*	Sheep, cattle, dog, horse, human	Deer, rodents	Taylor et al. (2016)
Anaplasmosis	*A. marginale* and *A. ovis*	Cattle, sheep, goats	Wild ruminants	Taylor et al. (2016)
Equine Granulocytic Anaplasmosis	*Anaplasma phagocytophilum*	Sheep, cattle, dog, horse, human	deer, rodents	Taylor et al. (2016)
Babesiosis	Babesia spp.	cattle, buffalo	deer (roe deer, elk, reindeer)	Taylor et al. (2016)
Malaria	Plasmodium	Humans	Livestock and wildlife	Hasyim et al. (2018)
Filariasis	Filaria	Humans	Livestock and wildlife	Orihel and Eberhard (1998)
NON-INFECTIOUS DISEASES				
Acute Carbohydrate Engorgement	Ingestion of large amounts of carbohydrates	Ruminants	Wild Ruminants	Trefz et al. (2012)
Myocardial Disease and Cardiomyopathy	Infectious and nutritional deficiency	Ruminants	Wild ruminants	Decloedt et al. (2012)

(Quinn et al. 2011; Constable et al. 2016). This disease is treated by closantel. Control strategies include avoidance or drainage of snail habitats and anthelmintic treatment of infected wildlife animals (Toledo et al. 2006; Foster et al. 2008; Dorny et al. 2011; Millar et al. 2012; Mason et al. 2012; Fuertes et al. 2015).

Fasciolosis (Liver Fluke Disease)

This disease is caused by *Fasciola hepatica* (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease include, weight loss, pallor, and submandibular edema (Bennema et al. 2011; Caminade et al. 2015). Pale friable liver is the characteristic lesion seen in the acute syndrome. Confirmatory diagnosis of this disease is based on the demonstration of immature flukes in liver parenchyma at necropsy in acute syndrome (Quinn et al. 2011; Constable et al. 2016), while in chronic syndrome, the confirmatory diagnosis is done through immunoassay and demonstration of characteristic eggs in feces. This disease can be treated by Triclabendazole (12 mg/kg orally) and Albendazole (10 mg/kg orally) (Bennema et al. 2011; Relf et al. 2011; Bloemhoff et al. 2015; Caminade et al. 2015).

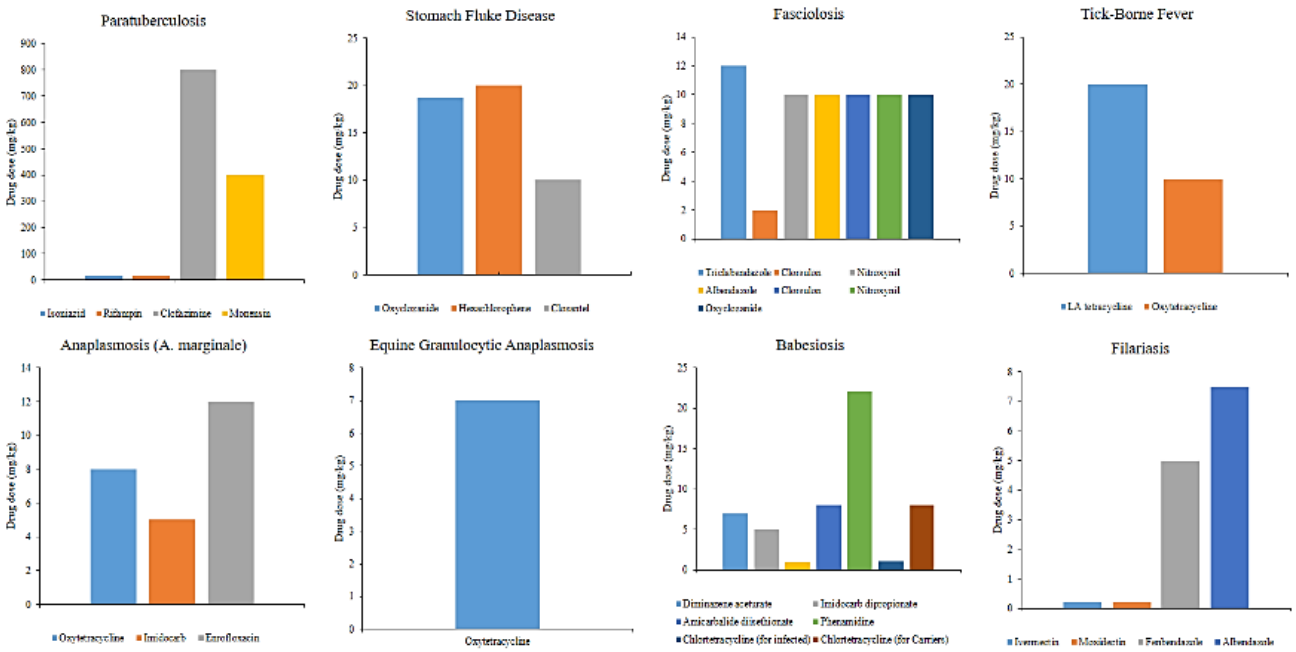

Fig. 1: Concentration of drugs required against various livestock disease of wildlife importance.

