Monograph

On

Fungal Diseases of Cats and Dogs

A guide for postgraduate students

By

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2016

Dedication

Today I am celebrating my 78th birth day and the uploading of my 12th Monograph. This monograph is dedicated to Prof Ahmed Hosny Mahmoud, Prof. Mahmoud Emad El-Gendy and Prof Hassan Gharib, the great professors of Medicine and Infectious Diseases, who impressed me, when I was undergraduate student, 1959, by their way of teaching and communication with the students.

This Monograph is also dedicated to my friend and classmate Mohamed Saleh, Prof. Emeritus in the Department of Animal Surgery, who was the first to establish an evening pet animal clinic in our faculty, more than 35 years ago

Prof. Dr. Mohamed K Refai
Cairo, 21. 4. 2016

Refai, M.K. *et al*. (2016). Monograph on fungal diseases of cats and dogs
A guide for postgraduate students,

https://www.academia.edu/21679188/
http://scholar.cu.edu.eg/?q=hanem/book/
https://www.researchgate.net/publication/293427976

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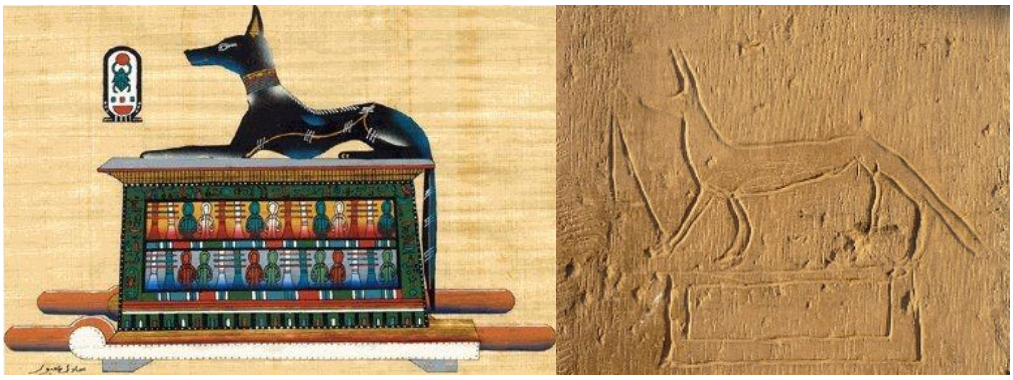
Introduction

In ancient Egypt society, cats and dogs held a prominent place in both life and the next world. Tens of millions of these animals were mummified, and some were placed within a pharaohs' tombs to rest forever in the companionship of their kings. Cats, for example, were considered the embodiment of Bastet, the goddess of joy and of music, in addition to being the protector of women, while the dog was considered the messenger of the god Anubis, portrayed in ancient Egypt as a man with a dog head.

Dogs are the earliest domesticated animals (maybe around 10 000 BC in the Near East). They were used as guardians, helper at hunts, and pets. There is some discussion about the ancestors of the dog, but it is most probably the wolf (canis lupus), because their social behaviour and anatomy are very similar. Dogs are attested in Egypt already from the Naqada Period, in paintings on pottery. Bones of dogs have been found at Merimde (Ancient Egyptian prehistoric settlement).

In the Old Kingdom (about 2686-2181 BC) the greyhound (Egyptian: Tsm - long narrow muzzle, nearly straight facial profile, long neck and limbs) was very common. From the Middle Kingdom (about 2025-1700 BC) onwards there is attested a greater variety of dogs (different types of ears, ring-tailed, saber-formed tails). Among pharaohs known to own greyhound-type dogs are Tutankhamen, Amenhotep II, Thutmose III, Queen Hatshepsut, and Cleopatra VII (of Antony and Cleopatra fame).

http://www.ucl.ac.uk/museums-static/digitalegypt/foodproduction/dog.html



Amazon.com: Egyptian Hand-Made Papyrus Painting - Anubis Dog: Oil Paintings: Oil Paintings. www.amazon.com, Graffiti on walls of Kom Ombo Temple, Ptolemaic Dynasty, Egypt,350-30BCwww.artsjournal.com

www.pinterest.com ancient Egyptian gods statues | Anubis Egyptian Dog God Small Statue by Ancient Treasures AT-

In catacombs south of Cairo, researchers have discovered burial sites filled with huge numbers of mummified animals — nearly 8 million of them, mostly dogs. The catacombs, at Saqqara, are dedicated to Anubis, the jackal-headed god of the afterlife.
Archaeologist and Egyptologist Salima Ikram, a professor at the American University in Cairo who has worked extensively at the site, writes that animal mummification began in ancient Egypt "to allow beloved pets to go on to the afterlife, to provide food in the afterlife, to act as offerings to a particular god and because some were seen as physical manifestations of specific gods that the Egyptians worshipped." Ikram explained that "each [animal] mummy would be a symbol of something a pilgrim had given as a gift to the god. So nowadays, people go to a church and light a candle. "But the Egyptians were in for the long haul, so instead of a candle, they would offer a mummy. So clearly, this means that there were a lot of very religious people out there who were asking Anubis for intercession and for help for a variety of things." **http://www.npr.org/2015/07/04/418079713/**

Dog Mummy, 305 B.C.E.-395 C.E. Animal remains, linen, painted. Brooklyn Museum - See more at:
Mummified dog, Taggart School Museum, Assuyt, Middle Egypt (Flickr) - See more at: http://www.ancient-origins.net/news-history-archaeology/mummifying-millions-canine-catacombs-and-animal-cult-industry-ancient-egypt-020386#sthash.ii1OK4Yg.dpuf

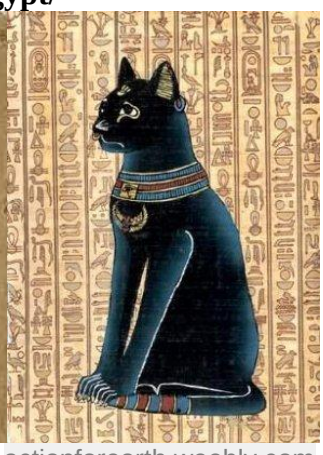
The origins of **cats (**domestic cats) are thought to have come from the African Wild Cat. The breed was domesticated in ancient Egypt to control vermin which was harming crops and causing diseases. The cats controlled the rat population which reduced disease and deaths and also allowed a larger

supply of food for the poor. This therefore changed the quality of living for the Egyptians and cats become a sacred creature representing life. They were associated with the goddesses Bastet, Isis and Pasht. By the time the Egyptian empire fell, cats were revered as master hunters and were worshipped like gods by all Egyptians including the pharaoh. If an Egyptian killed a cat they were immediately given the death penalty yet the fear of the all mighty cat itself made this a rare occurrence. The pharaoh's were mummified and buried with statues of cats. This represented good luck and safe companionship to the afterlife. Even today archaeologists are finding more and more hieroglyphics, statues and carvings of cats emphasising there importance in Ancient Egypt. Some cats were even mummified and their bodies left to lay in tombs and shrines. It was illegal to sell a cat outside of Egypt as they were such an important asset to their beliefs and society. The history of domesticated cats started in Egypt where they acclaimed their first home, but like all cats they didn't want to stay in one place to long!

Cats in the history of ancient Egypt were often identified with Ra. Probably such an honor cats ancient were awarded due to their eye structure. According to the Book of the Dead, the eyes of Ra changed depending on the time of day (the eye of Ra can be the sun or the moon). Cats, as we know, can do this “trick” too - in bright light their pupils constrict, becoming almost invisible slits. It was believed that during the day the cat eyes absorb the sunlight, and at night, they were giving it back – obviously the cat eyes night flicker was meant. Cats in ancient Egypt were considered messengers of Ra also because these animals hate snakes, destroying any settled in their territory. According to mythology, every night Ra descends into the underworld, where he kills his nemesis - snake Apophis, and then returns to the water of heavenly Nile (i.e., when the morning comes). Another sacred animal associated with Ra is the scarab beetle, which is read on the chest or forehead of a shorthair tabby cat (namely, striped and spotted cats lived in ancient Egypt and inherited this color from the wild ancestors). Sometimes god Ra in Egypt, killing Apophis acts in the form of a huge red cat (an animal hating snakes is a cat, and red is the colour of the sun).

http://pets-wiki.com/publ/cats/interesting/cats_in_ancient_egypt/

www.nedmartin.org


actionforearth.weebly.com

The Ancient Egyptians are known of the love that they had for cats. And posted them on the walls and on papyrus papers

Bastet Cat Goddess holding the flint knife or dagger used in ancient Egypt Khopwww.landofpyramids.org

Ancient Egyptian Gods: Bast (Bastet) www.ancientegyptonline.co.uk Cats were sacred to Bast, and to harm one was considered to be a crime against her and so very unlucky. Her priests kept sacred cats in her temple,

The Egyptian Mau cat is well known in legend and ancient past. It is believed that these cats are descendants of the sacred cats of Egypt, and their ancestry goes back at least 3,500 years. It is the only naturally occurring spotted cat, and it bears "M" mark on the forehead, sometimes called the scarab mark after the symbol the ancient Egyptians considered divine. As highly honoured cats, it was an offence in ancient times to hurt or kill one of these cats, and many have been found mummified in tombs. Mau is the Egyptian word for cat.

When a cat died, their human family would go into a deep mourning and shave their eyebrows. The cat would then be mummified and buried along with provisions such as milk, mice and rats. Cats were often taken to Bubastis to be buried, but tombs have also been discovered in Giza, Abydos, Denderah and Beni Hasan. For example, a tomb in Beni Hassan was discovered in 1888 which contained an estimated 80,000 feline burials. The deceased cat was wrapped in fine linen and taken to be embalmed. Diodorus recorded that the deceased cat was "treated with cedar oil and such spices as have the quality of imparting a pleasant odour and of preserving the body for a long time (J Hill 2010. http://www.ancientegyptonline.co.uk/cat.html).

J Hill 2010 , Source: http://ancientegyptonline.co.uk/cat.html, Sarcophagus for cat mummy .. factsanddetails.com

This Egyptian cat was mummified and offered to a god over 2,000 years agowww.justpetcats.com

Stray animals like cats and dogs are all around the streets of Egypt. We do not have any statistics on the numbers of strays. The estimated count in greater Cairo is around 35,000 dogs. However, these numbers cannot be verified." According to the *Egyptian Gazette*, there are approximately 5 million stray animals living on the streets of Egypt.

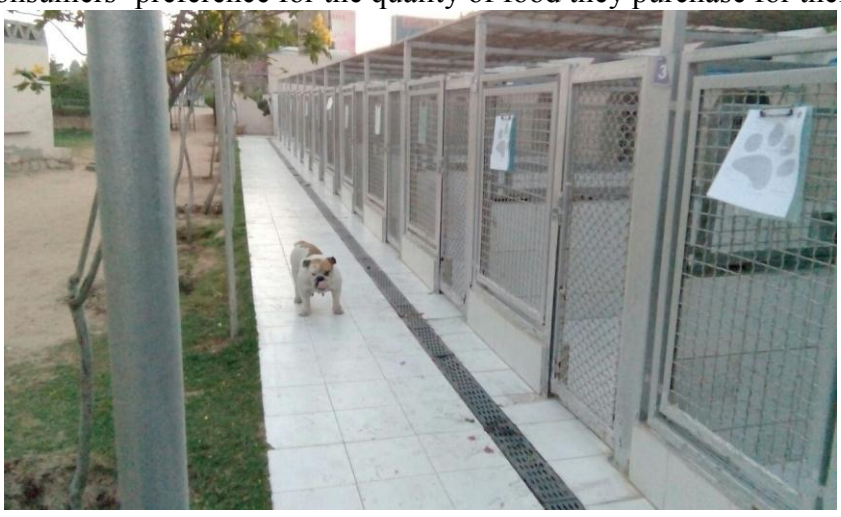

Stray cats and dogs

In Cairo, as well as in large cities in Egypt, the association between pet animals and humans has been changed throughout the last few years. The number of owned dogs and cats was dramatically and they are kept inside houses as common households.

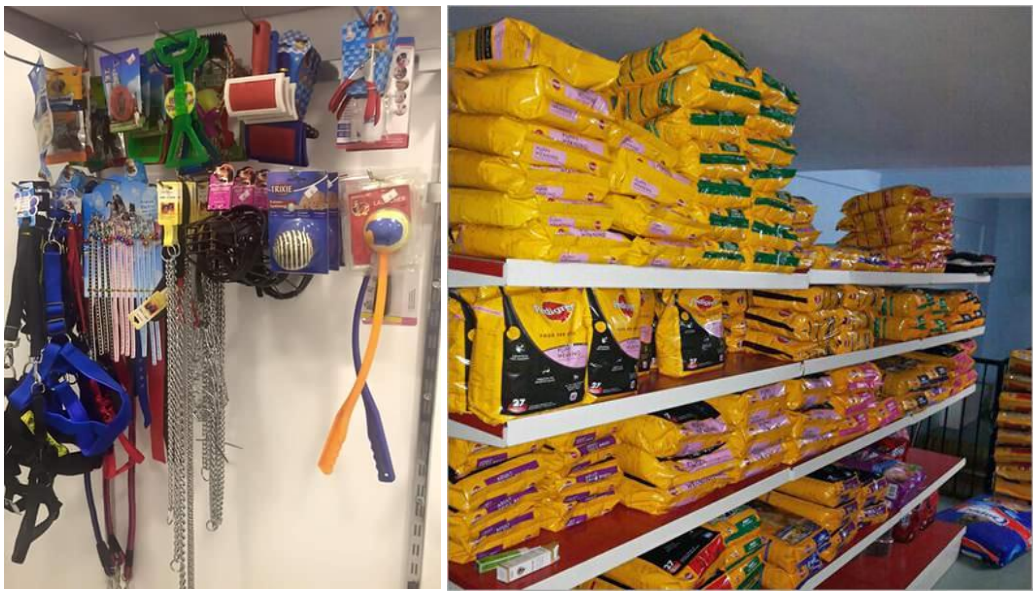
Maverick in Egypt Dog Show 2010, www.youtube.com

Granddaughters Farida and Laila Refai with their cats at home

The improved political and economic stability in Egypt affect positively the pet care market. Consumers are more apt to purchase pet care products from pet shops and hypermarkets with high availability. As the number of domesticated animals being kept in the country continues increasing, the numbers of pet breeders, veterinarians and retailers of pet care products are also increasing, and this has affected consumers' preference for the quality of food they purchase for their pets.



Shelters for dogs consisting of of cage for sleeping during nighttime and external corridor and a garden for a walk during the day



Pet accessories and foods in shops and supermarkets in Cairo

Private pet clinics in Cairo

Cats and dogs have a very important role in terms of human health and social benefits. In all cases, whether these pet animals are stray or owned, the close physical contact with such animals at high-frequency basis facilitates the transmission of pathogens between pets and human contacts. There are many diseases known to be transmitted from cats and dogs to human contacts, among them are the fungal diseases, which are the subject of the present monograph.

A. Fungal diseases of cats and dogs caused by dermatophytes

1. Ringworm (Dermatophytosis) in cats and dogs

1.1. Introduction

- Dermatophytosis is worldwide the most common and important fungal skin infections of cats and dogs.
- The disease is caused by several dermatophytes with *Microsporum canis* on the top. but other dermatophytes are also involved such as *Microsporum gypseum, Trichophyton mentagrophytes, Microsporum persicolor, Trichophyton verrucosum* and *Trichophyton quinckeanum.*
- *Microsporum canis* has been frequently isolated from apparently healthy cats and dogs, but it is not considered as part of the normal fungal flora of these animals. Arthrospores of *Microsporum canis* are transmitted through contact with sick or subclinically infected animals, mainly cats, but also dogs or other species, or indirectly through contaminated collars, brushes, toys, environments.
- *Microsporum canis* has been frequently isolated from human cases of tinea capitis and tinea corporis. The infection may be acquired from infected animals with cutaneous lesions but also from asymptomatic carriers or from the environment, as asymptomatic *M. canis* carriers are considered to be a critical factor in the epidemiology of dermatophytosis in human.
- ***Here are some reports:***

El-Bahay and Refai (1973) examined 113 apparently healthy stray cats and dogs in Cairo. The animals were examined clinically for loss of hairs or any skin lesions and were passed under UV light of a Wood´s lamp. All animals were free from any lesions and showed no fluorescence under the UV light. The mycological examination of the samples revealed the isolation of ***Microsporum canis*** from 5 samples. They concluded that the animals carried the fungal spores on their coat, without invasion of the skin or hairs.

Boyanowski e*t al.* (2000) collected samples from 2000 shelter cats from the Pacific western coastal USA in four different geographical regions to determine the fungal organisms most commonly found on the hair coat and the prevalence of these organisms. The overall prevalence of dermatophytosis was 5.5% (11 of 200 cats), with *Microsporum canis* isolated in 90.9% (10 of 11) of the samples from positive cats. This was a lower isolation rate or prevalence of dermatophytes than previous studies conducted on shelter cats in other regions of the USA. Ten of 11 of the cats were lesion free (either subclinical infection or mechanical carriage).

Mancianti *et al.* (2003) examined the environments inhabited by 30 symptomatic animals (21 cats and 9 dogs) infected by ***M canis*** by sampling both surfaces and indoor air. The surfaces were examined by means of contact plates; the air sampling was performed with AIR SAMPLER. Environmental contamination was detected in all households with cats, while only four out of nine houses harbouring dogs were found positive. The frequency of isolation in each sampling, and the results in terms of colony forming units per plate in the different houses appeared to be quite homogeneous. Heavily infected environments harboured kittens only. Infected owners were observed in eight households, in all of which at least one infected cat was present. No history of human dermatophytosis in households harbouring dogs was found. On the basis of these results results, infected cats appeared to cause substantial environmental contamination, and provoke a substantial presence of viable airborne fungal elements.

Mancianti *et al.* (2003) determined the load of ***M. canis*** arthrospores in households harbouring infected pets, in order to evaluate the infectivity of the animals versus the environment. The

environments inhabited by 30 symptomatic animals (21 cats and 9 dogs) infected by *M canis* were examined by sampling both surfaces and indoor air. The surfaces were examined by means of contact plates; the air sampling was performed with an AIR SAMPLER. Environmental contamination was detected in all households with cats, while only four out of nine houses harbouring dogs were found positive. The frequency of isolation in each sampling, and the results in terms of colony forming units per plate in the different houses appeared to be quite homogeneous. Heavily infected environments harboured kittens only. Infected owners were observed in eight households, in all of which at least one infected cat was present. No history of human dermatophytosis in households harbouring dogs was found. On the basis of our results, infected cats appear to cause substantial environmental contamination, and provoke a substantial presence of viable airborne fungal elements. Dogs seem to be of lower importance in the spread of *M canis*: they contaminated surfaces, but they never contaminated the air. The results of this study confirmed the potential leading role of the feline species in the environmental spread of M canis.

Cafarchia *et al.* (2006) investigated the relationship between the presence of dermatophytes on the hair coats of dogs and cats without cutaneous lesions and the occurrence of the disease in their respective owners. A total of 136 dogs and 248 cats were sampled from January 1999 to January 2005. Seventy-eight animals (22 dogs and 56 cats) belonged to individuals affected by tinea corporis caused by *M. canis* and 306 (114 dogs and 192 cats) to individuals without dermatophytosis. Dermatophytes were isolated from 20.5% of the dogs and 28.2% of the cats. *Microsporum canis* was isolated from 36.4% of dogs cohabiting with owners diagnosed with tinea corporis but it was never isolated from dogs whose owners had no lesions. By contrast, *M. canis* was isolated from 53.6% of cats cohabiting with owners diagnosed with tinea corporis and from 14.6% of cats whose owners had no signs of the disease. These results clearly indicate that both cats and dogs should be considered as a major source of pathogenic dermatophytes for humans even when they do not present clinical signs of dermatophytosis.

Frymus *et al.* (2013) reported that dermatophytosis, usually caused by ***Microsporum canis,*** is the most common fungal infection in cats worldwide, and one of the most important infectious skin diseases in this species. Many adult cats are asymptomatic carriers. Severe clinical signs are seen mostly in kittens or immunosuppressed adults. Poor hygiene is a predisposing factor, and the disease may be endemic in shelters or catteries. Humans may be easily infected and develop a similar skin disease. Infectious arthrospores produced by dermatophytes may survive in the environment for about a year. They are transmitted through contact with sick cats or healthy carriers, but also on dust particles, brushes, clothes and other fomites.

1.2. Prevalence

- The reported prevalence of *M canis* infection in cats and dogs is highly variable and depends on geographic region, the population sampled, whether or not culture status is correlated with diseases and criteria for data collection and reporting.
- Among various fungal culture surveys conducted in the USA and Europe over the past 20 years, the prevalence of culture-positive cats has ranged from 4–100%. However, these numbers can be very misleading and may overestimate actual disease prevalence due to fomite carriage by cats (Boyanowski *et al*., 2000, Iorio *et al*.,2007, Duarte *et al*., 2010).
- in one retrospective study in a shelter comparing screening cultures and post-culture examinations, data from 5644 cats over a 24 month period revealed 584 culture -positive (10.3%) cats, with skin lesions being noted at the time of admission in 381/5644 (6.75%)

cats.[10] However, only 94/5644 cats were both lesional and culture positive (1.6%); the remaining 490 culture-positive cats were fomite carriers (Verbrugge *et al*., 2006).

1.3. Transmission

Transmission of dermatophytosis is dependent on many factors including:

- The amount of infective material, frequency of exposure, global health of cats and dogs, and physiological stress.
- Exposure to infective spores via direct animal-to-animal contact is the most common and important route of transmission and represents the highest risk factor.
- Cats and dogs can also be exposed to infective spores via contact with contaminated blankets, bedding, toys, brushes, lab coats, leather gloves or even external parasites (Newbury *et al.*, 2010)
- Cats and dogs can also be exposed to infection via airborne transmission of spores (Mancianti *et al*., 2003)

1.4. Incubation period

- Incubation period is mostly cited in textbooks as being 2–4 weeks
- There is evidence that active infection develops much sooner. Contact between infective spores and the skin, and concurrent microtrauma are needed for disease development.

1.5. Pathogenesis

Adherence of *Microsporum canis* to feline corneocytes

The mechanisms and the kinetics of adherence have been investigated using different in vitro and ex vivo experimental models, most notably showing the role of a secreted serine protease from Microsporum canis in fungal adherence to feline corneocytes.

Invasion of keratinised structures

After germination of the arthroconidia, dermatophytes invade keratinised structures that have to be digested both secreted endoproteases and exoproteases into short peptides and amino acids to be assimilated. exoproteases, but their precise role in both fungal adherence and skin invasion should be further explored.

Reports:

Tabart *et al* (2008) reported on the use of a sophisticated model of reconstructed interfollicular feline epidermis (RFE), in which both the cornified layer and skin permeability resembled the in vivo situation. Using this same model, *M canis* arthro-conidia started to adhere to the RFE within 2 h and increased in numbers for up to 6 h post-inoculation. Sites were culture-positive and invasion was documented histologically within 5 days. A study using another experimental model of *M canis* infection in cats, found that hairs became infected and lesions developed at inoculation sites within 7 days post-inoculation.

Baldo *et al.* (2008) developed a model to study the adherence of M. canis to feline corneocytes through the use of a reconstructed interfollicular feline epidermis (RFE). In this model, adherence of arthroconidia to RFE was found to be time-dependent, starting at 2 h post-inoculation and still increasing at 6 h. Chymostatin, a serine protease inhibitor, inhibited M. canis adherence to RFE by 53%. Moreover, two mAbs against the keratinolytic protease subtilisin 3 (Sub3) inhibited *M. canis*

adherence to RFE by 23%, suggesting that subtilisins, and Sub3 in particular, are involved in the adherence process.

1.6. Predsposing factors

- young age (first 2 years of life),
- immunosuppression (including immunosuppressive treatment),
- other diseases,
- nutritional deficits (especially proteins and vitamin A),
- high temperature and high humidity
- skin trauma resulting from increased moisture,
- injury by ectoparasites or scratches due to pruritus,
- playing or aggressive behaviour, clipping, etc.
- poor hygiene
- overcrowding in catteries

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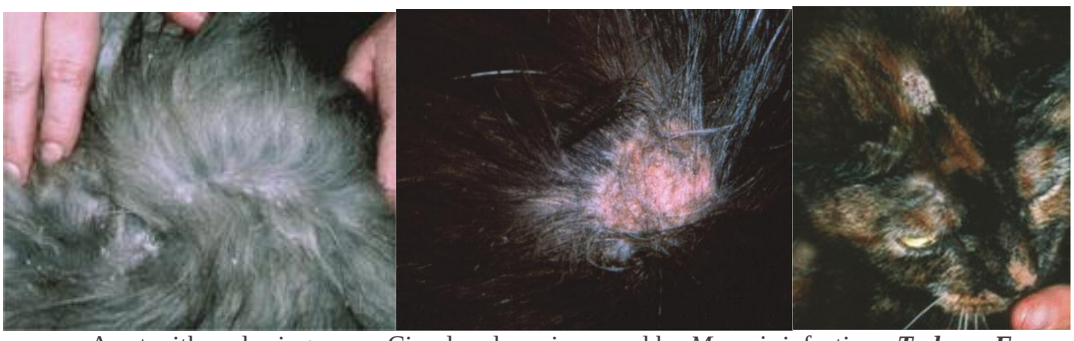
1.7. Immunity

- Naturally occurring ringworm is rarely recurrent, suggesting an effective and long-lasting immunity.
- Reinfections may occur, but require a much greater number of spores, and usually these subsequent infections are cleared more rapidly.
- Humoral and cellular immune response is induced.
    - Prominent activation of T helper type 2 (Th2) cells and the corresponding cytokine profile leads to antibody formation followed by chronic disease,
    - Activation of Th1 cells stimulates a cell-mediated response characterised by interferon-γ, and interleukins 12 and 2, and leads to recovery.
- Such cats are protected against reinfection.
- The role of the humoral response in dermatophytosis is unclear, although antibodies could have a fungistatic effect by means of opsonisation and complement activation.

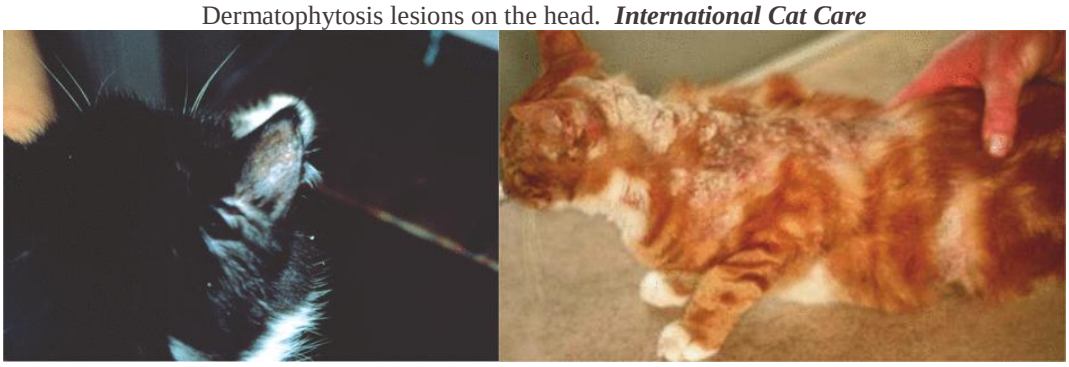
1.8. Clinical signs of ringworm

1.8.1. Clinical signs of ringworm in cats

- Regular and circular alopecia with hair breakage, desquamation and sometimes an erythematous margin and central healing. The lesions may be very small, but occasionally may have a diameter of 4–6 cm. Lesions may be single or multiple, and are localized mostly on the head (but also on any part of the body, including the distal parts of the legs and the tail.
- Young cats, in particular, display lesions localized at the bridge of the nose and then extend to the temples, the external sides of the pinnae and auricular margins Multiple lesions may coalesce. Lesions may appear as a papulocrustous dermatitis (‘miliary dermatitis’) affecting mainly the dorsal trunk. Extensive lesions with secondary bacterial involvement are sometimes associated with chronic ringworm, particularly in immunosuppressed cats. Such cats demonstrate atypical, large alopecic areas, erythema, pruritus, exudation and crusts. At this stage, dermatophytosis may mimic other dermatological conditions. Typical signs may be still visible at the margins of the lesions.



A cat with scaly ringworm, Circular alopecia caused by *M canis* infection. ***Tadeusz Frymus***
Dermatophytosis lesions on the head. ***International Cat Care***



Dermatophytosis of external sides of the pinnae of a cat. ***Tadeusz Frymus***
Generalized ringworm in a cat. ***International Cat Care***

Moriello (2014) grouped cats with dermatophytosis into 3 groups based on a global health assessment:

1. **Simple infection:** This group consists of cats or kittens with confirmed infections that are otherwise healthy and not under physiological stress. Lesions are obvious but limited in extent. Provided the cats/kittens remain healthy, and not stressed, and receive appropriate preventive care, they will respond well to therapy.
2. **Complicated infection:** This group consists of cats with widespread lesions, inflammatory lesions, long/ matted hair coats, other illnesses (most notably upper respiratory infections), a history of prior treatment and/or surrender for 'resistant dermatophytosis', as well as semi-feral or feral cats. In many cases, clipping of the hair coat reveals the true extent of the lesions. These cats are more complicated to treat because of the extent of their lesions, handling issues and/or other health factors. In some cases (eg, geriatric cats, cats with upper respiratory infection) antifungal therapy must be coordinated with treatment for pre-existing disease.

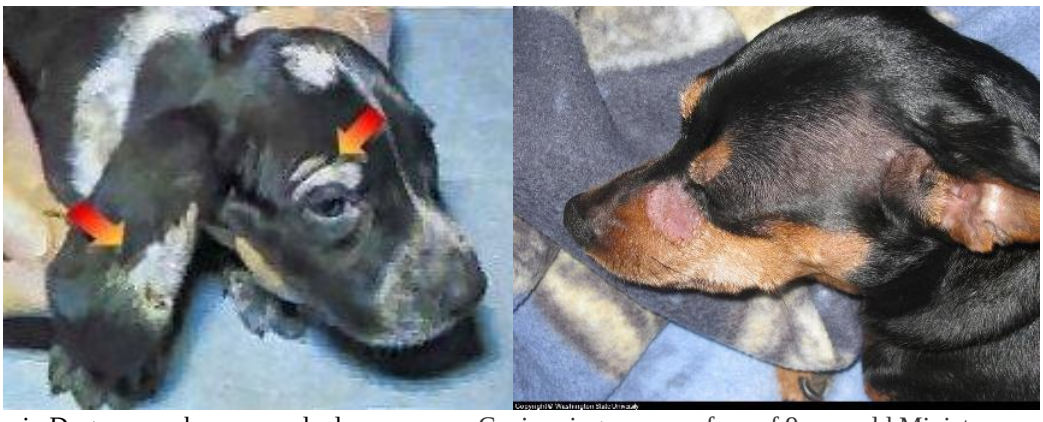
3. **Lesion-free/culture positive**: This group consists of cats that are mechanically carrying spores on their hair coat and/or cats with very early lesions that are not easily seen but mature enough to be shedding arthrospores. Colony forming units (cfu) on fungal culture, coupled with a re-examination under both room light (sometimes called 'white light') and a Wood's lamp, are helpful aids for differentiating fomite carriers ('dust mops') from cats with early lesions. A major risk these cats pose is contamination of the environment, which will confound fungal culture results; or, if truly infected, they are a source of infection for susceptible cats.
    i. ***Fomite carrier cats***: At the time of admission no lesions were noted and these cats were Wood's lamp negative. Upon re-examination, the cats were still lesion-free, Wood's lamp negative and culture negative. Typically these cats had fewer than 10 cfu/plate.
    ii. ***Infected cats***: At the time of admission, likewise, no lesions were noted and these cats were Wood's lamp negative. By the time culture results were available 7–14 days later, these cats were lesional, Wood's lamp positive and still culture positive. Lesions were

usually small and typically located in, on or near the ears, on the muzzle, between the digits, on the tail or in the axilla.

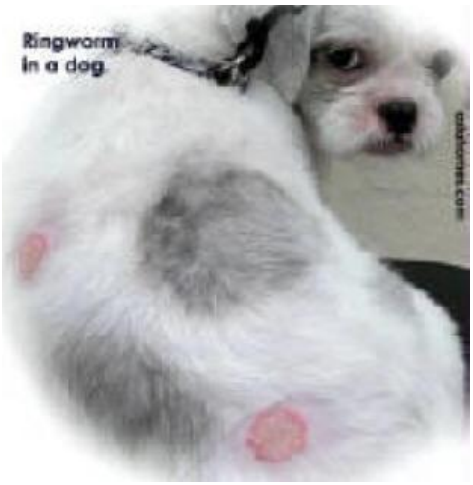
Focal lesion of dermatophytosis on an otherwise healthy cat. ***Dr Rebecca Stuntebeck*** Generalized dermatophytosis in a kitten with malnutrition, diarrhea and upper respiratory infection
Moriello (2014) J Feline Med Surg. 2014 May; 16(5):419-431

1.8.2. Clinical signs of ringworm in *dogs*

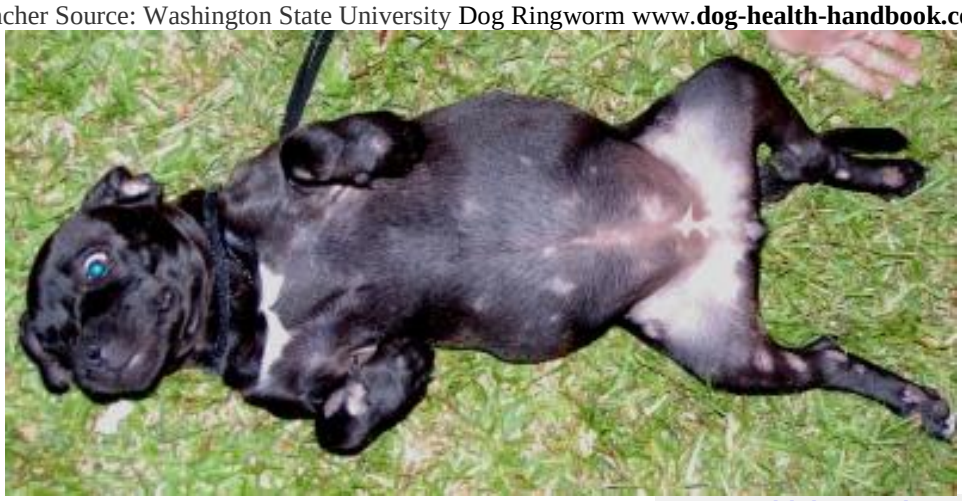
- Dermatophytosis is seen most often in puppies.
- The lesions frequently develop on the face and limbs, although they may occur on any part of the body.
- *M. canis* infection tends to appear as small circular areas of alopecia. The hairs are typically broken at the base, giving the appearance of having been shaved. The center of the lesion usually contains pale skin scales in the early stage, giving it a powdery appearance, and the edges are generally erythematous. Vesicles and pustules may also be seen.
- In later stages, the area is often covered by a crust and the edges swollen. Individual lesions may coalesce to form large, irregular patches.
- *T. mentagrophytes* and *T erinacei* cause lesions that tend to be more thickened and inflammatory than those caused by *M. canis*
- *M persicolor* typically causes localized or generalized scaling with little erythema and minimal alopecia.
- Other forms of dermatophytosis can include kerions (localized severe inflammation with swollen, boggy skin oozing pus) and pseudomycetomas.
- Onychomycosis may occur concurrently with dermatophytosis.



Ringworm in Dogs, www.homeremedyalmanac.com, Canine ringworm on face of 9 year old Miniature , **www.dog-health-handbook.com**



Pincher Source: Washington State University Dog Ringworm www.**dog-health-handbook.com**



Dog with Ringworm. This dog contracted ringworm from infected soil www.worldclassgsd.com

www.toapayohvets.com

Close-up of Charlie's ringworm sore.

1.9. Aetiology

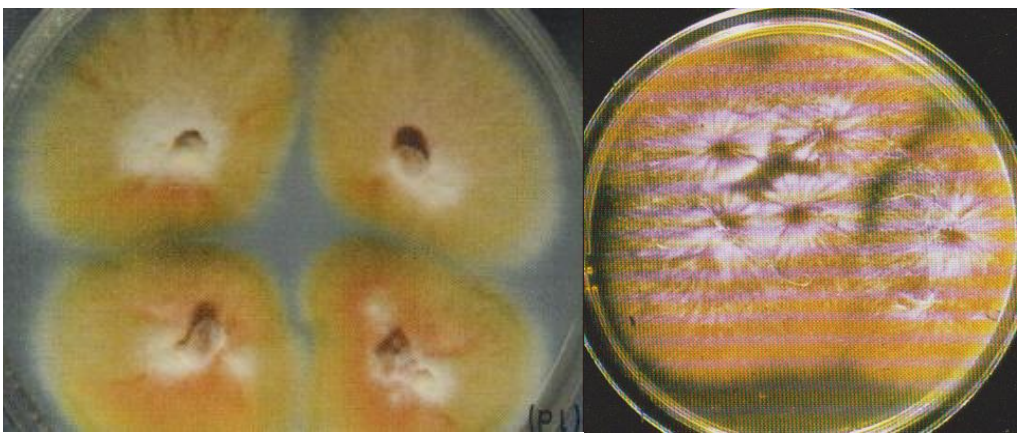
1.9.1. Microsporum canis Bodin, Les champignons parasites de l'homme, 1902.

Synonyms

= Microsporum audouinii Gruby var. canis Bodin in Besnier et al., 1900.
= Sabouraudites canis (Bodin) Langer., 1945.
= Microsporum felineum Mewborn, 1902.
= Microsporum lanosum Sabour., 1907.

= Sabouraudites felineus (Mewborn) Ota & Langer., 1923 as '(Fox & Blaxall, 1896)'.
= Sabouraudites lanosus (Sabour.) Ota & Langer., 1923.
= Closterosporia felinea (Mewborn) Grigoraki, 1925.
= Closterosporia lanosa (Sabour.) Grigoraki, 1925.
= Microsporum aurantiacum Conant, M. obesum Conant, M. pseudolanosum Conant, and =M. simiae Conant, 1941. Conant also considered M. equinum (Delacr. & Bodin) Gueguen

Colonies on Sabouraud's glucose agar are flat, spreading, 55-70 mm diam. after 2 weeks at 25°C, at first mostly submerged, surface very thin and strongly radiating, with a buff, granular to fluffy area in the centre where macroconidia are formed; rapidly mutating to produce patches of dense, fluffy, whitish to pale buff mycelium which eventually grows over the whole colony. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur.



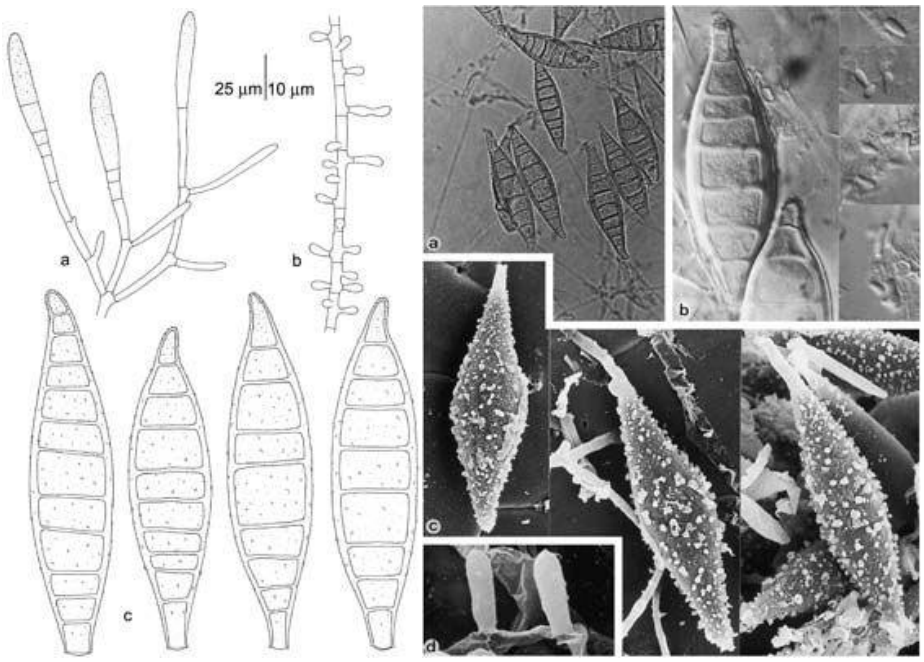
Colonies of *Microsporum canis*

Dysgonic strains are slow-growing, glabrous, brownish, usually confined to a very small area around the hair stumps from which they are growing; they are not stable and on sub-culture may give rise to colonies typical of *M. canis*.

Growth on Rice Grains: good growth of white aerial mycelium with production of yellow pigment.

Culture of dysgonic strain of *M. canis*, boiled polished rice grains to stimulate sporulation, **Mycology online**

Macroconidia most abundant in the centre of the colony, fusiform, variable in size, 35-110 x 12-25 µm, with up to 14 septa and thick (up to 4 µm thick at the centre of the cell), verrucose walls; ends remaining narrow and relatively thin-walled. Macroconidia borne terminally on short hyphae, usually at an acute angle along simple hyphae, occasionally on branched hyphae with up to 3 branches, themselves branched, arising at the apex of one cell. Microconidia rare on Sabouraud's glucose agar, more abundant on some other media, 3,5-8·5 x 1,5-3,5 µm, smooth-walled, non-septate or rarely 1-septate, sessile or on short pedicel, borne along the sides of simple hyphae.



Macroconidia of M. canis, Mycobank

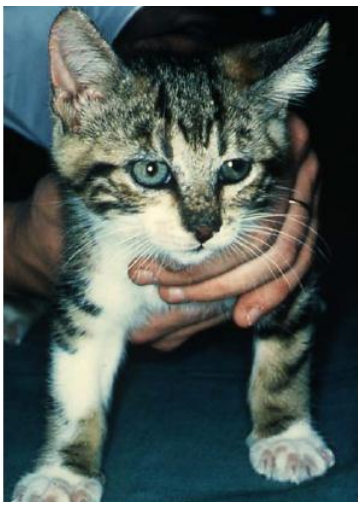
Geographical distribution: Africa (Algeria, Angola, Cape Verde Islands, Egypt, French W. Africa, Sahara, Tunisia, Union of S. Africa); Asia (Ceylon, India, Philippines, Turkey); Australasia & Oceania (Australia (N.S.W.), New Zealand); Europe, North America, Central America and West Indies (Costa Rica, Cuba, Guatemala, Mexico, Panama, Puerto Rico); South America (Argentina, Brazil (south of Pernambuco), Chile, Colombia, Ecuador, Peru, Uruguay, Venezuela).

Reports

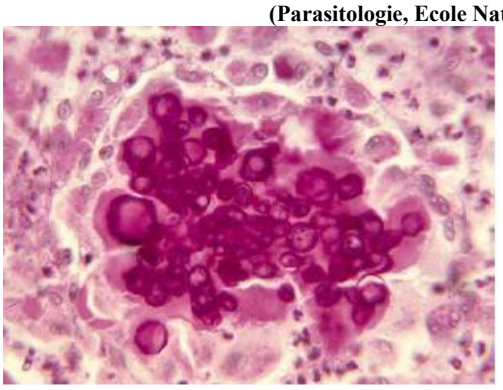
Dreisoerner *et al.* (1964) isolated ***Microsporum canis*** fro 17 out of 42 cats in a cattery suffering fromotitis externa.

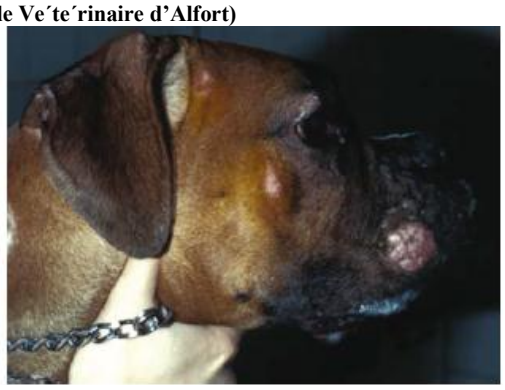
Kristensen and Krogh (1981) examined 774 specimens from dogs and 227 specimens from cats were for ringworm infection. Ninety-six (12.4%) of the samples from dogs and 66 (29.1%) of the samples from cats were positive by culture. ***Microsporum canis*** accounted for all infections in cats and for 95.8% of the infections in dogs.

Chermette *et al*. (2008) mentioned that ***Microsporum canis*** is largely predominant in cats with over 90% of the feline isolates in most of the surveys conducted worldwide. They described *Microsporum canis* infection in a kitten with lesions on the bridge of the nose, the ear margins and the digits. They also described histological lesion of the dermis in a mycetoma-like *M. canis* infection in a Persian cat, where a pyogranulomatous reaction could be seen around PAS-positive vesiculous fungal elements embedded in an eosinophilic mass. They also reported kerion in a dog due to *Microsporum canis* and a case of total alopecia in an extensive dermatophytosis due to *Microsporum canis* in a Yorkshire terrier following a corticotherapy.

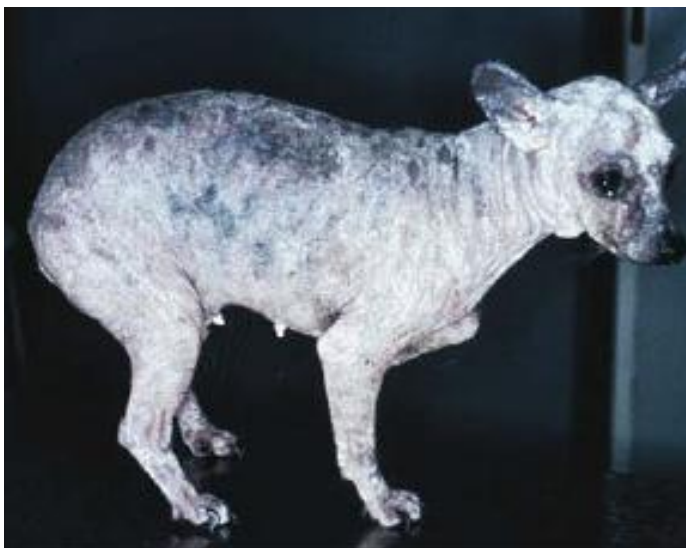


Microsporum canis infection in a kitten with lesions on the bridge of the nose, the ear margins and the digits **(Parasitologie, Ecole Nationale Ve´te´rinaire d'Alfort)**





Histological lesion of the dermis in a mycetoma-like M. canis infection in a Persian cat. A pyogranulomatous reaction can be seen around PAS-positive vesiculous fungal elements embedded in an eosinophilic mass. Kerion due to Microsporum canis in a dog (Parasitologie, Ecole Nationale Ve´te´rinaire d'Alfort)



a case of total alopecia in an extensive dermatophytosis due to *Microsporum canis* in a Yorkshire terrier following a corticotherapy **(Parasitologie, Ecole Nationale Ve´te´rinaire d'Alfort)**

Mancianti *et al.* (2002) examined dermatological specimens from 10.678 animals (7.650 cats and 3.028 dogs) for dermatophytes. All the animals presented clinical signs of ringworm. Two thousand-four hundred fifty-six of the 10.678 (23%) examined animals scored positive for dermatophytes, 566 out of 3.028 canine (18.7%) and 1890 out of 7.650 feline specimens (24.7%). ***Microsporum canis*** constituted 83% and 97% of the isolated dermatophytes respectively in dogs and cats.