Table 2: Treatment of wildlife diseases (Table 2 and Figure 1 are adopted from references cited with each disease in Table 1)

Disease	Drug	Route of administration
Paratuberculosis	Isoniazid	q 24 h, PO, for life
	Rifampin	q 24 h, PO, for life
	Clofazimine	mg/animal, q 24 h, PO for life
	Monensin	mg/lactating animal, q24h, PO, for life
	Dietzia Probiotic	$3x10^{11}$ CFU/animal, q24h PO, long term
		$1.5x10^{11}$ CFU/calf q24, PO for the first 60 days of life
PPR	Hyperimmune Serum/PPR Vaccine	
Stomach Fluke Disease	Oxyclozanide	two doses q48h, PO
	Hexachlorophene	PO
	Closantel	PO
Fasciolosis	Triclabendazole	PO
	Albendazole	PO
	Clorsulon	SC
	Nitroxynil	SC
	Oxyclozanide	PO
Tick-Borne Fever (*A. phagocytophila*)	LA Tetracycline	IM at early stages
	Oxytetracycline	IV daily for 5 days at early stages
Anaplasmosis (*A. marginale*)	Oxytetracycline	IM, daily for 3 Days
	Imidocarb	IM twice, 7 days apart
	Enrofloxacin	SC twice, 48 hours apart
Equine Granulocytic Anaplasmosis	Oxytetracycline	12hours for 7 days
Babesiosis	Diminazene Aceturate	IM first dose
	Imidocarb Dipropionate	SC
	Amicarbalide Diisethionate	IM
	Phenamidine	
	Chlortetracycline	PO
Filariasis	Ivermectin	SC
	Moxidectin	SC or PO
	Fenbendazole	PO, every day for 7 days
	Albendazole	PO

Tick-Borne Fever (Anaplasma Phagocytophila)

The tick-borne fever is a disease of sheep and cattle, caused by *Anaplasma phagocytophilum*, and transmitted by *Ixodes ricinus* and *Ixodes scapularis* (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease are fever, lethargy, and fall in milk production in cattle, and abortion (Troese et al. 2011; Severo et al. 2012; Kahlon et al. 2013). Laboratory test that can aid in the diagnosis of this disease is thrombocytopenia (Quinn et al. 2011; Constable et al. 2016). Confirmatory diagnosis is based on the demonstration of *Anaplasma*

phagocytophilum. This disease can be treated by oxytetracycline. It is controlled by oxytetracycline during risk period and tick control. Dose of long-acting tetracycline should be 20 mg/kg intramuscularly at early stages (Estrada-Peña et al. 2009; Troese et al. 2011; Al-Khedery et al. 2012; Severo et al. 2012; Kahlon et al. 2013; Sharma et al. 2017).

Anaplasmosis

This disease is caused by *Anaplasma marginale*, is mostly prevalent in tropical regions (Quinn et al. 2011; Constable et al. 2016) and is transmitted by ticks. Clinical signs of Anaplasmosis are death or severe debility, anemia and jaundice. Laboratory tests that can aid in the diagnosis are serology and PCR. Post-mortem findings of diagnostic importance in this disease include anemia and demonstration of the organism. Confirmatory diagnosis is done through detection of the organism (De La Fuente et al. 2007; Kocan et al. 2010; Cabezas-Cruz et al. 2013). Anaplasmosis is treated (Quinn et al. 2011; Constable et al. 2016), and controlled by tetracycline, as it provides temporary or prolonged protection (De La Fuente et al. 2007; Zivkovic et al. 2007; Estrada-Peña et al. 2009; Kocan et al. 2010; Chávez et al. 2012; Cabezas-Cruz et al. 2013).

Equine Granulocytic Anaplasmosis

It is caused by *Anaplasma phagocytophilum* and can infect humans, cattle, horses, cats, wild ruminants, including deer and other mammalian species (Quinn et al. 2011; Constable et al. 2016). It is characterized by icterus, limb edema, and ataxia (Radostits et al. 2007; Dzięgiel et al. 2013; Pusterla and Madigan 2013; Stuen et al. 2018). Clinical signs associated with this disease are rapid edema development on the neck, inflamed tongue and conjunctivae (Quinn et al. 2011; Constable et al. 2016). This disease is treated by supportive treatment, as it has no specific treatment (Radostits et al. 2007; Dzięgiel et al. 2013; Pusterla and Madigan 2013; Stuen et al. 2018).