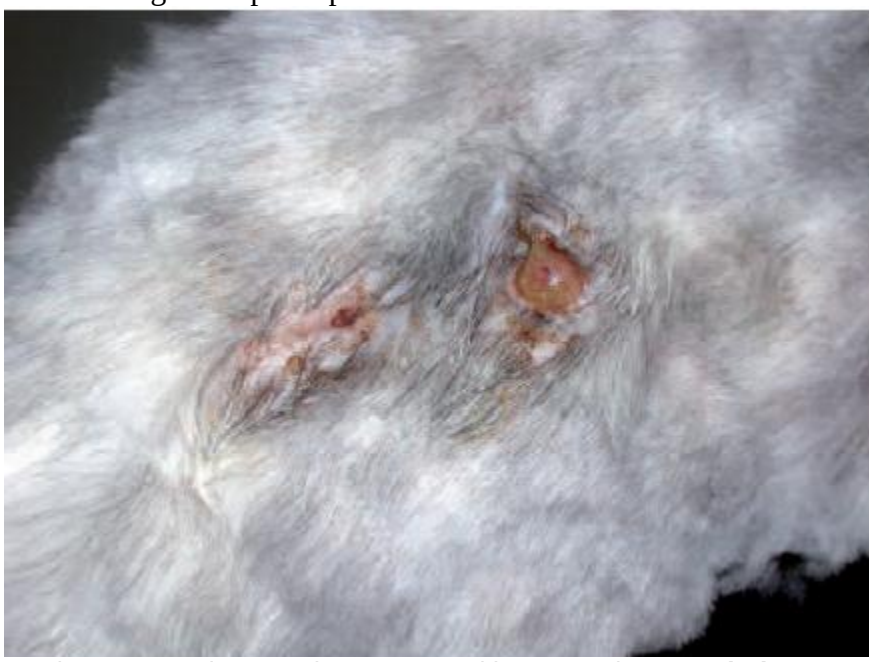
Cafarchia *et al.* (2004) examined 424 animals (268 dogs and 156 cats) with skin lesions (alopecia and peripheral scaling), of which 99 (23.3%) yielded a positive culture (20.5% of the dog samples and 28.2% of the cat samples). ***Microsporum canis*** was the most common dermatophyte isolated from dogs and cats (77.7%). Young dogs and cats, especially those younger than 1 year, showed a statistically significant higher prevalence of *M. canis* infection than older animals. No statistically significant association was found between infection and sex in cats, while male dogs were more affected by dermatophytes. Among breeds, Yorkshire terriers showed the highest positivity (50%) caused mainly by *M. canis* (46.6%), while no differences were noticed for cats. A significantly higher prevalence of positive samples was registered in summer and in autumn for cats.

Pinter and Štritof (2004) reported on the examination of 3854 dogs with different dermatological disorders, in the period from 1970 to 2002, in Croatia. *Microsporum canis* was diagnosed in 840 cases (21.8%). difficult, together with duration of infection and reappearance due to persisting spores.

Iorio *et al.* (2007) collected 200 hair/skin samples from 2002 to 2004 from two groups of cats (privately owned and stray cats from a shelter) Thirteen of the 100 privately owned cats (13%) and 100% of the stray cats were positive. ***Microsporum canis*** was the most common dermatophyte isolated in both cat groups.

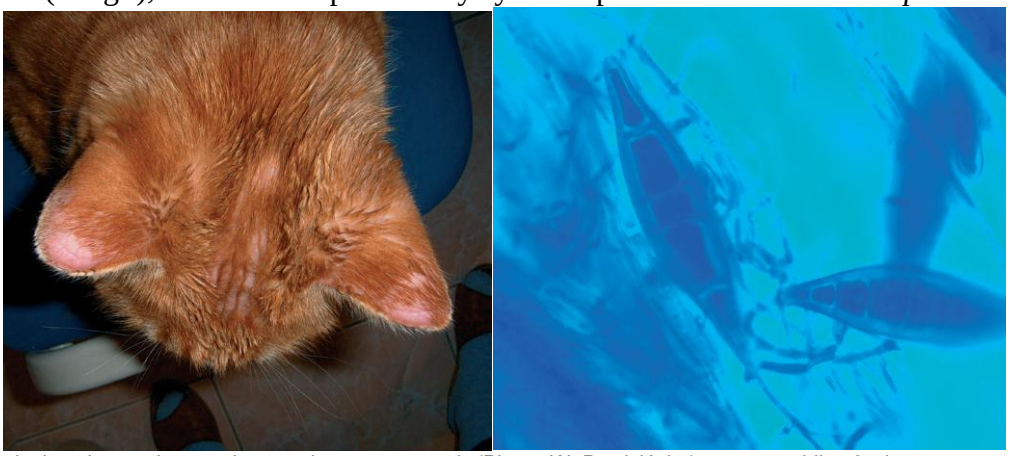
Yahyaraeyat *et al.* (2009) isolated dermatophytes from 54 (43.5% of 124 cats in Tehran, Iran. ***M. canis*** constituted 94.7 % of the isolated dermatophytes. The positive cats were between 1-48 months years old, cats less than I year old significantly suffered from dermatophytosis. Of 292 dogs examined, 63 yielded dermatophytes (21.5%), of which 88.8% were M. canis.The positive dogs were between 2 weeks and 11 years old.

MORETTI *et al.* (2013) mentioned that *M. canis* is the most frequently isolated dermatophyte which has its natural reservoir in the cat. This dermatophyte is found in over 90% of fungal infection in cats. Typical lesions observed in kittens are non-inflammatory alopecic areas, with central desquamation, which are surrounded by brittle or easy to extract fur. Lesions localize preferentially on areas that are most in contact with the healthy carrier mother cat while feeding, *i.e.*, face, ears and legs. Other forms are characterized by small, crusted scaly, sometimes itchy, lesions. Other aspects are miliary-like dermatitis andring-shaped lesions with inflammation or papules on the periphery and fur regrowth in the center. Single or multiple cutaneous nodules, firm and painless at palpation, are usually found on the back and neck. Nodules are in blue or purplish colour, without alopecia or erythema and may result in "scutula formation. This form of dermatophytosis is mainly found in Persian cats because of their genetic predisposition to it.



Pseudomycetoma in a Persian cat caused by *M. canis*, **Dr. Federico Leone**

Dąbrowska *et al.* (2014) examined samples collected from 8 cats and 7 dogs suspected of ringworm by direct microscopy and cultured on Sabouraud dextrose agar (SDA). Ringworm was identified in all specimens. Culture on Sabouraud dextrose agar supplemented with chloramphenicol (0.05 g /l) and cycloheximide (0.4 g/l), at 30°C for up to 14 days yielded pure cultures of *Microsporum canis.*



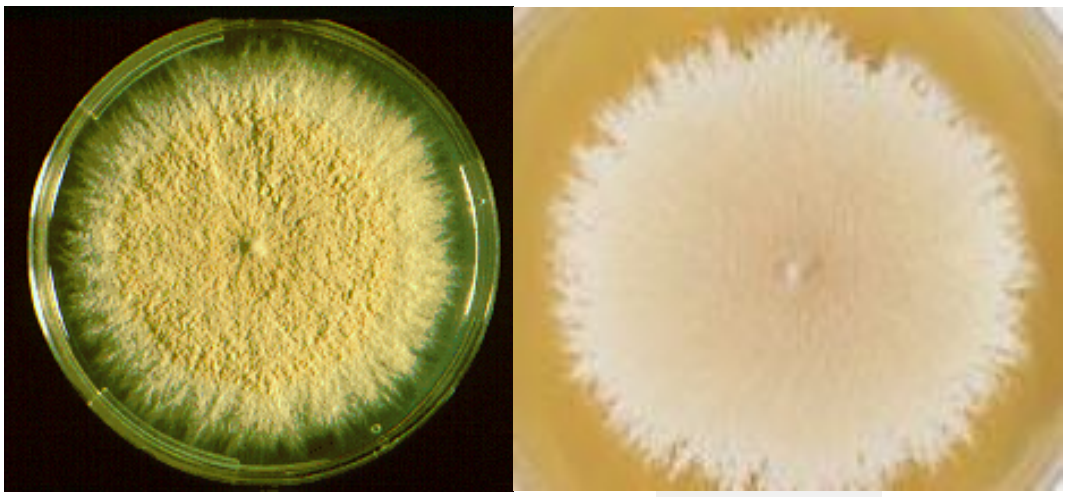
Typical ringworm lesions in a cat's ears due to *Microsporum canis* (Photo: W. Dardzińska), macroconidia of *Microsporum canis*. Microconidia typically are absent. Macroconidia are fusoid, verrucose, and thick walled. They have a recurved apex and contain 5–15 cells.
Dąbrowska *et al.* (2014)

Proverbio *et al.* (2014) conducted a study to determine the prevalence of dermatophytes in stray cats with and without clinical lesions from different colonies in rural and urban areas of Milan and surroundings in northern Italy. Stray cats (273) were caught during a trap-neuter-release (TNR) program conducted in different colonies of northern Italy in both rural and urban areas. Each cat was examined in dark environment with a Wood's lamp prior to sample collection. Hair or scales exhibiting typical fluorescence were removed with a pair of sterile hemostats and cultured. The hair of all cats was then sampled by Mackenzie modified brush technique regardless of the presence or absence of skin lesions attributable to dermatophytosis. All the hair samples were subjected to fungal culture. 15 cats were positive (5.5%). ***Microsporum canis*** was the most common dermatophyte isolated (13/15).

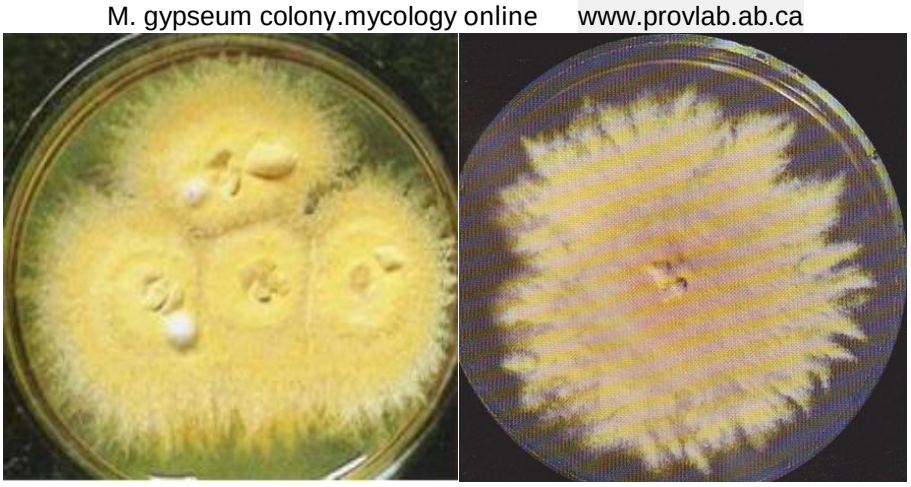
1.9.2. Microsporum gypseum (E. Bodin) Guiart & Grigoraki, Lyon Médical 141: 377 (1928)

≡Trichophyton gypseum E. Bodin, Les champignons parasites de l'homme: 115 (1902)
≡Achorion gypseum (E. Bodin) E. Bodin, Annales de Dermatologie et Syphilis 8: 585 (1907)
≡Sabouraudites gypseus (E. Bodin) M. Ota & Langeron, Annales de Parasitologie Humaine Comparée 1: 328 (1923)
≡Closterosporia gypsea (E. Bodin) Grigoraki, Annales des Sciences Naturelles Botanique 7: 411 (1925)
≡Trichophyton mentagrophytes var. gypseum (E. Bodin) Kamyszek, Med. Weteryn.: 146 (1945)
=Microsporum flavescens Horta, Memórias do Instituto Oswaldo Cruz 3 (2): 301-308 (1912)
=Microsporum scorteum Priestley, Ann. Trop. Med. Parasit.: 113 (1914)
=Microsporum xanthodes Fischer, Dermatol. Wochenschr.: 214-247 (1918)
=Favomicrosporon pinettii Benedek, Mycopathologia et Mycologia Applicata 31 (2): 111 (1967)

On Sabouraud's dextrose agar, colonies are usually flat, spreading, suede-like to granular, with a deep cream to tawny-buff to pale cinnamon coloured red surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral boarder. A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains.

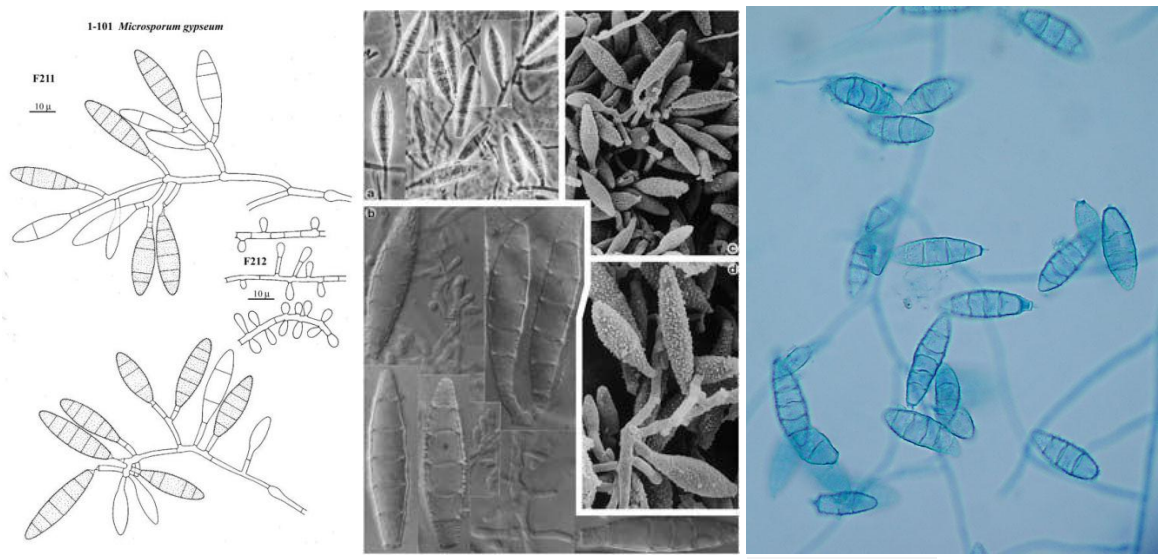


M. gypseum colony.mycology online www.provlab.ab.ca



M. gypseum colonies on Kimmig agar, Rieth

Cultures produce abundant, symmetrical, ellipsoidal, thin-walled, verrucose, 4-6 celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncate. Numerous clavate shaped microconidia are also present, but these are not diagnostic.



Mycobank labmed.ucsf.edu

Reports:

Okoshi and Hasegawa (1957) described the clinical and mycological findings in 3 cases of cats ringworm caused by ***Microsporum gypseum***. The 3 cats had been born and reared separately in Tokyo. Lesions occurred on the head, scrotum, paw, and pad; some showed mild scaling with loss of hair, and others crust formation and inflammation.

Kano *et al.* (2001) reported a 1- to 2-month-old female cross-breed cat presented with alopecia, erythema and many crusts on the tail. Microscopic examination of crusts from the tail disclosed epithelial debris, exudate, mycelium, and arthrospores. ***Microsporum gypseum*** was cultured from the crusts on a Sabouraud glucose agar at 27°C for 1 week.

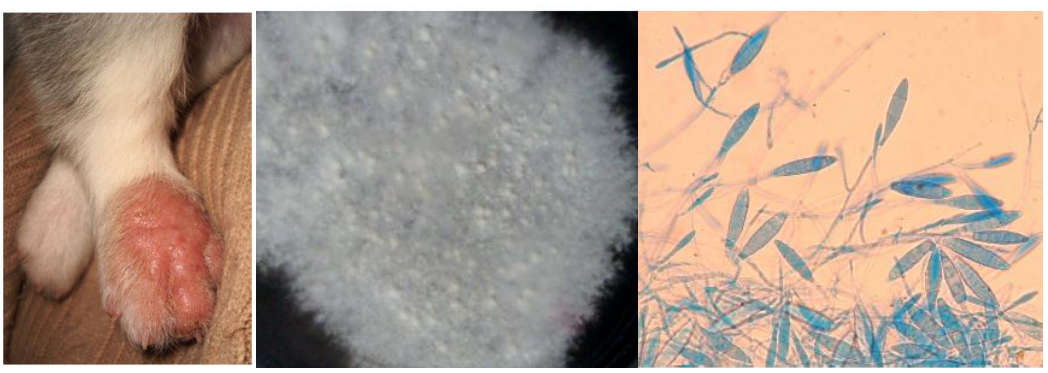
Mancianti *et al.* (2002) examined dermatological specimens from 10.678 animals (7.650 cats and 3.028 dogs) for dermatophytes. All the animals presented clinical signs of ringworm. Two thousand-four hundred fifty-six of the 10.678 (23%) examined animals scored positive for dermatophytes, 566 out of 3.028 canine (18.7%) and 1890 out of 7.650 feline specimens (24.7). M. gypseum represented 13%. Microsporum gypseum was mostly recorded from sporting (hunting) breeds. The annual distribution of the infections in dogs showed a significantly higher incidence for M. gypseum in summer versus winter and spring.

Andrino *et al.* (2003) described a case of severe canine onychomycosis. The aetiological agent was identified as ***Microsporum gypseum***. The incidence of this fungus in this kind of pathology was discussed, with special attention to the successful treatment with topic enilconazole and systemic griseofulvin.

Pinter and Štritof (2004) reported on the examination of 3854 dogs with different dermatological disorders, in the period from 1970 to 2002, in Croatia. *Microsporum gypseum* was isolated in 38 dogs (1.0%).

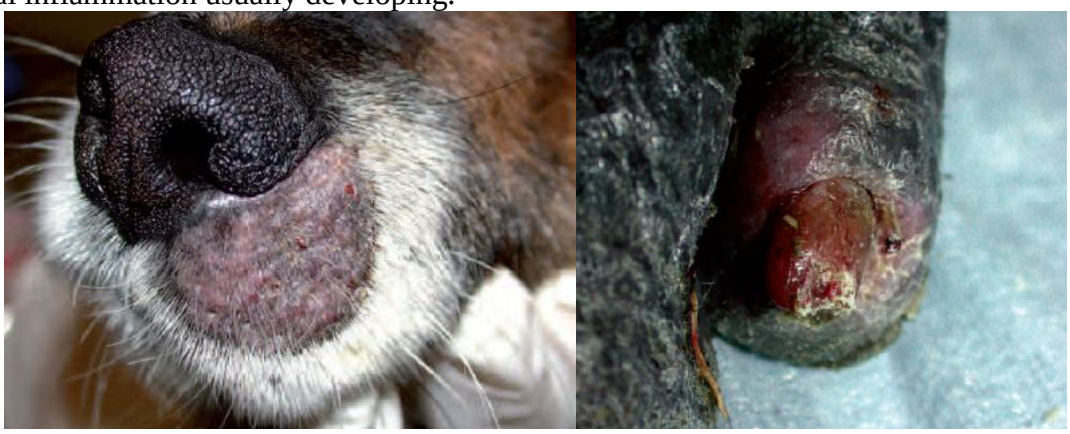
Yahyaraeyat *et al.* (2009) isolated dermatophytes from 54 (43.5% of 124 cats in Tehran, Iran. ***M. gypseum*** was recovered from 2.6% of the cases. Of 292 dogs examined, 63 yielded dermatophytes (21.5%), of which 3.7% were M. gypseum.

Madrid *et al.* (2012) reported an outbreak of canine neonatal dermatophytosis caused by ***Microsporum gypseum***. Seven puppies with 20 days old-age were submitted to clinical examination, where five showed regions of alopecia, erythema and scaling in the hindlimb and/or tail. Dermatophytosis was confirmed by isolation of *M. gypseum* and topical antifungal therapy was prescribed to all animals. Two animals had spontaneous clinical cure of the lesions and the others were treated for 30 days with ketoconazole or miconazole. Fungal cultures were negative after the end of the treatment



Presence of swelling, erythema and alopecia right hind limb of puppy affected by ringworm
C and macroconidia of Microsporum gypseumolony, **Madrid *et al.* (2012)**

MORETTI *et al.* (2013) mentioned that *M. gypseum* often cause kerion which presents as a deep, infiltrated inflammatory swelling, with a damp, ulcerated pus exuding surface and is often associated with secondary bacterial infection. These infections frequently develop on the face and limbs of hunting and truffle dogs that spend a lot of time outdoors in contact with the ground. Onychomycosis is very rare in dogs and usually caused by *M. gypseum*. The nail becomes brittle, loses its shape with periungual inflammation usually developing.



Kerion in a dog presents as a deep, infiltrated inflammatory swelling, with a damp, ulcerated pus exuding surface caused by *M. gypseum*, Onychomycosis in a dog caused by *M. gypseum*, the nail becomes brittle, loses its shape with periungual inflammation, **MORETTI *et al.* (2013)**

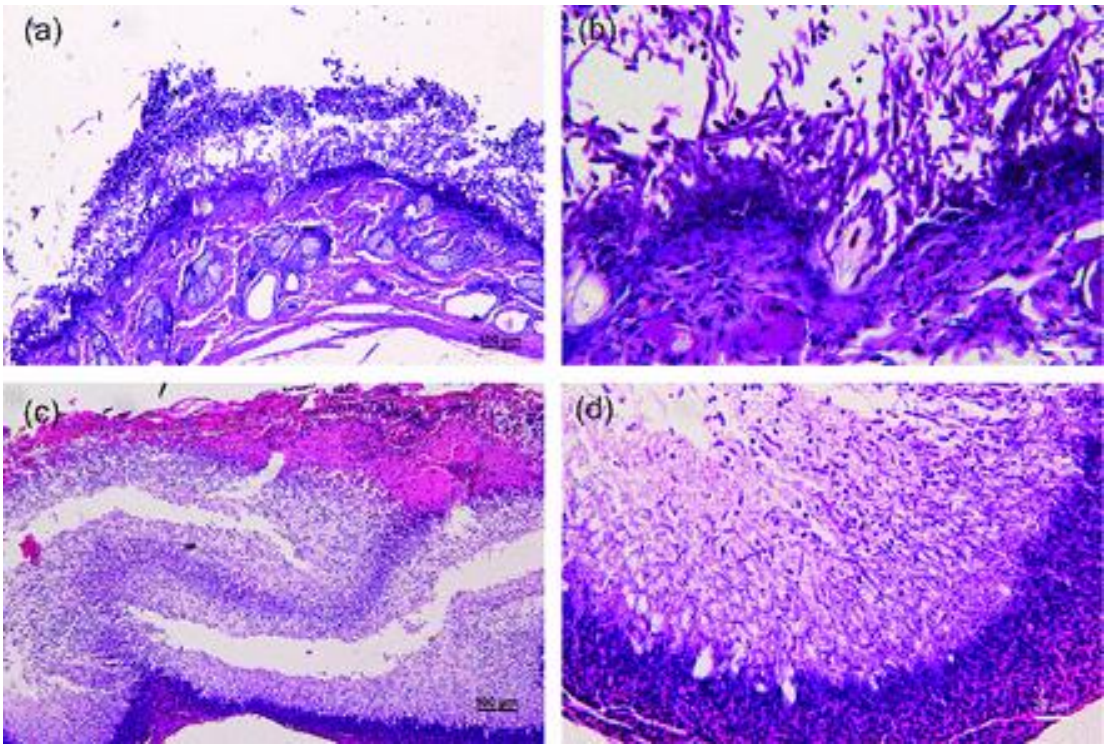
Nardoni *et al.* (2013) evaluated the occurrence of infection by *Microsporum gypseum* retrospectively in dermatological specimens from 15,684 dogs and cats dermatologically diseased from Italy. One hundred and eighty-five specimens out of 15,684 (1.1%) scored positive for ***Microsporum gypseum***.

Sun *et al.* (2014) examined four cat favus cases, focusing on clinical presentations and histopathological features. Physical examination revealed a waxy, yellow scutulum surrounded by broken hairs adherent to the ears in all cases cases, and yellow favic scutulum with a waxy surface on the right hind leg of one of the cases. After the scutulum was carefully removed, a figurate ulcerated base was revealed. Microscopic examination of the waxy crust after it was pretreated with 20% potassium hydroxide (KOH) revealed many arthroconidia and hyaline hyphae. Wood's light examination was negative. In all cases the etiologic agent was identified as ***M. incurvatum*** based on its morphological characteristics and sequences of internal transcribed spacers (ITS) of nuclear ribosomal DNA.

Left:Favus in a kitten showing a waxy, yellow scutulum surrounded by broken hairs adherent to the ear. Middle:yellow scutula on the external aspect of the right ear, Right:yellow favic scutulum with a waxy surface on the right hind leg , **Sun *et al.*, 2014**

The histopathology of the cases elucidated the development of favic lesions. The fungus gained entry to the skin from the stratum corneum of the epidermis and then invaded horizontally and downward into hair follicles. The hair shafts within the follicles were also infected. Hyphae then proliferated very quickly between the stratum corneum and Malpighian layer, forming the main body of scutula. The hyphae that grew upward between the two tissue layers and became fragmented into

arthroconidia. Histological staining revealed alternate horizontal dense and loose zones of fungal growth.

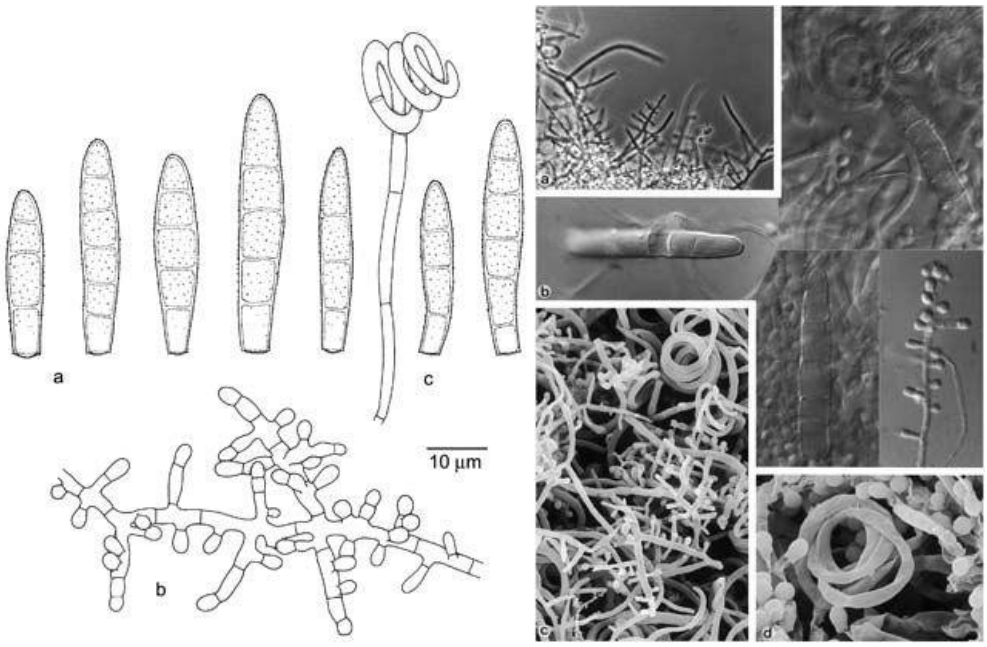


The scutulum was composed of a thin layer of stratum corneum, main fungal portion, and a destructed, necrotic lower epidermis. Hair follicles and hairs were invaded by fungal hyphae (100X, PAS stain) (b) Higher magnification of Fig. 2(a). (c) Horizontal zonations of fungal portion of scutulum (100X, H&E stain). (d) Destruction of lower epidermis by fungal hyphae (400X, H&E), **Sun *et al.*, 2014**

Watanabe (2014) reported two cases of dermatophytosis caused by ***Microsporum gypseum.*** One case was a 59-year-old healthy woman who complained of itchy annular erythema on her right forearm. We isolated ***Microsporum gypseum*** from scales on the forearm. The other case was a 73-year-old midwife who had developed infiltrated erythema on her face for 6 months. ***Microsporum gypseum*** was isolated from scales of the nose. Both women liked gardening and ***Microsporum gypseum*** was isolated from the garden soil of these women by a hair-baiting technique. The first case had a cat, a mouse and an owl, and the second had a dog. Hairbrush culture of these pets, however, was negative. So we concluded both cases were infected with ***Microsporum gypseum*** from garden soil. They isolated ***Microsporum gypseum*** from soil collected in Chigasaki city. Of the 7 fungal cultures from 10 samples, 2 cultures were identified as ***Microsporum gypseum***.

1.9.3. Microsporum persicolor (Sabour.) Guiart & Grigoraki, Lyon Médical 141: 377 (1928)

≡Trichophyton persicolor Sabour., Maladies du Cuir Chevelu 3: 632 (1910) [MB#119354]
≡Ectotrichophyton persicolor (Sabour.) Castell. & Chalm., Manual of Tropical Medicine: 1005 (1919)
≡Sabouraudites persicolor (Sabour.) M. Ota & Langeron, Annales de Parasitol Hum Comp 1: 329 (1923)
≡Closteroaleurosporia persicolor (Sabour.) Grigoraki, Annales des Sciences Naturelles Botanique 7: 412 (1925)
≡Sabouraudites mentagrophytes var. persicolor (Sabour.) M. Ota & Kawats. (1933) [MB#416556]
≡Ctenomyces persicolor (Sabour.) Nann., Repert sistem dei miceti dell' uomo e degli animali 4: 154 (1934)
≡Epidermophyton persicolor (Sabour.) C.W. Dodge, Medical mycology.: 486 (1935)
≡Langeronites persicolor (Sabour.) Ansel (1957)
≡Trichophyton mentagrophytes var. persicolor (Sabour.) Ueckert, Zentralblatt für Bakteriologie und Parasitenkunde Abteilung 1 176: 127 (1959)
≡Microides persicolor (Sabour.) De Vroey, Annales de la Société Belge de Médecine Tropicale 50 (1): 25 (1970)



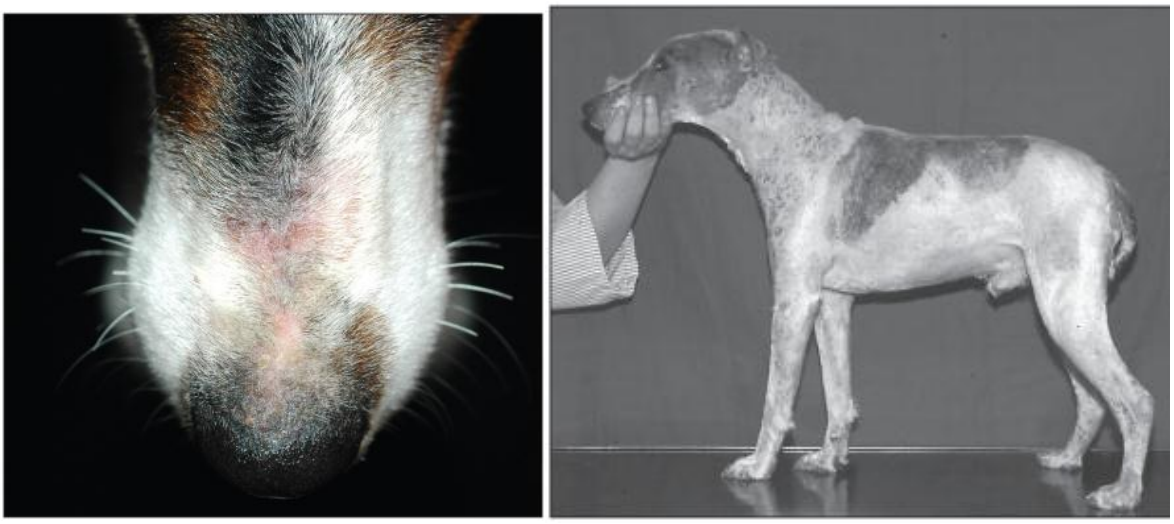
Mycobank

Reports

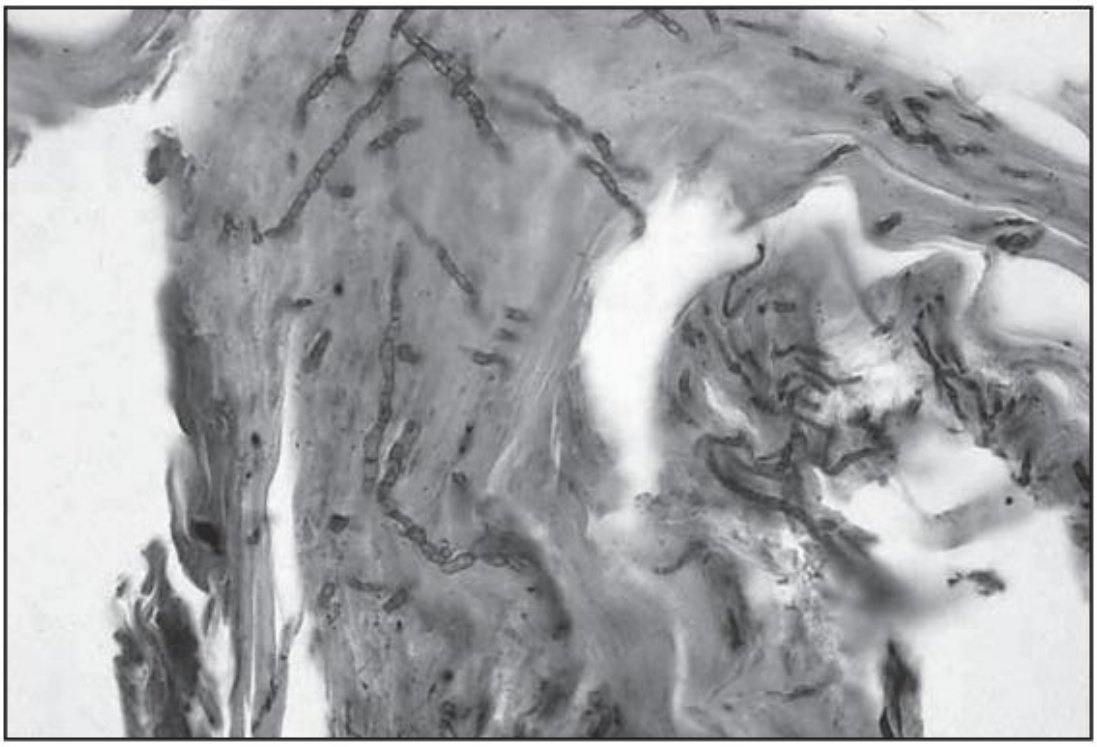
Bond *et al. (1992)* *mentioned that* ***Microsporum persicolor***, a rare zoophilic dermatophyte, was isolated from three dogs with skin disease of between three and five years duration. Skin lesions consisted of scaling with minimal alopecia or erythema. Severe inflammatory changes were not observed clinically and pruritus was absent or mild. The face was affected in all three cases and more widespread lesions were found in two. The diagnosis of dermatophytosis was confirmed in each case by the demonstration of fungal hyphae in the epidermal stratum corneum on examination of skin biopsies. However, hair shaft invasion was not observed in either skin scrapings or histological sections. Of the three dogs, one partially improved following repeated courses of treatment, a second completely recovered with 11 weeks of combined topical and systemic therapy. Response to therapy could not be assessed in the remaining case.

Pinter and Štritof (2004) reported on the examination of 3854 dogs with different dermatological disorders, in the period from 1970 to 2002, in Croatia *Microsporum persicolor* was diagnosed only twice (0.1%).

Muller *et al.* (2011) conducted a retrospective study of 16 cases of dermatophytosis due to ***Microsporum persicolor*** in dogs is reported. Hunting dogs were overrepresented (12/16). Skin lesions were observed on the face in all cases, but also on other locations (limbs, neck). The lesions included alopecia (15/16), erythema (13/16), scales (14/16), and crusts (13/16). Histopathology was performed in 10 cases and showed folliculitis and a lichenoid interface dermatitis. Fungal culture was positive in all cases and clinical resolution was achieved with standard antifungal agents (enilconazole, ketoconazole, griseofulvin). Two recurrences were observed (new contacts with rodents).



Alopecia, scaling, and crusting on the nose of a fox terrier. Alopecia and generalized erythema on a smooth-haired fox terrier, **Muller *et al.* (2011**



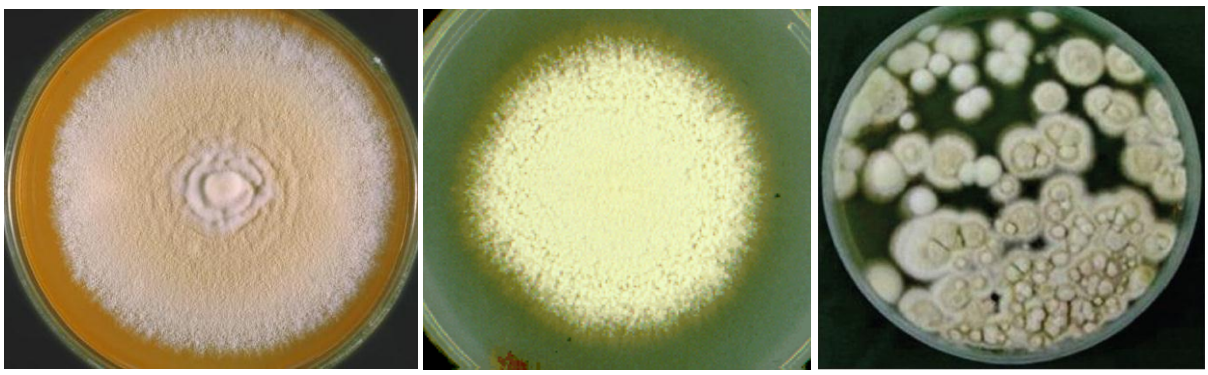
Mycelial filaments without arthrospores in epidermal and follicular keratin. (H & E 800×), **Muller *et al.* (2011**.

1.9.4. Trichophyton mentagrophytes (C.P. Robin) R. Blanch., Traité de Pathologie Générale 2: 912 (1896)

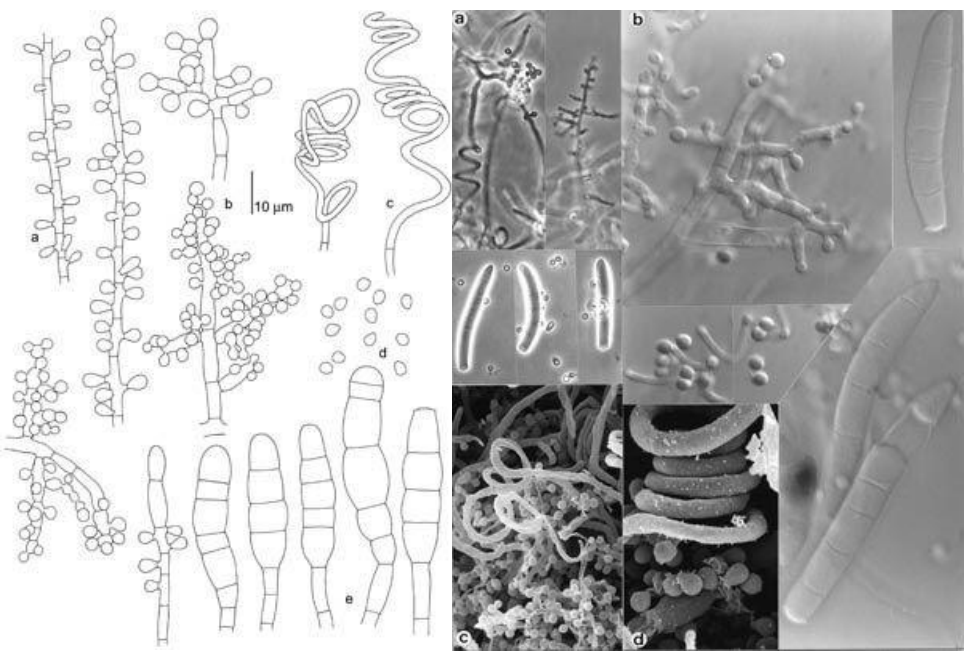
≡Microsporum mentagrophytes C.P. Robin, Histoire naturelle des végétaux parasites qui croissent sur l'homme et sur les animaux vivants: 129 (1853)
≡Sporotrichum mentagrophytes (Robin) Sacc., Sylloge Fungorum 4: 100 (1886)
≡Ectotrichophyton mentagrophytes (Robin) Castell. & Chalm., Manual Trop Med (1919)
≡Ctenomyces mentagrophytes (Robin) Langeron & Miloch., Annls Parasit. hum..: (1930)
≡Spiralia mentagrophytes (Robin) Grigoraki, Compt. Rend. Soc. Biol., Paris: 186 (1932)
≡Sabouraudites mentagrophytes (Robin) M. Ota & Kawats. (1933) [MB#450836]
≡Microides mentagrophytes (Robin) De Vroey, Annales Soc Belge de Méd Trop (1970)
=Trichophyton mentagrophytes var. mentagrophytes

=Oidium quinckeanum Zopf, Die Pilze in morphol, physiol , boil system Bez : 481 (1890)
=Trichophyton granulosum Sabour., Rev. Gén. Méd. Vét.: 561 (1909)
=Trichophyton asteroides Sabour., Maladies du Cuir Chevelu 3: 347 (1910)
=Trichophyton denticulatum Sabour., Maladies du Cuir Chevelu 3: 374 (1910)
=Trichophyton lacticolor Sabour., Maladies du Cuir Chevelu 3: 362 (1910)
=Trichophyton radians Sabour., Maladies du Cuir Chevelu 3: 374 (1910)
=Trichophyton depressum MacCarthy, Ann. Dermatol. Syph.: 190 (1925)
=Grubyella langeronii E.A. Baudet, Annls Parasitol. Humaine Comp.: 417 (1930)
=Trichophyton papilliosum Lebasque (1933) [MB#253799]
=Trichophyton papillosum Lebasque, Les Champignons des Teignes 72 (1933)
=Trichophyton sarkisovii L.G. Ivanova & I.D. Poljakov, Mikologiya i Fitopatologiya:1983

On Sabouraud's dextrose agar, colonies are generally flat, white to cream in colour, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous single-celled microconidia are formed, often in dense clusters. Microconidia are hyaline, smooth-walled, and are predominantly spherical to subspherical in shape, however occasional clavate to pyriform forms may occur. Varying numbers of spherical chlamydoconidia, spiral hyphae and smooth, thin-walled, clavate shaped, multicelled macroconidia may also be present.



T. mentagrophytes ww.wikiwand.com Mycology online w www.e-ijd.org



Mycobank

Reports

Connole (1968) described a case of ringworm due to Trichophyton mentagrophytes in a dog

Chatterjee *et al.* (1980) reported an association of **Trichophyton mentagrophytes** and Demodex canis in a mongrel dog with multiple kerions

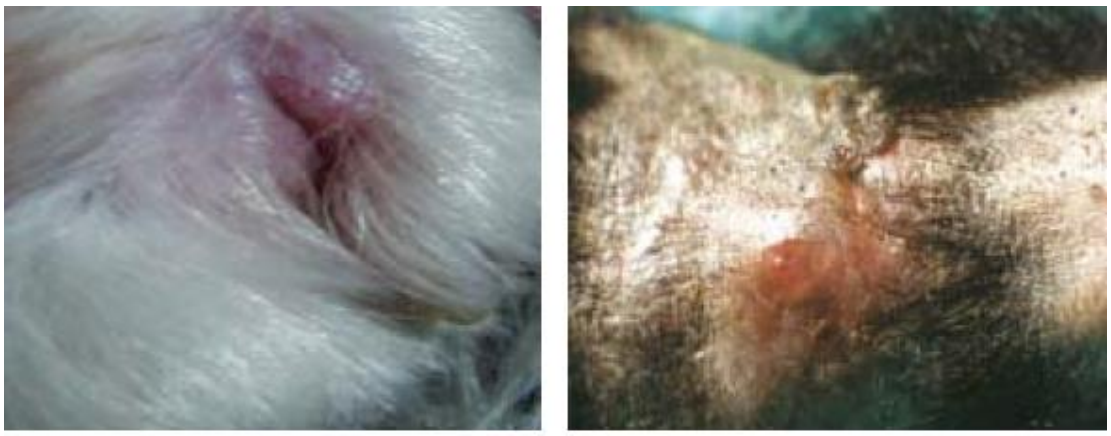
BERGMAN *et al.* (2002) described multiple, dermal and subcutaneous nodules in a young female Manchester Terrier dog that had a chronic history of superficial dermatophytosis. Skin biopsy specimens of the nodules revealed granulomatous inflammation in the deep dermis and subcutis with branching fungal organisms. Cultures of multiple biopsy specimens from the nodules all yielded ***Trichophyton mentagrophytes***. The lesions in this dog were similar to granulomatous dermatophytosis, a skin disease that has been reported in Persian cats and one Yorkshire Terrier dog.

Kristensen and Krogh (1981) examined 774 specimens from dogs and 227 specimens from cats were for ringworm infection. Four dogs (4.2%) were infected with ***Trichophyton mentagrophytes.*** Three fourths of the infections with M. canis were diagnosed during August through January. Ringworm infections can be diagnosed by direct microscopy of hair and scrapings. Wood's lamp examination, skin biopsy, and culture. Of these, the latter method is the most reliable.

Mancianti *et al.* (2002) examined dermatological specimens from 10.678 animals (7.650 cats and 3.028 dogs) for dermatophytes. All the animals presented clinical signs of ringworm. Two thousand-four hundred fifty-six of the 10.678 (23%) examined animals scored positive for dermatophytes, 566 out of 3.028 canine (18.7%) and 1890 out of 7.650 feline specimens (24.7%). ***T. mentagrophytes*** constituted 5.5% and 0.2%. T. mentagrophytes was mostly recorded from sporting (hunting) breeds

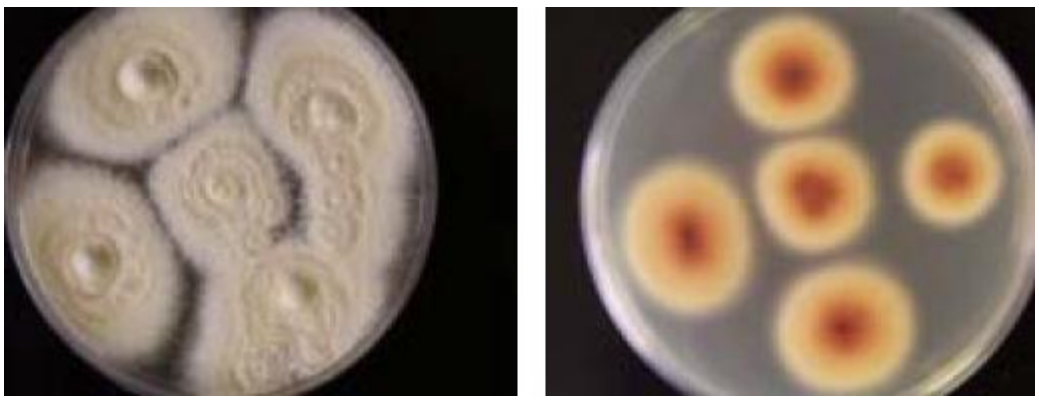
Pinter and Štritof (2004) reported on the examination of 3854 dogs with different dermatological disorders, in the period from 1970 to 2002, in Croatia. Clinical and laboratory examinations of all skin and hair samples yielded 66 (1.7%) isolates of ***Trichophyton mentagrophytes***. A retrospective study of trichophytosis due to *T. mentagrophytes* was performed in order to present different clinical aspects in dogs. All 66 dogs showed clinical evidence of skin lesions, and four groups with different symptoms were identified. The majority of dogs 42 (63.6%) with *T. mentagrophytes* infection had lesions typical of dermatophyte infection. The remaining 24 dogs (36.4%) were without lesions typical of dermatophyte appearance. The clinical picture included multifocal to diffuse appearance in 12 dogs (18.2%), severe inflammatory lesions in 10 (15.2%) or granulomatous lesions resembling pseudomycetoma in 2 dogs (3.0%). Considering the veterinary and public health importance of canine ringworm, attention was focused on *T. mentagrophytes* due to variations in clinical appearance which might make early diagnosis very difficult, together with duration of infection and reappearance due to persisting spores.