Babesiosis

Babesiosis is caused by Babesia spp. andis mostly prevalent in tropical and subtropical countries. Transmission of Babesiosis occurs through blood-sucking ticks (Quinn et al. 2011; Constable et al. 2016). Clinical signs are hemoglobinuria, anemia, fever and jaundice, with high case-fatality rate. Laboratory tests that can aid in the diagnosis of this disease are demonstration of parasites in stained blood smear and positive serology. Polymerase chain reaction (PCR) can be used for the detection of parasites in blood. Wildlife infections include babesiosis of elk, and caribou having hemoglobinuria, fever, and sudden death. Elk may not show any clinical signs (Brown et al. 2006; Gohil et al. 2013). Necropsy lesions include watery blood and jaundice. Confirmatory diagnosis of this disease is through demonstration of parasites in blood smear. This disease can be treated by diminazene aceturate and imidocarb, and controlled by tick eradication and vaccination (Quinn et al. 2011; Constable et al. 2016). Desert bighorn sheep and red deer are also susceptible to babesiosis infection (Brown et al. 2006; Uilenberg 2006; Hunfeld et al. 2008; Gohil et al. 2013).

Malaria

Malaria is caused by plasmodium that is present with the mosquito and is transmitted to the other animals by the bite of female anopheles mosquito.The life cycle of plasmodium involves colonization in the liver, followed by the red blood cells. It can infect different wildlife that contributes to the increased incidence of malaria in human population. It is controlled by eradication of vectors. There is no effective vaccine against malaria. It is treated by artemisinin in humans (Hasyim et al. 2018).

Filariasis

Filariasis is caused by the nematode parasite, named as the *Dictyocaulus filaria*, and commonly called as the lung worm. Filariasis mostly infests small ruminants like sheep and goats. Lambs between 4 and 6 months of age are more vulnerable to be infected, but generally small ruminants of all ages can be infected with filariasis. Relatively low infection in lambs aged 1-4 weeks may be attributed to the passive immunity acquired by feeding of colostrum of dams by kids. Infections may or may not be symptomatic. Parasites are found in eye orbit or conjunctivae, heart and CNS. *Microfilaria semiclarum* and *Microfilaria bolivarensis* have zoonotic importance. It is probable that almost any filaria parasitizing in animals can infect humans (Orihel and Eberhard 1998). This can be detected by the detection of larvae, having a conical tail, in the faeces of suspected cases, but the number of larvae is not the indication for the extent of infestation. Filariasis is differentially diagnosed from bronchitis in calves at necropsy, and it presents exudates and patches of consolidation, calcified nodules having live or dead worms (Votypka et al. 2020). This infestation has been treated successfully with avermectins and benzidimidazoles. This treatment can readily kill the adult worms but may not have any effect on the eggs and larval stages. To achieve the goal of killing all of the stages of the worm, it is recommended to feed the albendazole orally for at least two weeks at the dose rate of 1 mg per kilogram body weight of the animal (Rebollo et al. 2017; Fang et al. 2019; Juwita et al. 2020). Live attenuated vaccine against this disease is also available in some countries (Shey et al. 2019).

Non-Infectious Diseases

Acute Carbohydrate Engorgement of Ruminants

This disease is caused by consuming large amounts of carbohydrates (Quinn et al. 2011; Constable et al. 2016). Clinical signs associated with this disease are anorexia, dehydration and ruminal stasis, leading to recumbency (Gozho et al. 2007; Li et al. 2012; Kleen et al. 2013).

Laboratory tests that can aid in the diagnosis of this disease are ruminal fluid pH below 5, absence of protozoa in rumen, hemoconcentration and sloughing of ruminal mucosa. Confirmatory diagnosis associated with this disease is ruminal fluid pH below 5 (Quinn et al. 2011; Constable et al. 2016). This disease should be differentially diagnosed from simple indigestion. Rumen lavage or rumenotomy can be used for the treatment of the disease (Gozho et al. 2007; Li et al. 2012; Kleen et al. 2013). Infected animals should be provided with palatable hay. The disease can be controlled by preventing accidental access of animals to grains (Radostits et al. 1983; Gozho et al. 2007; Bramley et al. 2008; O'Grady et al. 2008; Kleen et al. 2009; Quinn et al. 2011; Li et al. 2012; Trefz et al. 2012; Kleen et al. 2013).

Myocardial Disease and Cardiomyopathy

This disease causes capture myopathy in wild ruminants. The main clinical sign of this disease is reduction in cardiac reserve (Quinn et al. 2011; Constable et al. 2016). Post mortem findings of diagnostic importance in this disease include myocarditis and myocardial degeneration. This disease is treated by specific therapy, if available (O'Toole et al. 2009; Hughes et al. 2009; Brihoum et al. 2010; Snider and Stern 2011; Brihoum et al. 2011; Decloedt et al. 2012).

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