Canine dermatophytosis due to *T. mentagrophytes* and its cultural appearances. Left: classic crusting, erosive, alopecic well demarcated lesion on the tip of the nose. Right: Irregular erythematous lesions resemble bacterial hypersensitivity and bacterial hypersensitivity-like lesions on the dog head. **Pinter and Z. Štritof:, 2004**



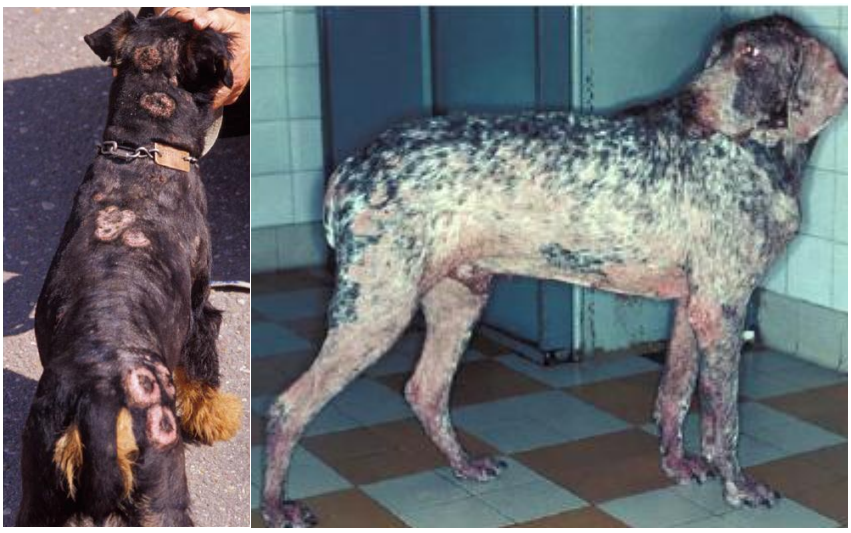
Left: Pododermatitis (severe initerdigital edema). Right: Crusting, erosive, alopecic dermatitis with numerous draining tracts from the dog with generalised *T. mentagrophytes* infection. **Pinter and Z. Štritof:, 2004**

Left: Fungal (*Trichophyton mentagrophytes)* nodules on the muzzle of a dog. The lesion is firm, raised and alopecic. Right: Painful nodular lesion on the dogs digit caused by long-lasting *T. mentagrophytes* infection. **Pinter and Z. Štritof:, 2004**



Left: *T. mentagrophytes* culture on Sabouraud dextrose agar makes a colony with a powdery or cottony-flat surface.Right:The reverse side of *T. mentagrophytes* colonies is usually brown. **Pinter and Z. Štritof:, 2004**

Chermette *et al*. (2008) described cases of ringworm with numerous suppurative lesions due to ***Trichophyton mentagrophytes*** in a hunting dog. Regrowth of hairs was visible on the centre of lesions situated on the right hip and chronic and extensive dermatophytosis due to a mixed *Microsporum canis* and *Trichophyton mentagrophytes* infection in another dog.



Numerous suppurative lesions due to Trichophyton mentagrophytes in a hunting dog. Regrowth of hairs is visible on the centre of lesions situated on the right hip Facial, Chronic and extensive dermatophytosis due to a mixed Microsporum canis and Trichophyton mentagrophytes infection in a dog **(Parasitologie, Ecole Nationale Ve´te´rinaire d'Alfort)**

Yahyaraeyat *et al.* (2009) examined 292 dogs with ringworm in Tehran, Iran, of which 63 dogs yielded dermatophytes (21.5%), of which 88.8% were M. canis and 7.4% were *T. mentagrophytes*.

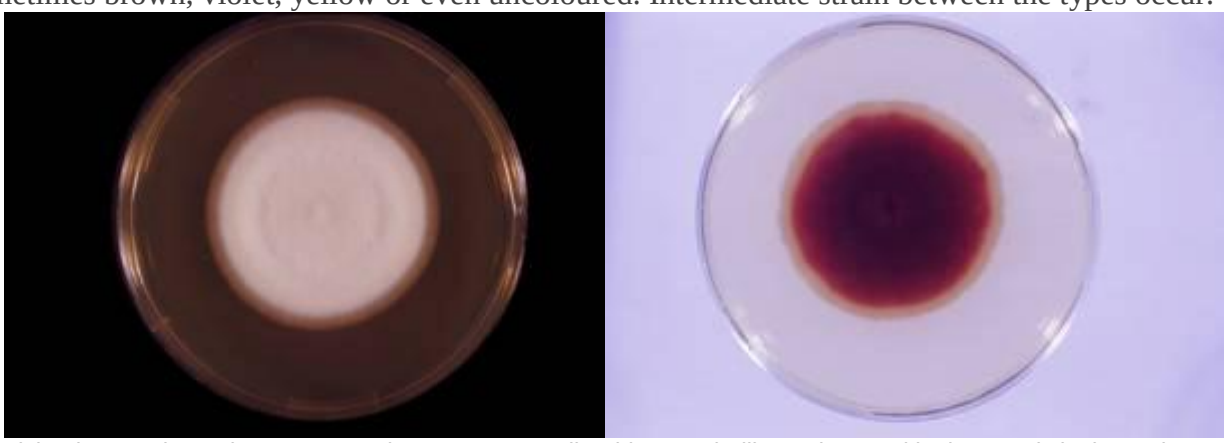
Proverbio *et al.* (2014) conducted a study to determine the prevalence of dermatophytes in stray cats with and without clinical lesions from different colonies in rural and urban areas of Milan and surroundings in northern Italy. Stray cats (273) were caught during a trap-neuter-release (TNR) program conducted in different colonies of northern Italy in both rural and urban areas. Each cat was examined in dark environment with a Wood's lamp prior to sample collection. Hair or scales exhibiting typical fluorescence were removed with a pair of sterile hemostats and cultured. 15 cats were positive (5.5%). ***Trichophyton mentagrophytes*** ftom 2 cases.

1.9.5. Trichophyton rubrum (Castell.) Sabour., British Journal of Dermatology: 389 (1911)

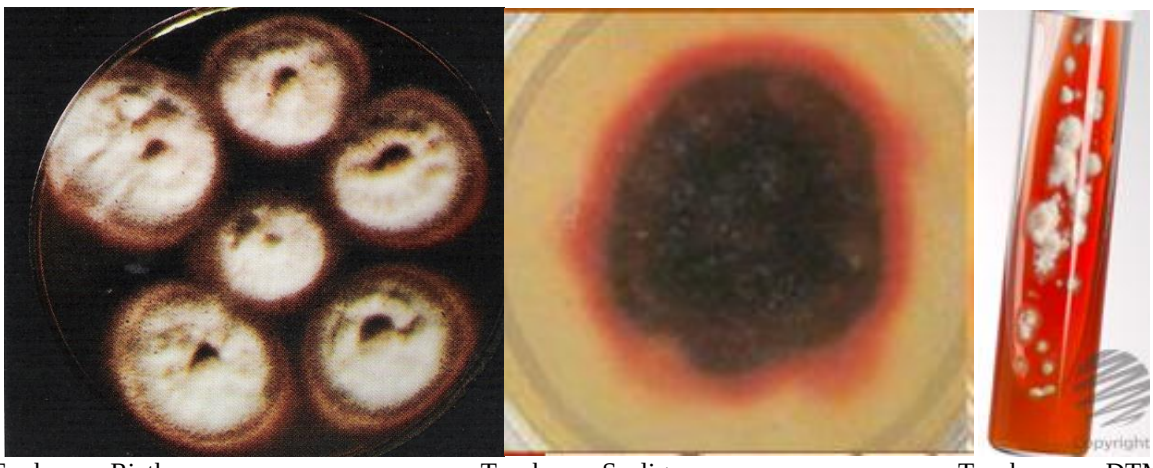
≡Epidermophyton rubrum Castell., Philippine Journal of Science Section B Medical Science 5 (2): 203 (1910)
≡Sabouraudites ruber (Castell.) M. Ota & Langeron, Annal Parasitol Humaine Comparée 1: 328 (1923)
≡Sabouraudiella rubra (Castell.) Boedijn, Mycopathologia et Mycologia Applicata 6 (2): 125 (1953)
=Trichophyton rosacea Sabour. (1894)
=Trichophyton rosaceum Sabour., Trichoph. Hum. F.: 92 (1894)
=Trichophyton megninii R. Blanch., Traité de Pathologie Générale 2: 915 (1895)
=Trichophyton roseum E. Bodin, Les champignons parasites de l'homme: 120 (1902)
=Epidermophyton pernettii Castell., Br. J. Derm. Syph.: 148 (1910)
=Trichophyton circonvolutum Sabour., Maladies du Cuir Chevelu 3: 320 (1910)
=Trichophyton purpureum H. Bang, Ann. Dermatol. Syph.: 238 (1910)
=Trichophyton vinosum Sabour., Maladies du Cuir Chevelu 3: 386 (1910)
=Trichophyton rubidum Priestley, Med. J. Aust.: 474 (1917)
=Trichophyton marginatum Muijs, Ned. Tijdschr. Geneesk.: 2205 (1921)
=Trichophyton pedis M. Ota, Bull. Soc. Pathol. Exot.: 594 (1922)
=Epidermophyton lanoroseum MacCarthy, Ann. Dermatol. Syph.: 53 (1925)
=Epidermophyton plurizoniforme MacCarthy, Ann. Dermatol. Syph.: 37 (1925)
=Trichophyton coccineum Y. Katô, Trans. 6th Congr. Far East Assoc. Trop. Med., Tokyo: 861 (1925)

=Trichophyton multicolor O. Magalh. & J.A. Neves, Memórias do Instituto Oswaldo Cruz 20 (2): 271 (1927)
=Trichophyton kagawaense H. Fujii, Jap. J. Dermatol. Urol.: 305-357 (1931)
=Trichophyton pervesi Catanei (1937)
=Trichophyton pervesii Catanei, Archives de l'Institut Pasteur d'Algerie 15: 267 (1937)
=Trichophyton rodhaini Vanbreus. (1949)
=Trichophyton rodhainii Vanbreus., Annales de Parasitologie Humaine Comparée 24: 244 (1949)
=Trichophyton gourvilii var. intermedium Biguet et al., Annls Parasitol. Humaine Comp.: 419
=Trichophyton kuryangei Vanbreus. & S.A. Rosenthal, Annal Parasitol Humaine Comparée 36: 802 (1961)
=Trichophyton rubrum var. nigricans Frágner, Ceská Mykologie 20 (1): 27 (1966)
=Trichophyton fluviomuniense Pereiro, Sabouraudia 6: 315 (1968)
=Trichophyton fischeri J. Kane, Sabouraudia 15: 239 (1977)
=Trichophyton raubitschekii J. Kane, Salkin, Weitzman & Smitka, Mycotaxon 13 (1): 260 (1982)
=Trichophyton kanei Summerb., Mycotaxon 28 (2): 511 (1987)

Colonies appear in various shades of white, yellow, brown, and red. It may also be found in various textures, being waxy, cottony, or smooth. Two types may be distinguished: *T. rubrum* downy type and *T. rubrum* granular type. On Sabouraud glucose agar, growth is slow to moderately rapid, texture downy, sometimes powdery. Colour white to pale pink on the surface; reverse typically wine red, sometimes brown, violet, yellow or even uncoloured. Intermediate strain between the types occur.

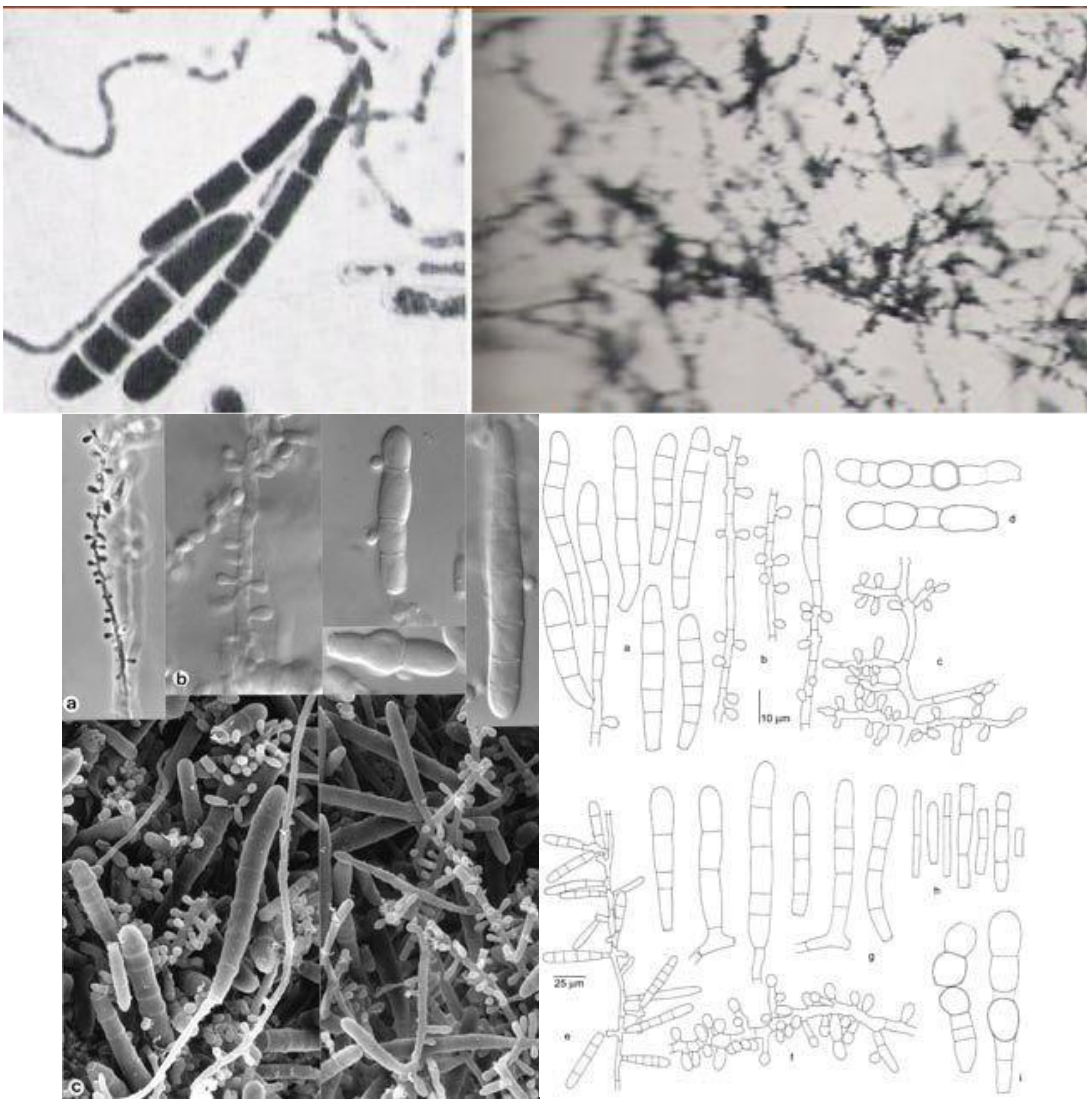


Trichophyton rubrum downy type. Cultures are generally white, suede-like to downy with characteristic deep wine-red reverse pigment.,Life



T rubrum , Rieth T. rubrum, Seeliger T. rubrum on DTM

Microscopically, microconidia are numerous to rare, club – shaped to pyriform, may be found solitary along the hyphae or sometimes in clusters, and are unicellular; and microconidia are frequently absent; pencil – to cigar – shaped, and are multi - septate.



Mycobank

Reports

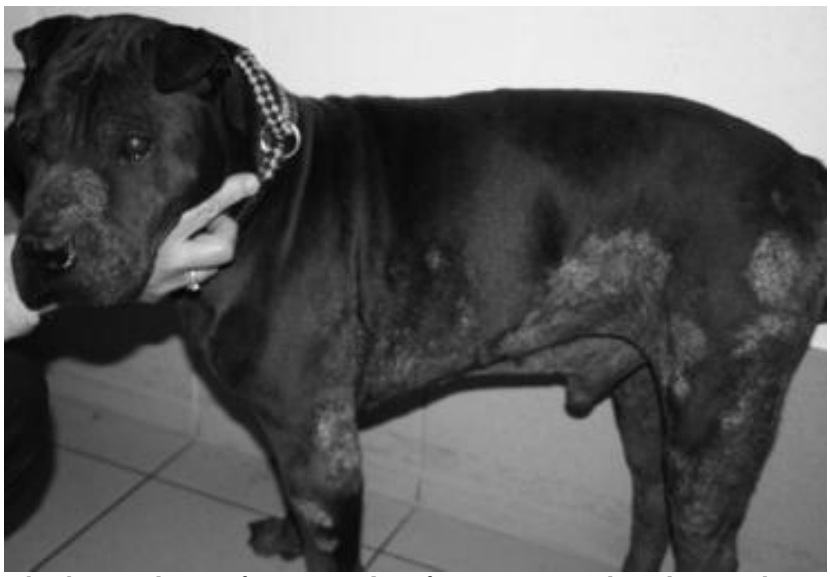
Kushida snd Watanabe **(1975)** diagnosed a case of ringworm in a 2-year-old male Dachshund caused by ***Trichophyton rubrum***. The owner of this dog had tinea pedis probably caused by the same fungus. The authors believed that this is the first authenticated case of T. rubrum infection in a dog recorded in Japan, the infection probably having been acquired from man.

Kano *et al.* (2010) reported an 11-year-old male Yorkshire terrier with pyoderma, alopecia, papules, and eruptions on the face, dorsal area of the neck and the legs. Although microscopic examination of skin scrapings from the lesions did not show hyphae or arthroconidia of dermatophytes, white, flat and powdery colonies developed from the skin samples inoculated onto dermatophyte test medium (DTM) after 2-week incubation at 24°C. Microscopically, the isolate did not produce macro- or microconidia. Itraconazole was orally administered at 7 mg/kg once a day. After 3 months of treatment, the skin lesions were cured and the fungus could not be recovered from the dog's skin. The subcultured colony of the clinical isolate was white, flat and granular with an elevated center, and formed a red pigment when grown on SDA at 24°C for 4 weeks Microscopic examination of portions of the colonies revealed branched hyphae, abundant macro-conidia which were long and slender in shape, and variably shaped microconidia. The urease test was positive after 7 days, growth on lactose agar was restricted and the isolate did not perforate hair. Sequence analysis of genomic DNA extracted from the isolate for detection of *CHS1* (chitin synthase gene 1) and ITS1-5.8S-ITS2 was performed. The PCR products from the samples were sequenced 3 times by the dideoxy chain

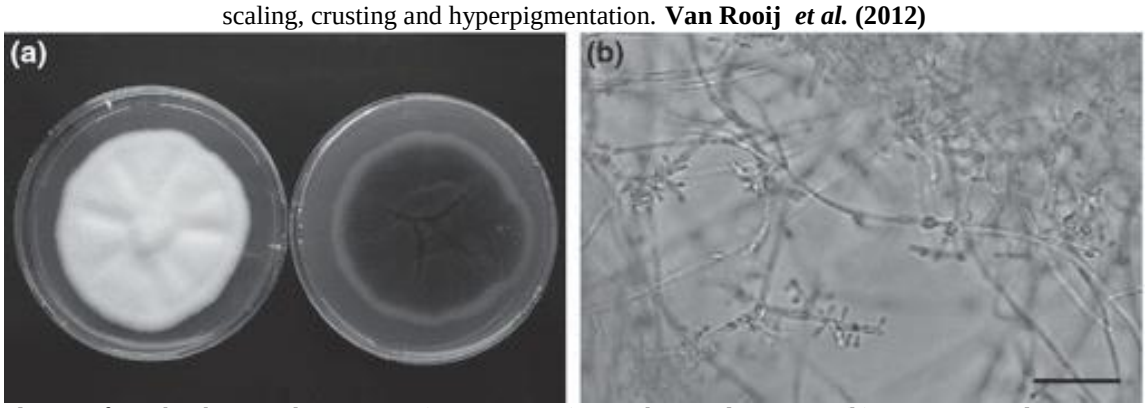
termination method using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences reported in this paper have been deposited in the GenBank database [accession nos. AB517617 (*CHS1*) and AB517618 (ITS1-5.8S-ITS2)]. Comparative sequence analysis within GenBank revealed that the queried*CHS1*sequence was 99% identical to both *T. rubrum* var. *raubitschekii* and *T. rubrum* (GenBank accession no. AB011055 and AB005793, respectively) and <96% identical to *T. mentagrophytes* (GenBank accession no.AB005794), and that the queried ITS1-5.8S-ITS2 sequence was 99% identical to both *T. rubrum* var. *raubitschekii* and *T. rubrum* (GenBank accession no. AF170470 and AJ270798, respectively) and <96% identical to *T. mentagrophytes* (GenBank accession no. AB246678 and AB246679). Therefore, the isolate was identified as ***T. rubrum* var. *raubitschekii*** by both mycological and molecular analyses.

Left:Pyoderma with alopecia, papules, and eruptions on the face, dorsal area of the neck and the legs of the dog. Middle:White, flat and granular colony of ***T. rubrum* var. *raubitschekii*** with an elevated center and red pigmentation when cultured on SDA, Right**:**Abundant macroconidia are long and slender in shape by microscopic examination when cultured on SDA, **Kano *et al.* (2010)**

Van Rooij *et al.* (2012) reported a 9-year-old intact male Shar-pei with unresponsive pruritic dermatitis. The dog had a 5-year history of initial seasonal pruritic skin disease. Well-demarcated areas of extensive hair loss, hyperpigmentation, scaling and crusting were were present around the nose, on the legs, on the feet with hyperkeratosis of the pads, on the trunk and tail. Direct microscopic examination of a diff-quik stained tape strip demonstrated arthroconidia and hyphae. A periodic acid-Schiff (PAS) stain of a skin biopsy demonstrated fungal elements within the hair follicles and the stratum corneum. Skin scrapings were initially inoculated on Sabouraud glucose agar enriched with 2 mg ml^{-1} actidione and 0.5 mg ml^{-1} chloramphenicol. Identification was performed on Sabouraud and diluted Sabouraud glucose agar. The obtained culture was velvety to slightly lanose, white to cream-coloured with a red-brown verso.Microscopic examination revealed abundant pyriform microconidia arranged along the hyphae, and in groups.No macroconidia were observed. Growth on Bromocresol purple medium (BCP) did not change the indicator colour and urease testing was negative. The isolate was identified as ***T. rubrum*** and sequencing of the ITS1–5.8S–ITS2 region was additionally performed for species confirmation



Clinical aspect of a *Trichophyton rubrum* infection in a dog after treatment with antibiotics, showing multifocal alopecia, scaling, crusting and hyperpigmentation. **Van Rooij *et al.* (2012)**

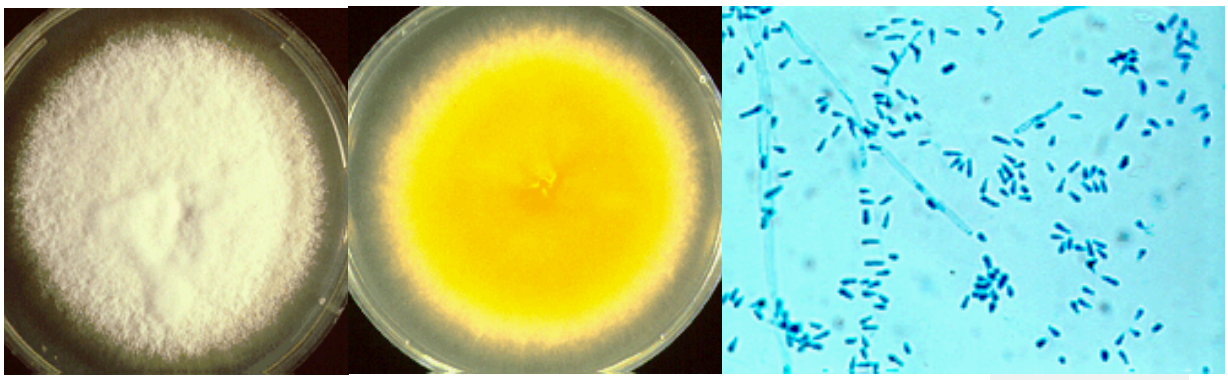


(a) Colonies of *Trichophyton rubrum* strain (IHEM 22409) on Sabouraud at 25 °C. (b) Microscopical examination *T. rubrum*strain showing abundant piriform microconidia; **Van Rooij et al. (2012)**

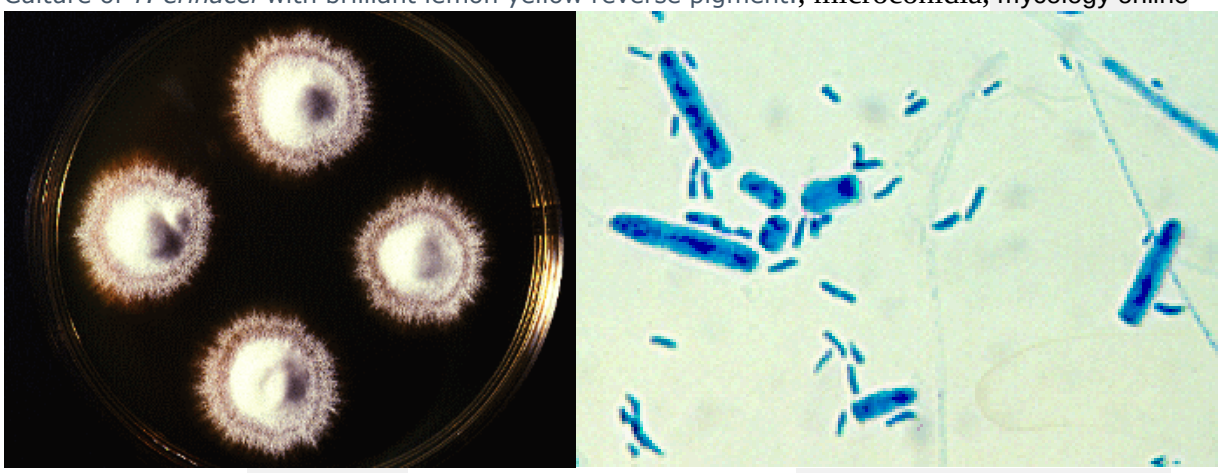
1.9.6. Trichophyton erinacei (J.M.B. Sm. & Marples) Quaife, Journal of Clinical Pathology 19: 178 (1966)

≡Trichophyton mentagrophytes var. erinacei J.M.B. Sm. & Marples, Sabouraudia 3 (1): 9 (1963) [MB#353915]
=Trichophyton proliferans M.P. English & Stockdale, Sabouraudia 6: 267 (1968) [MB#340394]

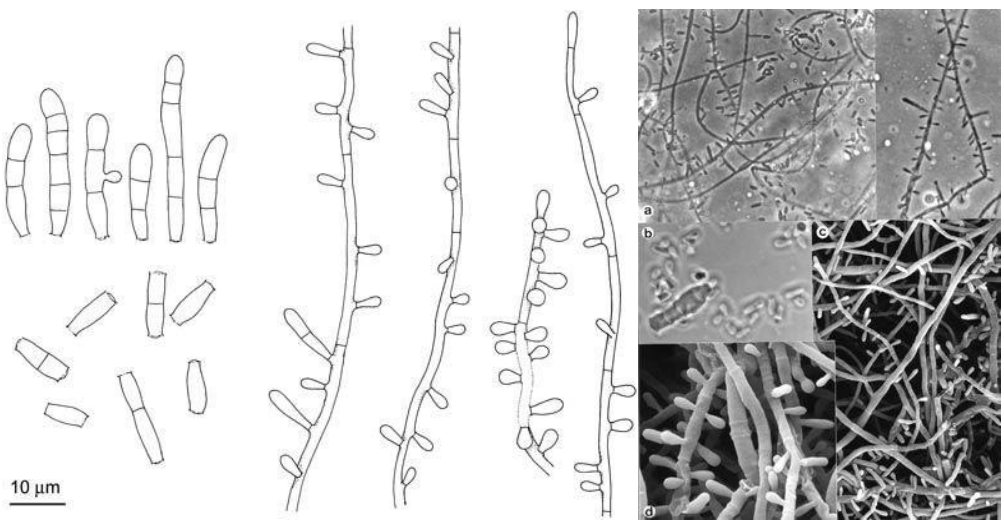
Colonies (SDA) are white, flat, powdery, sometimes downy to fluffy with a brilliant lemon yellow reverse. Numerous large clavate microconidia are borne on the sides of hyphae. Macroconidia are smooth-walled, two- to six-celled, clavate, variable in size, and may have terminal appendages. Macroconidia are much shorter than those seen in *T. mentagrophytes*.



Culture of *T. erinacei* with brilliant lemon yellow reverse pigment., microconidia, mycology online



Trichophyton erinacei www.gla.ac.uk Microconidia and macroconidia www.mycology.adelaide.edu.au



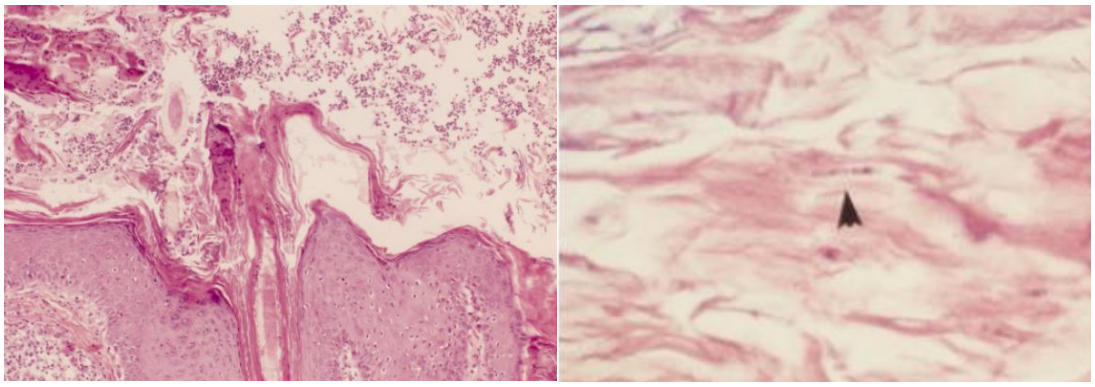
***Trichophyton erinacei*, Mycobank**

Trichophyton erinacei is rarely isolated from dogs with dermatophytosis. It is a zoophilic dermatophyte transmitted by hedgehogs and, in contrast to other dermatophyte species, is characterised by a severe suppurative and inflammatory response known as kerion.

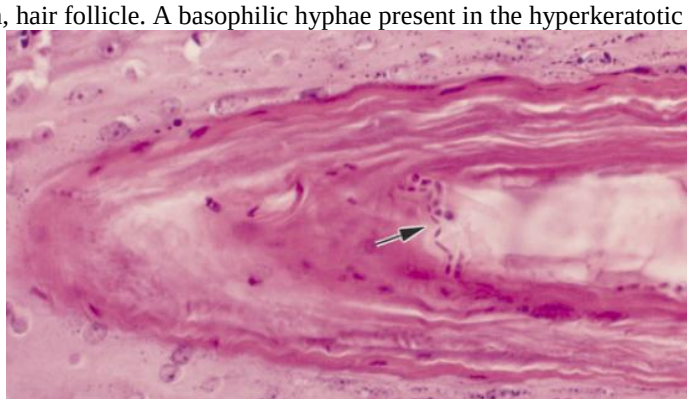
Reports

Fairley (2001) reported a retrospective study of the histological features of four cases of canine ***Trichophyton erinacei*** infection. In all four dogs the initial lesions affected the dorsal muzzle and in two dogs the lesions spread to more distant sites on the body. Clinically, the lesions were characterized by scaling, crusting and hair loss. Histologically, the main lesions were characterized by acanthosis, epidermal, ostial and infundibular hyperkeratosis, serocellular crusting, mural folliculitis and furunculosis. Fungal hyphae were usually sparse and often difficult to see in haematoxylin and

eosin stained sections. When visible they were seen in the epidermal, ostial and infundibular scale and, less frequently, within hair shafts.



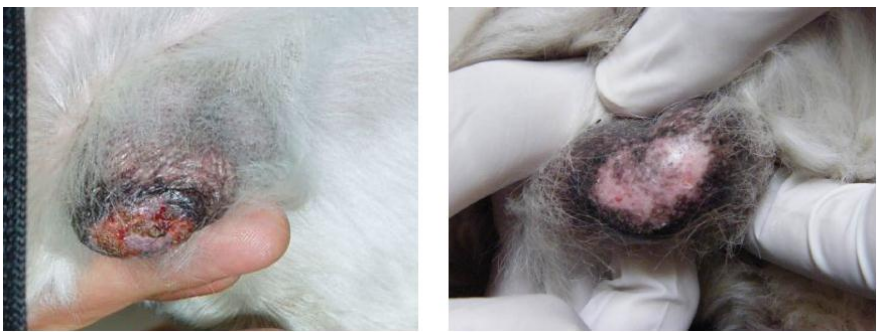
Severely affected nasal skin, with epidermal and infundibular acanthosis and hyperkeratinosis with condensed scale enveloping. Nasal skin, hair follicle. A basophilic hyphae present in the hyperkeratotic scale, **Fairley (2001)**



Nasal skin, hair follicle, basophilic hyphae breaking up into arthrospores in the cornified scale, **Fairley (2001)**

Piérard-Franchimont *et al.* (2008) reported 3 related cases of human dermatophytosis and 1 dog dermatophytosis likely caused by contact with a European hedgehog. ***Trichophyton erinacei*** was isolated from stratum corneum samples. This type of zoophilic dermatophytosis is rare in south-east Belgium and probably in the rest of the country as well.

Kurtdede *et al.* (2014) reported a 5-year-old male mongrel dog a history of a localised pruritic and suppurative alopecic lesion on the scrotum. Routine blood tests, peripheral blood smears, multiple skin scrapings and bacteriological culture did not reveal any abnormalities. However**, *Trichophyton erinacei*** was isolated from the scrapings. The presence of hedgehogs around the daily walking areas of the dog suggested the possibility of direct or indirect contact of the dog with hedgehogs. Fungicidal treatment was implemented with oral itraconazole (5 mg/kg once daily) and topical application of clotrimazole (twice daily) for a month. The scrotal lesion healed completely and hair grew back within a month. No recurrence occurred during a 4 month follow-up. They stressed that T. erinacei should be included in the differential diagnosis of suppurative scrotal skin lesions of dogs, which have come into possible contact with hedgehogs.



Left: A well-circumscribed, painful, circular and suppurative skin lesion on the scrotum with hair loss
Right: Healing of the lesion within 19 days, **Kurtdede *et al.* (2014)**

1.9.7. Trichophyton terrestre

Reports

Aho *et al*. (1987) isolated Trichophyton terrestre from twenty of 276 cats examined (7.2%) in seven catteries. The catteries that gave positive isolations of ***T. terrestre*** were: a) three catteries that bred mainly Persian cats, but also had one or more outdoor-indoor European shorthair cats, b) one cattery that bred European shorthair cats and colorpoint Persians, c) one cattery that bred only European shorthairs, d) one that bred only Persian cats, and e) one cattery that bred four different breeds including European shorthairs as well as Persian cats. The isolation of T. terrestre was significantly more often achieved from European shorthairs than from Persian cats, and from the group of European shorthairs and Persians kept together than from an other breeds. The hairbrush technique was found to be the most reliable method of sampling especially when the cats were asymptomatic. None of the 276 cats examined yielded Microsporum canis. Diluted Sabouraud dextrose agar containing chloramphenicol and cycloheximide was the medium of choice for the isolation of T. terrestre. Of the 21 isolates, three produced creamy white, downy colonies, while 18 developed red-pigmented, granular colonies. Microconidia were numerous. They were 1-celled, cylindric to clavate and were borne singly. Four isolates also produced smoothwalled, cylindric to cigar-shaped, 2–4–celled macroconidia. Spiral hyphae were observed. In addition, three isolated produced Arthroderma-type peridial hyphae but none developed pseudo- or fertile gymnothecia.

Guzman-Chavez *et a*l. (2000) collected two hundred samples from dogs and one hundred from cats by using the MacKenzie's tooth brush technique. They isolated 67 and 90 keratinophilic strains from cats and dogs samples, respectively. The most commonly fungi isolated in pure culture in this study were Chrysosporium spp (25%), followed by ***Trichophyton terrestre*** (22%), Microsporum gypseum (5%), M. canis (4%), as well as mixed cultures like Chrysosporium spp. & M. gypseum (2%) and T. terrestre & T. mentagrophytes (1%). Keratinophilic fungi were found in higher numbers in the cat haircoat (67%) than in the dog's (45%) and the same was true with regard to dermatophytes with 12 isolates out of a 100 samples in cats and 7 Isolates out of 200 samples from dogs. This may represent a health risk for humans in contact with a dermatophyte infected cat or dog.

1.9.8. Trichophyton quinckeanum

In 1879, Smith described five human favus cases, two of which may have been contracted from sick cats. Later in 1957, Von Zezschwitz published a case of cat favus with scutulum on its right ear. No attempt was made to culture the samples from these human and feline cases. During the climax of *T. quinckeanum* endemics in Europe, which lasted from the 1940s until the 1960s, numerous infected mice transmitted this pathogen to humans and domestic animals, including cats. Infections by this fungus can give rise to various clinical presentations; the most well known is the favic type, commonly called “mouse favus” to describe such infections. Photo documentation of cat favus caused by *T. quinckeanum* were presented by Szathmary and La Touche.

1.9.10. Trichophyton tonsurans

Report:

Brilhante *et al.* (2006) reported a 2-year-old female Doberman Pinscher with suspected dermatophytosis. The animal showed a rounded lesion of 3 cm in diameter, patches of scalp hair loss and scaling. The lesion was not inflamed, and it was in the distal portion of the right femoral region of the leg. Direct microscopic examinations of the epidermal scales, using 30% KOH, were negative for mites, but showed hyaline-septated arthroconidiate hyphae suggesting dermatophyte infection. Ectothrix or endothrix parasitism was not observed in the hair. Cultures of the clinical specimens, placed on blood agar, Sabouraud dextrose agar, Sabouraud with chloramphenicol and Mycosel agar, showed a colony which suggested ***T. tonsurans***.

1.9.11. *Epidermophyton floccosum*

Reports:

STENWIG and TAKSDAL (1984) reported the isolation of ***E. floccosum*** from an 8-year-old Boxer bitch with a small skin lesion on the middle of the left flank. The 5x5 cm lesion was characterized by alopecia. There were no crusts, scaling or erythema in the central part of the lesion. In the periphery the hairs were easily removed and the skin was erythematous and crusty. During the previous 3 years the dog had occasionally shown symptoms of pruritus, dry coat and scaling, but the cause of these symptoms was not established. During the same period the dog was treated with B-vitamins and thyroxin. It received antibiotics when the skin lesions was observed. Corticosteroids were given in the same period because the dog had lameness in a hind leg. A skin scraping was cultivated on Sabouraud dextrose agar (SDA) containing yeast extract (5 g 1 -~) and 5 /~g chloramphenicol ml -~, on Mycobiotic agar (MBA) (Difco) containing 5/tg chloramphenicol ml-~ and on blood agar. The SDA and MBA plates were incubated at 30°C for 3 weeks and examined weekly. The blood agar plate was incubated aerobically at 37°C overnight. Direct microscopic examination of samples in 3.6 M KOH revealed septate fungal hyphae. The hyphae were found in close connection with epithelial cells, but were not found within or in close connection with the hairs. The diameter of the hyphae was 1.5-2.0/~m. No chains of arthrospores were observed.

Terreni et al. (1985) isolated *Epidermophyton floccosum* from a lesion of dermatophytosis on a dog with hyperadrenocorticism. This report is the first unequivocally documented case of canine infection due to *Epidermophyton floccosum* in the United States.

1.10. Zoonotic hazard

Ringworm is probably the most common zoonosis of cats and dogs and can be spread by direct contact or by spores in the environment. *Microsporum canis* is the most common cause

of ringworm in cats and dogs and it has been frequently isolated from human cases of tinea capitis and tinea corporis. The infection may be acquired from infected animals with cutaneous lesions but also from asymptomatic carriers or from the environment, as asymptomatic *M. canis* carriers are considered to be a critical factor in the epidemiology of dermatophytosis in human. All other dermatophytes isolated from cats and dogs as M. gypseum, T. mentagrophytrs etc are also of zoonotic importance.

Reports:

Winkler (1970) reported 195 cases of ringworm due to *M. canis* infection (10 men, 46 women, 70 boys, 69 girls) in SW-Finland during a period of 13 years (1955–1968). These figures probably represent only part of the total number of cases. The yearly distribution and seasonal incidence show great variations. The youngest patient was a 5-months-old baby, the oldest a grandfather, 76 of age. The majority of the lesions were on the glabrous skin. In adults the lesions were most often found on the arms, in children on the scalp (in 50%) and face. Some of them tended to kerion formation. Lesions on the scalp were rarely seen in adults (5 cases). Most of the patients acquired the infection from cats or kittens. There was only one dog in this material. Five cases of occupational infection — 3 nurses and 2 laboratory technicians — occurred. Reinfection was seen only once, in a 13-year old boy, infected 1964 and 1967. ***M. canis*** was, as the only species, cultivated from all examined patients and from 36 cats or kittens and from one dog. Fifteen of twenty-one stray-cats caught in the autumn 1967 in Turku were infected with *M. canis.* This fungus was cultivated from dust and soil inside and outside a cellar haunted by cats.

Katoh *et al.* (1991) reported a 19-year-old female student who purchased a puppy from a pet shop four weeks earlier. At the time of her first examination, an annular edematous erythema with adherent scales and vesicles surrounding its margin was seen on the left forearm. On direct examination of the vesicles, fungal elements were detected, and ***Microsporum canis*** was isolated. The puppy was a Pomeranian and was kept in the house at all times. No clinical lesions were seen on the puppy, and the Wood's lamp test was negative. However, *M. canis* was isolated from the animal by the hairbrush method. Symptoms disappeared after the patient was treated topically with terbinafine cream for three weeks. Although the dog received no treatment whatsoever, there was no evidence of the disease on the pet. Results of the hairbrush method performed on the pet two and three weeks later were negative, but, at five weeks, it was again positive. Human infection with *M. canis* from an asymptomatic dog was demonstrated in this case.

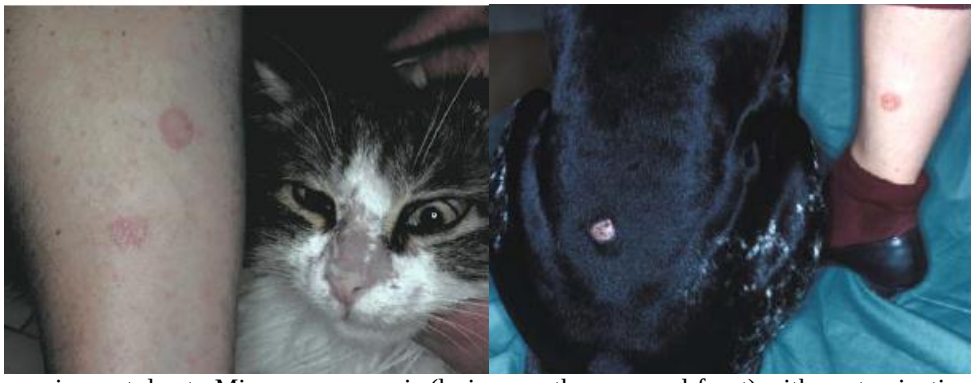
Drusin *et al*. (2000) reported an outbreak of nosocomial ringworm involved five infants in a neonatal intensive care unit. The index case was a nurse infected with Microsporum canis by her cat. After standard infection control measures were initiated, the outbreak was resolved successfully by an interdisciplinary professional collaboration of physician and veterinary dermatologists and infection control personnel.

Cafarchia *et al.* (2006) investigated the relationship between the presence of dermatophytes on the hair coats of dogs and cats without cutaneous lesions and the occurrence of the disease in their respective owners. A total of 136 dogs and 248 cats were sampled from January 1999 to January 2005. Seventy-eight animals (22 dogs and 56 cats) belonged to individuals affected by tinea corporis caused by M. canis and 306 (114 dogs and 192 cats) to individuals without dermatophytosis. Age,

sex, breed, habitat and season were recorded for each animal and examined as potential risk factors. Dermatophytes were isolated from 20.5% of the dogs and 28.2% of the cats. Microsporum canis was isolated from 36.4% of dogs cohabiting with owners diagnosed with tinea corporis but it was never isolated fromdogs whose owners had no lesions. By contrast, M. canis was isolated from 53.6% of cats cohabiting with owners diagnosed with tinea corporis and from 14.6% of cats whose owners had no signs of the disease. These results clearly indicate that both cats and dogs should be considered as a major source of pathogenic dermatophytes for humans even when they do not present clinical signs of dermatophytosis.

Iorio *et al.* (2007) collected 200 hair/skin samples from 2002 to 2004 from two groups of cats (privately owned and stray cats from a shelter) and 165 samples were obtained during the same period from persons in whom dermatophyte infection was highly suspected. The epidemiological data were statistically evaluated. Thirteen of the 100 privately owned cats (13%) and 100% of the stray cats were positive; of the 165 human samples examined 109 (66%) were positive for dermatophytes. *Microsporum canis* was the most common dermatophyte isolated in both cat groups while *Trichophyton mentagrophytes* was the most common in humans. Interestingly, a geophylic dermatophyte species (*Microsporum gypseum*) was found to be present and associated with clinical signs. Living in the countryside proved to be a risk factor for dermatophytoses in privately owned cats while in humans the main risk factor for *M. canis* was contact with animals followed by young age.

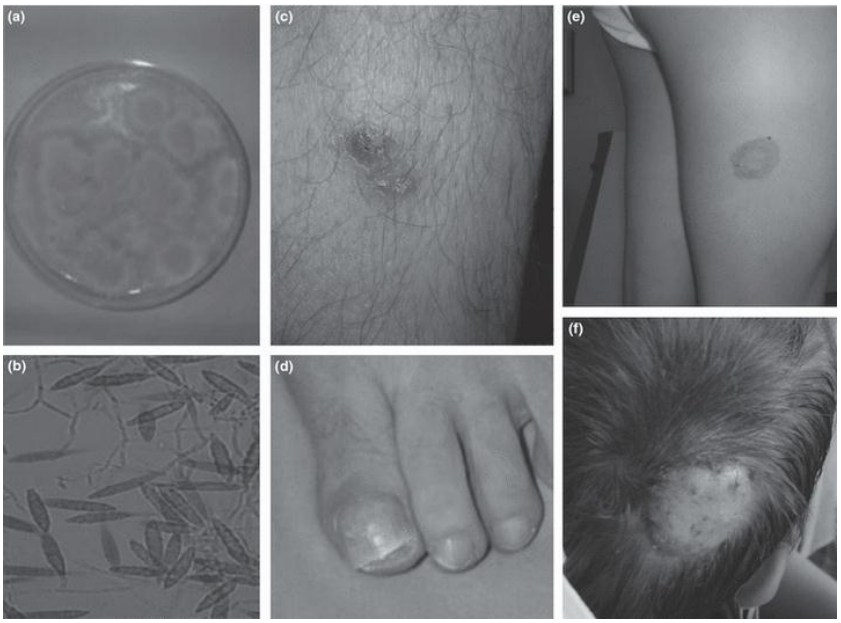
Chermette *et al.* (2008) reported 2 cases of ringworm in cats and dogs with transmission of infection to their owners. In the first case, the cat had lesions on the nose and front and contamination of the owner on her forearm and in the second cases the dog had a typical isolated patchy lesion of dermatophytosis on the back of a dog, and contamination of the owner on her leg. ***Microsporum canis*** was isolated from both animals and their owners a



Ringworm in a cat due to Microsporum canis (lesions on the nose and front) with contamination of the owner on her forearm), Typical isolated patchy lesion of M. canis dermatophytosis on the back of a dog, and contamination of the owner (**Chermette *et al.* (2008)** Parasitologie, Ecole Nationale Ve´te´rinaire d'Alfort)

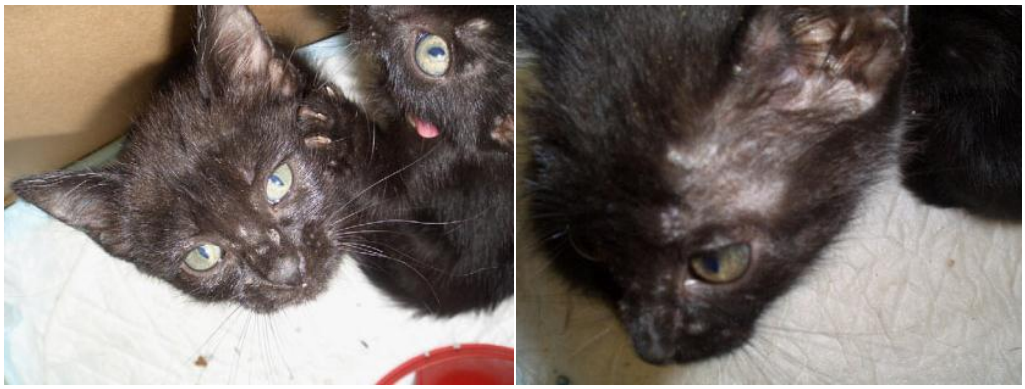
Romano *et al.* (2009) reported 14 cases of dermatophytosis caused by ***Microsporum gypseum***, representing 6.8% of all dermatophytic infections reported, in Siena, Italy, between 2005 and 2006. There were as follows: six cases of tinea corporis, one case of tinea corporis associated with tinea capitis, one case of tinea corporis associated with tinea barbae, one kerion on the head, one tinea cruris, one tinea faciei, one tinea barbae, two onychomycosis. In the three subjects with tinea corporis, the

clinical appearance was impetigo-like, psoriasis-like and pityriasis rosea-like respectively. In six cases, the source of infection was a cat, whereas in the others it was contact with soil.

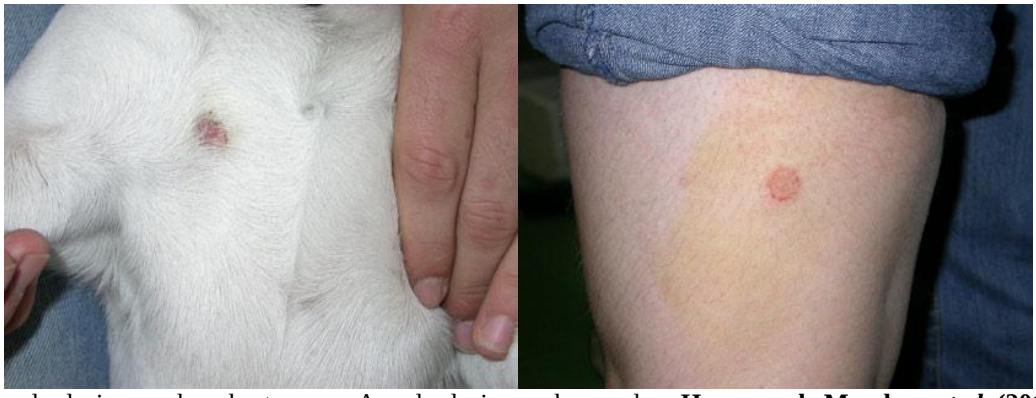


Clinical pictures of infections and *Microsporum gypseum.* (a) Macroscopic appearance of the colony; (b) microscopic view of *M. gypseum*: ellipsoidal macroconidia with four to six septa (Cotton Blue 400×); (c) impetigo-like tinea corporis; (d) onychomycosis; (e) tinea-imbricata-like tinea corporis; (f) kerion, **Romano *et al.* (2009)**

Hermoso de Mendoza *et al.* (2010) described an outbreak of zoonotic ringworm carried by a litter of stray cats. Four veterinary students, four dogs, and six cats living in five separate locations were affected. All had direct or indirect contact with the infected kitten litter. They tried to identify the causal dermatophyte. Microscopic features of scrapings and hairs treated with 20% KOH strongly suggested a ***M. canis*** etiology, and a diagnosis of ringworm was empirically supported by successful treatment of humans and animals. Nevertheless, cultures failed to show the expected morphology.



Kitten facial lesions and dirty poor fur. Infected litter kitten**, Hermoso de Mendoza *et al.* (2010)**



Annular lesion on dog chest Annular lesion on human leg**, Hermoso de Mendoza *et al.* (2010)**

Kaneko *et al*. (2011) presented a 52-year-old female patient with multiple annular erythemas on the trunk and extremities. The patient had a cat with hair loss suggestive of a fungal infection. Culture of the patient's scrapings and the hair of the patient's cat yielded rapid growth of cottony, pale-yellow, colonies on an agar plate. Microscopy revealed a lot of spindle-shaped macroconidia identified as ***M. canis***.

Lower limb of the patient with multiple annular erythema, M. canis colony,Macroconidia, **Kaneko *et al*. (2011)**

López *et al.* (2012) examined 45 samples from cats with and without dermatological lesions. These samples were collected through skin scraping, hair removal and Mackenzie brush, respectively. The frequency of dermatophytes isolated in this preliminary study was 13.3%. There were not statistically significant differences by source, age, sex, race or dermatological condition. Zoonotic dermatophytes were found in 2 household cats out of the 21 that had direct contact with children or the elderly. ***M. canis*** was isolated in 83.3% cases.

Frymus *et al.* (2013) stated that, an infected cat represents a notable zoonotic hazard. Although individual *M. canis*-infected cats are certainly capable of disseminating the infection to human beings in the same household, the cat population in general is often incriminated as a reservoir of human infection.

Segundo *et al.* (2004) studied cases ringworm in man animals from January 1994 to December 2002. A total of 46 clinical cases of M. canis infections were recorded, 26 female and 20 males: tinea capitis 21, tinea corporis 17, tinea pedis five, onychomycosis two, and only one case with tinea faciei. The 46 cases with positive culture yield 42 positive samples in KOH. Six out of 461 dogs were KOH positive (1%) and 23 (4.98%) were culture positive: 21 M. canis, one M. gypseum and one

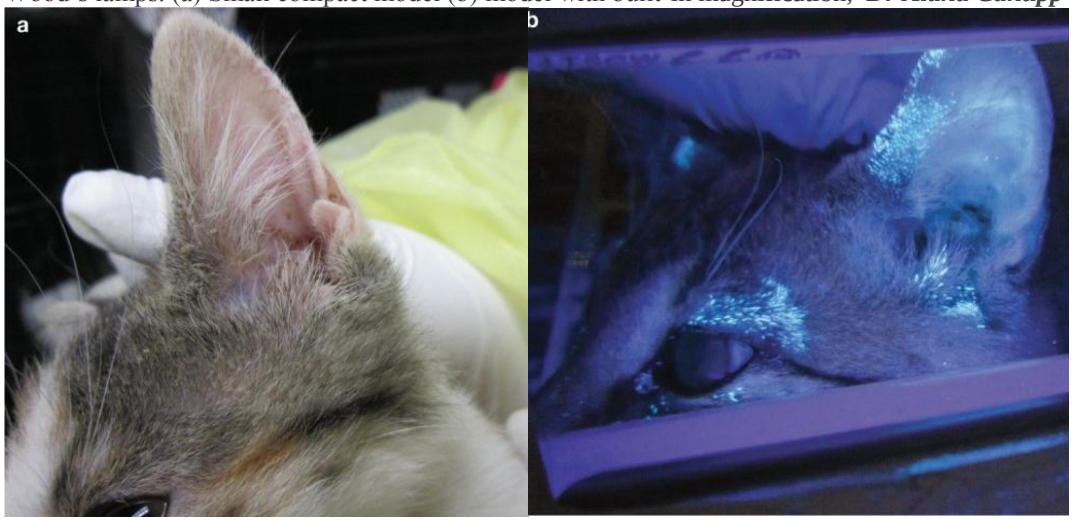
Trichophyton spp. From the 68 samples of cats, eight (11.76%) were positive to KOH and 26 (38.23%) were positive for M. canis isolates.

1.11. Diagnosis:

1.11.1. Wood's lamp examination

- An inexpensive and simple screening tool for *M canis* infection.
- it is not very sensitive: only about 50% of *M canis* strains fluoresce and other dermatophytes do not fluoresce at all.
- debris, scale, lint and topical medications (eg, tetracycline) can produce false-positive results.
- Wood's lamp findings should be confirmed by other methods.

Wood's lamps. (a) Small compact model (b) model with built-in magnification, ***Dr Alana Canupp***

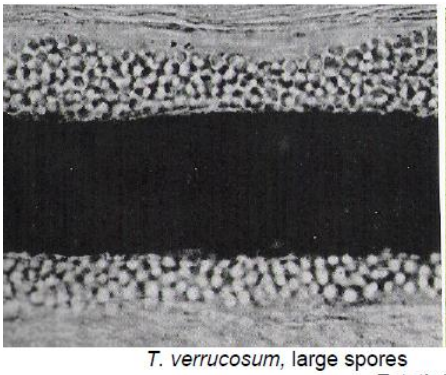


Ear of a cat with dermatophytosis. Note the limited lesion extent observed in room light (a) versus how, under Wood's lamp examination (b), the extent of the lesions is highlighted. ***Dr Alana Canupp***

1.11.2. Direct microscopic examination

- It is recommended to pluck hairs for this purpose under Wood's lamp illumination, or from the edge of a lesion.
- The sample should be cleared with 10–20% potassium hydroxide solution before examination.

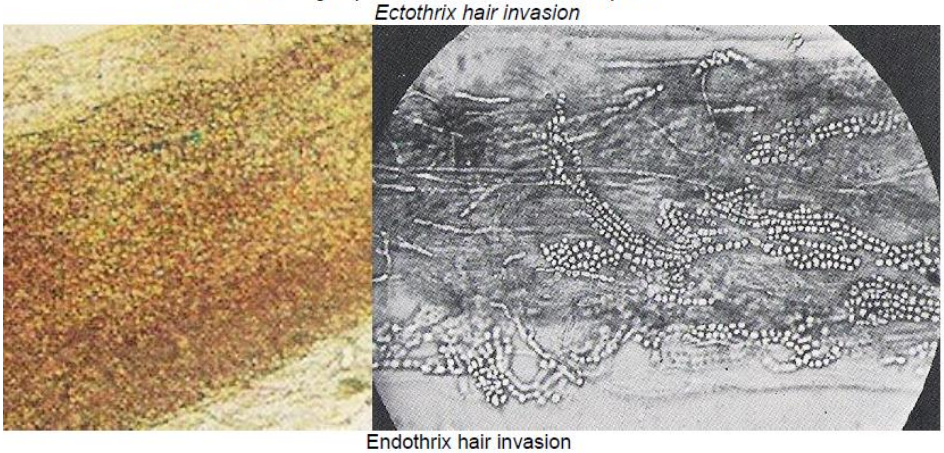
- direct microscopic examination may give false-positive results, especially if saprophytic fungal spores are present or debris is interpreted as fungal elements
- sensitivity of this technique is relatively poor and has been assessed as 59%.
- higher sensitivity (76%) has been achieved by fluorescence microscopy with calcafluor white – a special fluorescent stain that binds strongly to structures containing cellulose and chitin.



T. verrucosum, large spores

M. canis, small spores

Ectothrix hair invasion

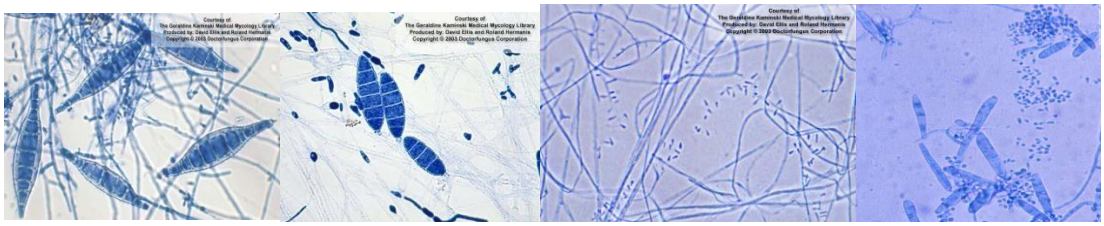


Endothrix hair invasion

1.11.3. Culture on Sabouraud dextrose agar

Part of the samples is embedded into Sabouraud dextrose agar with chloramphenicol and actidione then incubated at 30^{o}C for 1-4 weeks. Identification characters include colony texture, pigmentation, growth rate and distinctive morphological structures such as macroconidia, microconidia, spirals, chlamydospores, etc.

Colonies od dermatopytes on Sabouraud dextrose agar



Microscopic features of dermatophytes

1.11.4. Molecular diagnosis: (Ziółkowska *et al*., 2015)

a. Polymerase chain reaction (ITS-PCR)

- Amplification is carried out on one of the conserved regions of the genome containing a fragment of the rDNA gene (including parts of 18S and 28S rDNA, as well as the whole of ITS1, 5,8S rDNA and ITS2).
- PCR is conducted using the universal primers

  ITS 1: 5′-TCCGTAGGTGAACCTGCGG-3′

  ITS4: 5′-TCCTCCGCTTATTGATATGC-3′.
- ITS-PCR is carried out in a T Personal thermal cycler (Biometra GmbH, Goettingen, Germany), with 25 µl of reaction mixture composed of 12.5 µl Qiagen Taq PCR Master Mix (2.5 U Taq DNA Polymerase, 200 µmol of each nucleotide and 1.5 mmol l^{-1} $MgCl_2$) (Qiagen, Hilden, Germany), 10 pmol of each primer (Genomed S.A, Warsaw, Poland) and 1 µl of DNA template.
- The thermal cycler reaction conditions :

  initial cycle at 95 °C for 3 min, followed by 30 cycles at 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min and then an extension cycle of 72 °C for 10 min.
- Electrophoretic separation of PCR products is carried out in 2% agarose in 1xTBE buffer (Tris Borate EDTA buffer) (Sigma-Aldrich, Seelze, Germany). The gels are documented and analysed in GelDoc 2000 (BIO-RAD, Hercules, California, USA).

b. Restriction fragment length polymorphism of the ITS region (ITS-RFLP)

- Restriction analysis of the PCR product is carried out using four enzymes, *Mva*I, *Hinf*I, *Hha*I and *Eco*RI (ThermoScientific®, Waltham, USA) at 37 °C for 60 min in 20 µl of a reaction mixture containing 8 µl PCR product, 6U endonuclease, 2 µl reaction buffer and 10 µl water (Sigma-Aldrich).
- The digestion products are subjected to electrophoresis in 6% polyacrylamide gel.
- The size of the ITS-RFLP restriction fragments is analysed using BIO-GENE 11.01 software (Vilber-Lourmat, Paris, France).
- The ITS size profiles obtained for each of the clinical isolates following digestion with endonucleases areconverted to a binary matrix.
- The similarity between the ITS patterns is calculated using Nei and Li's algorithm, and strains were grouped by UPGMA on the basis of the DNA patterns obtained.

- Computer programs are used in these stages of the analysis: FENAL 1.0 beta and NTSYS-pc 2.02 g.

i. Determination of ITS sequences

- The ITS sequencing reaction is carried out using a BigDye® Terminator Cycle Sequencing Kit (Life Technologies, Carlsbad, California, USA) and the primers ITS1 and ITS4.
- The PCR mixture (10 µl) contains the following:
  2 µl 2.5× concentrated Ready Reaction Premix, 1 µl 5× concentrated BigDye Sequencing Buffer, 0.25 µl primer at a concentration of 5 pmol (initially 100 pmol), DNA amplicon at a concentration of 50 ng and sterile distilled water at a final volume of 10 µl.
- Two separate reactions are carried out for primers ITS1 and ITS4.
- PCR is performed in a T Personal cycler (Biometra GmbH) with the following conditions:
  initial denaturation for 1 min at 96 °C, denaturation for 10 s at 96 °C, annealing of primers for 5 s at 50 °C and elongation for 4 min 60 °C. The final three stages, i.e. denaturation, annealing of primers and elongation, are repeated 25 times.
- The PCR product is purified using an ExTerminator kit (A&A Biotechnology, Gdynia, Poland), and then the DNA sequence was read in a 3500 Genetic Analyser from Life Technologies, Carlsbad, California, USA.

ii. Phylogenetic analysis of ITS sequences

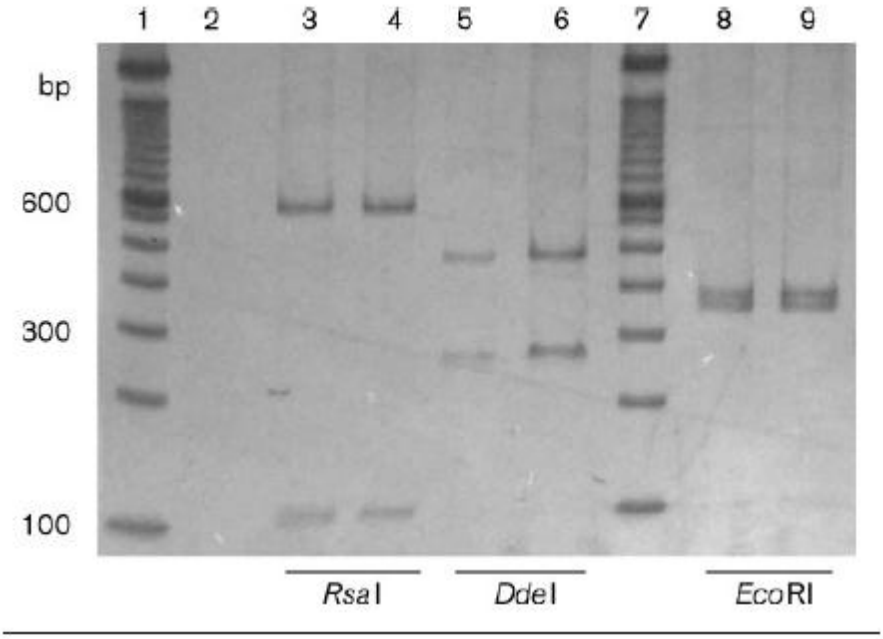
- Taxonomic identification based on ITS sequences is performed using Nucleotide Archive, available in the databases.
- The phylogenetic analysis is based on the ITS sequences of the clinical isolates and dermatophytes representing known genera and species, taken from databases.
- he ITS nucleotide sequences included in the analysis are compared in pairs and the degree of similarity is determined using ClustalX software.
- A phylogenetic tree is constructed using the maximum likelihood (ML) method.

Reports:

Brilhante *et al.* (2006) reported a 2-year-old female Doberman Pinscher with suspected dermatophytosis. The animal showed a rounded lesion of 3 cm in diameter, patches of scalp hair loss and scaling. The lesion was not inflamed, and it was in the distal portion of the right femoral region of the leg. Direct microscopic examinations of the epidermal scales, using 30% KOH, were negative for mites, but showed hyaline-septated arthroconidiate hyphae suggesting dermatophyte infection. Ectothrix or endothrix parasitism was not observed in the hair. Cultures of the clinical specimens, placed on blood agar, Sabouraud dextrose agar, Sabouraud with chloramphenicol and Mycosel agar, showed a colony which suggested ***T. tonsurans***.

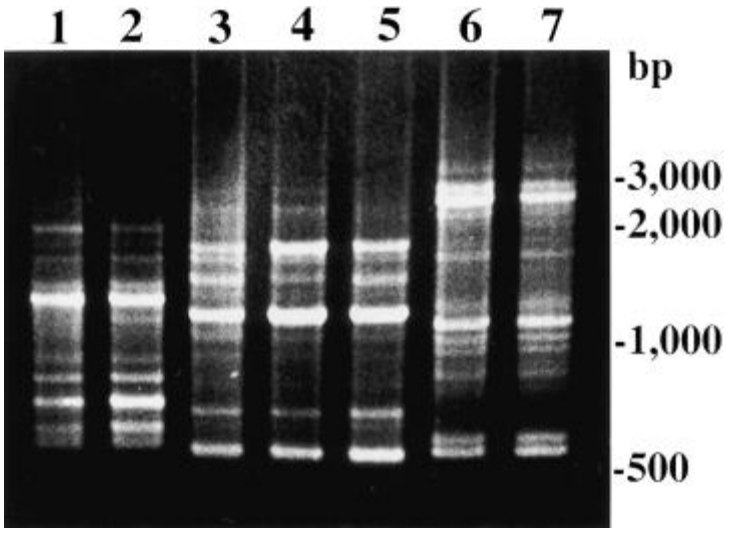
The PCR assay detected an amplicon of approximately 720 bp. The Sau3A-digested product consisted of four fragments of 45, 60, 280 and 335 bp. DdeI, RsaI and EcoRI cut the amplicon regions into two fragments with the following lengths: 120 and 600

bp for RsaI; 280 and 440 bp for DdeI; 380 and 330 bp for EcoRI. The results obtained from the animal strain and from the human strain were similar to the expected digestion pattern of the ITS sequences for T. tonsurans strains registered in GenBank. The results obtained from the PCR assay with T. mentagrophytes and with T. tonsurans strains were similar.



PCR-enzyme restriction patterns in PAGE (6% gel) of a human control strain of T. tonsurans (lanes 4, 6 and 9) and the T. tonsurans strain obtained from a dog (lanes 3, 5 and 8). A 100 bp ladder was used to estimate the product sizes (lanes 1 and 7). Lane 2 is a negative (no template) control

Kano *et al.* (2001) reported a 1- to 2-month-old female cross-breed cat presented with alopecia, erythema and many crusts on the tail. Microscopic examination of crusts from the tail disclosed epithelial debris, exudate, mycelium, and arthrospores. ***Microsporum gypseum*** was cultured from the crusts on a Sabouraud glucose agar at 27°C for 1 week. The isolate of *M. gypseum* from the cat was examined by **random amplification of polymorphic DNA (RAPD), chitin synthase 1 gene (CHS1) sequence** and mating experiments. The RAPD band patterns of the clinical isolate of *M. gypseum* was identical to those of tester strains of *Arthroderma gypseum*. Nucleotide sequence analysis of the CHS1 gene fragments from the isolate and a tester strain of *A. gypseum* showed 100% similarity. The mating experiments on the clinical isolate of *M. gypseum* completely agreed with the results from RAPD and CHS1 gene sequence. The isolate from the cat was confirmed to be *A. gypseum* (–) mating type, which was consistent with the result of mycological examination by molecular analyses

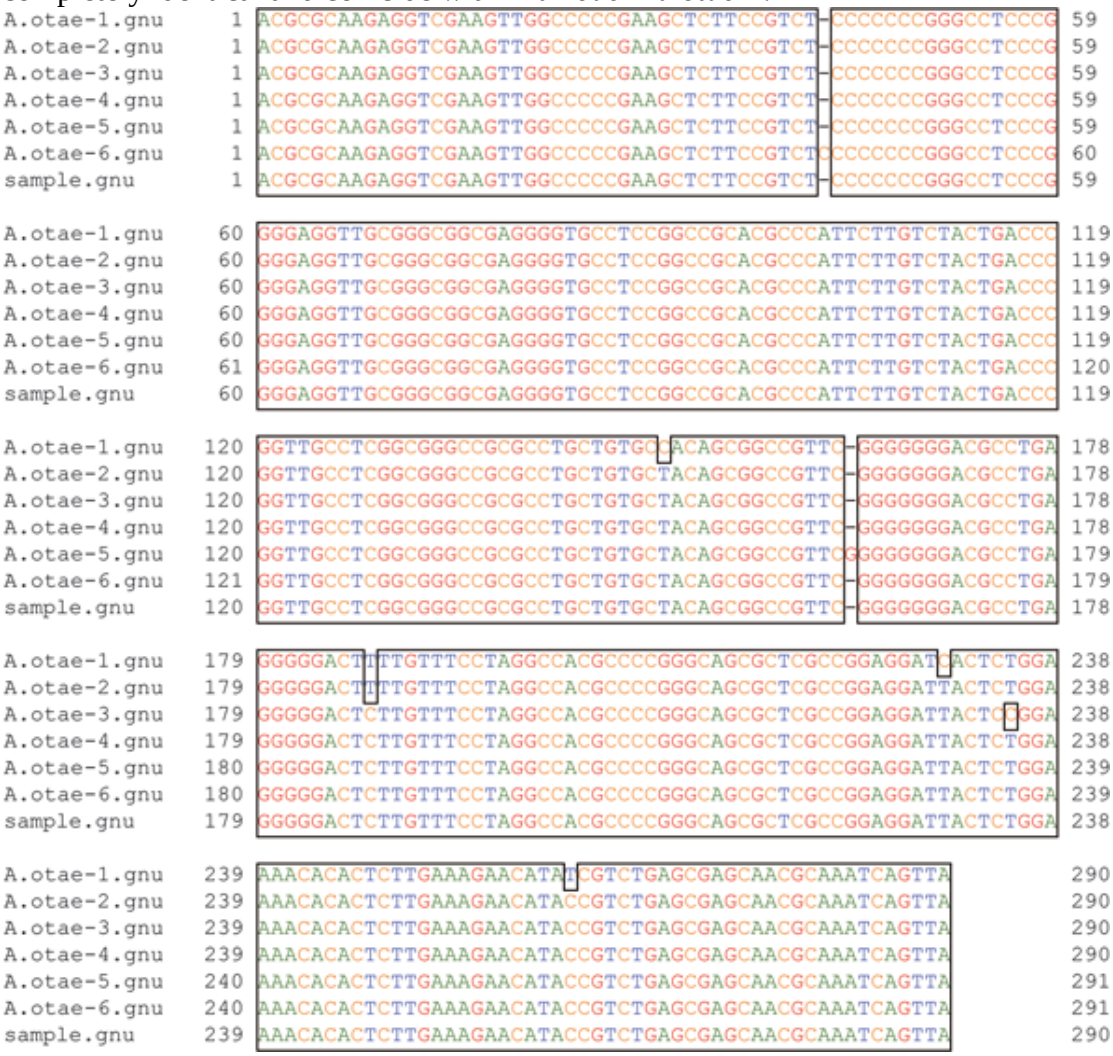


RAPD patterns of the tester strains of *A. fulvum* (VUT-4006 and VUT-4007) *A. gypseum* (VUT-4004 and VUT-4005), *A. incurvatum* (VUT-4002 and VUT-4003), and a clinical isolate of *M. gypseum* (VUT-99011). Lanes: 1, VUT-4006; 2, VUT-4007; 3, VUT-4004; 4,VUT-4005; 5, VUT-96011; 6, VUT-4002; 7, VUT-4003. The genomic DNA samples were amplified with a 21-mer primer (FM1).

Sharma et al. (2007) developed two microsatellite markers were and used them to analyse a global set of 101 ***M. canis*** strains to reveal patterns of genetic variation and dispersal. Using a Bayesian and a distance approach for structuring the ***M. canis*** samples, three populations could be distinguished, with evidence of recombination in one of them (III). This population contained 44 % of the animal isolates and only 9 % of the human strains. Population I, with strictly clonal reproduction (comprising a single multilocus genotype), contained 74 % of the global collection of strains from humans, but only 23 % of the animal strains. From these findings, it was concluded that population differentiation in ***M. canis*** is not allopatric, but rather is due to the emergence of a (virulent) genotype that has a high potential to infect the human host. Adaptation of genotypes resulting in a particular clinical manifestation was not evident. Furthermore, isolates from horses did not show a monophyletic clustering.

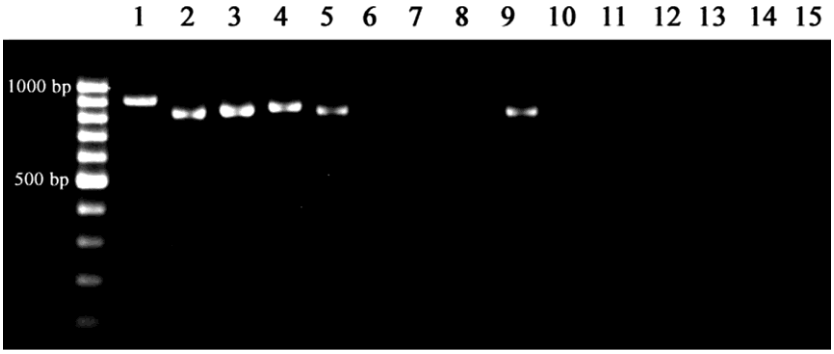
Kaneko *et al*. (2011) verified that the ITS1 sequences of the fungal species isolated from a patient and his cat were completely identical and coincided with *A. otae*-4. They emphasized that, the molecular analysis provided useful information that not only *M. canis* was the same pathogen, but also the same fungal strain involved in both infections. In their study, they used the oligonucleotide primers for ITS1-specific PCR previously designed by Makimura (Makimura K *et al*. *J Clin Microbiol* 1998; **36**: 2629–33) were as follows: for 18 SF1, 5′-AGGTTTCCGTAGGTGAACCT-3′; and for 58 SR1, 5′-TTCGCTGCGTTCTTCATCGA-3′. PCR was performed under the following conditions: 25 cycles at 94 °C for 1 min, 60 °C for 15 s, and 72 °C for 15 s. Thermal cycling was terminated by polymerisation at 72 °C for 10 min. The products were then stained and visualised by UV irradiation. Both strands of the PCR products were directly sequenced with primers – 18 SF1 and 58 SR1. The ITS1 sequences of *M. canis* isolated from the patient and cat were aligned by using the CLUSTAL W computer program and the GENETYX-MAC 10 software, respectively. We verified that the ITS1 sequences of these species were completely identical and coincided with *A. otae* ITS1 genotype 4. Alignments indicated that the internal transcribed

spacer 1 sequences of *Microsporum canis* isolated from the patient and cat were completely identical and coincide with *Arthroderma otae*-4.

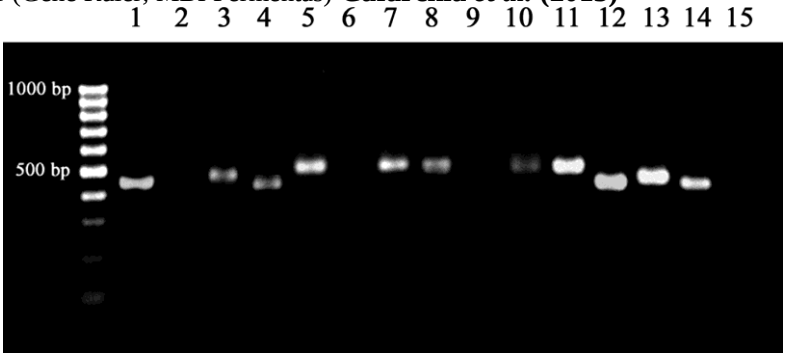


Alignments indicating that the internal transcribed spacer 1 sequences of *Microsporum canis* isolated from the patient and cat are completely identical and coincide with *Arthroderma otae*-4. **Kaneko *et al.* (2011)**

Cafarchia *et al.* (2013) established and evaluated a PCR-based approach employing genetic markers of nuclear DNA for the specific detection of dermatophytes on such specimens. Using 183 hair samples, they directly compared the test results of one-step and nested-PCR assays with those based on conventional microscopy and in vitro culture techniques (using the latter as the reference method). The one step-PCR was highly accurate (AUC > 90) for the testing of samples from dogs, but only moderately accurate (AUC = 78.6) for cats. A nested-PCR was accurate (AUC = 93.6) for samples from cats, and achieved higher specificity (94.1 and 94.4%) and sensitivity (100 and 94.9%) for samples from dogs and cats, respectively. In addition, the nested-PCR allowed the differentiation of *Microsporum canis* from *Trichophyton interdigitale* and *Microsporum gypseum* or *Trichophyton terrestre*, which was not possible using the one step-assay. The PCRs evaluated here provide practical tools for diagnostic applications to support clinicians in initiating prompt and targeted chemotherapy of dermatophytoses and culture techniques.



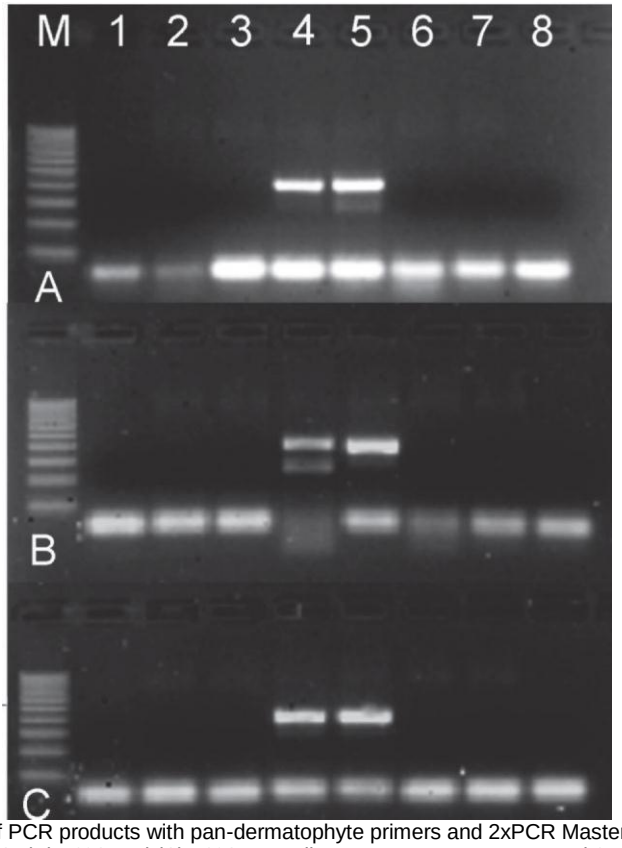
Results of PCR amplification of ITS + from genomic DNA samples carried out using primers DMTF18SF1 and DMTF28SR1.*Microsporum canis, M. fulvum, M. gypseum, Trichophyton interdigitale* (zoophilic), *T. terrestre* (lanes 1–5), species of*Alternaria, Aspergillus, Cladosporium* (lanes 6–8), *Chrysosporium*(lane 9), *Malassezia, Mucor, Penicillium, Rhizopus, Scopularopsis*(lanes 10–14) and no-DNA control (lane 15). Amplicons were sized by comparison with a 100 bp ladder (Gene Ruler, MBI Fermentas) **Cafarchia *et al.* (2013)**



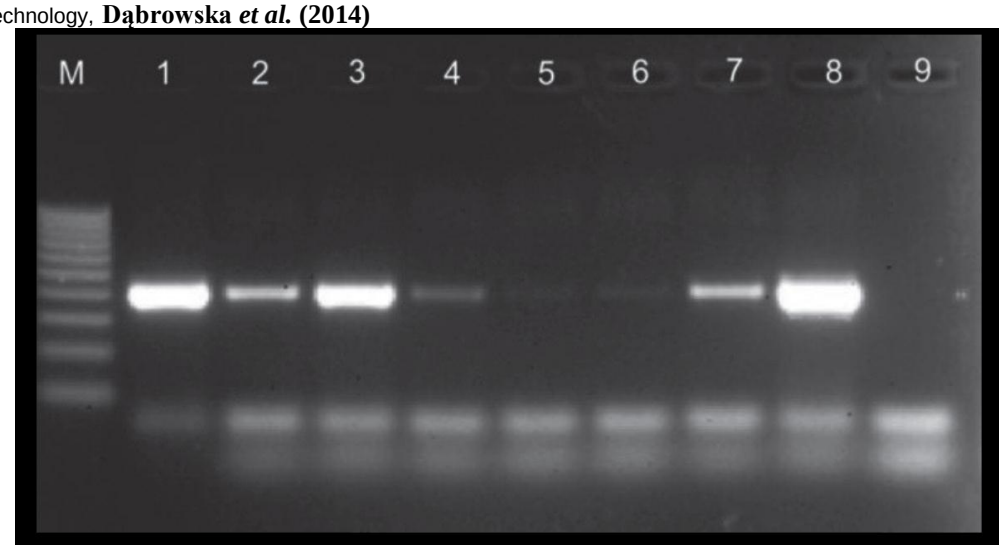
Selected results of the nested-PCR testing of genomic DNAs from hair samples of dogs or cats (lanes 1–10), as well as from *M. canis, M. gypseum, T. interdigitale* (zoophilic) and *T. terrestre*and no-DNA control samples (lanes 11–15, respectively). Amplicons were sized by comparison with a 100 bp ladder (Gene Ruler, MBI Fermentas). **Cafarchia *et al.* (2013)**

da Costa *et al*. (2013) investigated the genetic variability of *M. canis* isolates from different animal species using two microsatellite markers, namely, McGT(13) and McGT(17). The study included a global set of 102 *M. canis* strains, including 37 symptomatic cats, 35 asymptomatic cats, 19 human patients with tinea, 9 asymptomatic dogs and 2 symptomatic dogs. A total of 14 genotypes were identified, and 6 large populations were distinguished. There was no correlation between these multilocus genotypes and the clinical and epidemiological data, including the source, symptomatology, clinical picture, breed, age, sex, living conditions and geographic location. These results demonstrated that the use of microsatellite polymorphisms is a reliable method for the differentiation of *M. canis* strains

Dąbrowska *et al.* (2014) applied a method of extraction of fungal DNA (Brillowska-Dabrowska and coworkers (2007) and PCR amplification with pan-dermatophyte primers for (5'GAAGAAGATTGTCGTTTGCATCGTCTC3') and panDerm_rev (5'CTCGAGGTCAAAAGCACGCCAGAG3') to confirm the presence of dermatophytes.The time-temperature profile for PCR was; initial denaturation for 3 min at 95°C followed by 45 s at 94°C, 45 s at 54°C or 56°C or 58°C and finally 45 s at 72°C for a total of 35 cycles. The presence of a specific PCR product of approximately 366 bp was determined by electrophoresis on a 2% agarose gel containing ethidium bromide. PCR assay confirmed correct identification of strains as dermatophytes, i.e. seven representatives of the *Trichophyton mentagophytes* complex strains and eight *Microsporum canis.*



Electrophoretic patterns of PCR products with pan-dermatophyte primers and 2xPCR Master Mix Plus High GC (A&A Biotechnology) at: (**A**) 54°C, (**B**) 56°C and (**C**) 58°C annealing temperature. Lanes 1 and 8: negative control with water, lines 2 and 3: *Microsporum canis*, lines 4–7: *Trichophyton mentagrophytes*, line M: 100–1000 bp Ladder (A&A Biotechnology, **Dąbrowska *et al.* (2014)**



Electrophoretic patterns of PCR with pandermatophyte primers and at 58°C annealing temperature and homemade PCR mix. Lanes 1 and 8: *Trichophyton mentagrophytes,* lines 2–7: *Microsporum canis,* line 9 negative control with water, line M: 100–1000 bp Ladder (A&A Biotechnology). **Dąbrowska *et al.* (2014)**

Ziółkowska *et al.* (2015) conducted a study on 24 isolates recovered from humans and various animal species with clinical symptoms of dermatophytosis. The analysis included phenotypical identification methods and molecular methods: internal transcribed spacer sequencing and ITS-restriction fragment length polymorphism (RFLP) with multi-enzyme restriction. ITS sequence analysis identified the isolates to species - Trichophyton interdigitale, Arthroderma benhamiae and A.

vanbreuseghemii, and ITS-RFLP detected six different genotypes. Genotypes I, II and III characterised strains belonging to A. benhamiae, genotype IV characterised the A. vanbreuseghemii strain, and genotypes V and VI occurred only within the species T. interdigitale. Strains isolated from guinea pigs were dominant within genotype I, while genotype II was found mainly in strains from foxes. Multi-enzyme restriction analysis of this region enables intraspecific differentiation, which may be useful in epidemiological research, particularly in determining the source of infections.

1.12. Treatment of ringworm in cats and dogs

- In immunocompetent cats, isolated lesions disappear spontaneously after 1–3 months and may not require medication. However, treatment of such cases will reduce the disease course as well as the risk for other animals and humans, and contamination of the environment.
- Topical treatment is generally less effective in cats compared with humans due to poor penetration of the medicines through the hair coat, lack of tolerance of this treatment by many cats and the possible existence of unnoticed small lesions.
- therapeutic measures should include a combination of systemic and topical treatment, maintained for at least 10 weeks. Generally,
- cats should be treated not only until the lesions completely disappear, but until the dermatophyte can no longer be cultured from the hairs on at least two sequential brushings 1–3 weeks apart.

1.12.1. Topical therapy

- In cats with a limited number of lesions, hairs should be clipped away from the periphery of lesions incorporating a wide margin. Clipping should be gentle to avoid spreading the infection due to microtrauma.
- Spot treatment of lesions may be of limited efficacy; instead, whole body shampooing, dipping or rinsing is recommended.
- In patients with generalised disease, longhaired cats and for cattery decontamination, clipping the entire cat is useful to make topical therapy application easier and to allow for better penetration of the drug.
- Topical whole body treatment with a 0.2% enilconazole solution performed twice weekly. or 2% miconazole with or without 2% chlorhexidine as a twice weekly body rinse or shampoo.

1.12.2. Systemic therapy

Itraconazole

- itraconazole is currently the preferred drug in feline dermatophytosis and is licensed for this indication
- A pulse administration of 5 mg/kg/day for 1 week, every 2 weeks for 6 weeks has been suggested.

- three cycles of treatment consisting of 1 week with treatment (5 mg/kg) and 1 week without. Such a treatment schedule (3 x 7 days of dosing) provides actual coverage of at least 7 weeks.

Terbinafine

- An alternative is terbinafine administered orally 30–40 mg/kg once daily.
- It seems also suitable for pulse therapy.
- Occasional vomiting and intensive facial pruritus has been observed as side effects.

Ketoconazole

- Ketoconazole has been used orally at 2.5–5 mg/kg twice daily.
- cats are relatively susceptible to side effects with this drug, which include liver toxicity, anorexia, vomiting, diarrhoea and suppression of steroid hormone synthesis.
- Ketoconazole is also contraindicated in pregnant animals.

Griseofulvin

- It is administered orally for at least 4–6 weeks at 25–50 mg/kg q12–24h.
- Griseofulvin is poorly soluble in water and micronised formulation as well as administration with fatty meals enhance absorption.
- Adverse reactions include anorexia, vomiting, diarrhoea and bone marrow suppression, particularly in Siamese,
- The use of griseofulvin is contraindicated in kittens younger than 6 weeks of age and in pregnant animals as the compound is teratogenic, particularly during the first weeks of gestation.

Reports:

Angarano and Scott (1987) used Ketoconazole, an antifungal imidazole derivative , to treat Tricophyton mentagrophytes infection in a dog. The drug was administered orally (11 mg/kg of body weight, q 24 h) and continued for 90 days. Though ketoconazole is not licensed currently for veterinary purposes, it has been used successfully to treat dermatophyte infections as well as intermediate and deep fungal diseases in both dogs and cats. In this case, ketoconazole was found to be nontoxic and less expensive than griseofulvin in the treatment of dermatophytosis.

Carlotti *et al.* (2010) treated enzootic dermatophytosis in a shelter with approximately 140 cats according to a protocol combining identification, isolation and treatment of subclinical carrier and affected animals in accordance with a three-area system: healthy animals (no lesions and negative cultures), subclinical carrier animals (no lesions but with positive cultures) and clinically affected animals (lesions and positive cultures). The cats were examined and inspected under a Wood's lamp and had samples taken for fungal culture every 2 weeks. Thirty-three per cent of the cats had a positive fungal culture at the start of the study. Clinically affected animals and carriers were treated with a 0.2% enilconazole lotion (Imaverol) twice a week and given itraconazole (Itrafungol) 5 mg/kg SID orally every other week. The environment was treated once a day with a 1% bleach solution and once a week with a 0.6% enilconazole (Clinafarm) solution. Treated animals were considered cured after

two consecutive negative fungal cultures. All cats were cured within 56 days. Prophylactic measures against dermatophytosis were implemented for new arrivals consisting of individual quarantine and the systematic taking of fungal cultures. No relapses were observed based on the fungal cultures taken from the animals and the environment over the first 10 months.

Nardoni *et al.* (2013) evaluated the occurrence of infection by *Microsporum gypseum* retrospectively in dermatological specimens from 15,684 dogs and cats dermatologically diseased from Italy. Clinical outcome after treatment with griseofulvin combined with topical enilconazole was evaluated in 41 dogs and, out of label, 10 cats. Furthermore, in vitro susceptibility to griseofulvin and enilconazole was evaluated on 31 clinical isolates of *Microsporum gypseum*. One hundred and eighty-five specimens out of 15,684 (1.1%) scored positive for *Microsporum gypseum*. The treatment failed to achieve both mycological and clinical cure in 16 dogs (39%) and four cats (40%), as well as fungal isolates demonstrated a very poor in vitro sensitivity when tested versus griseofulvin: the MIC value was 150 μg/mL. The ED50 value was calculated at 66 μg/mL. They concluded that blind treatments with griseofulvin in ringworm due to *Microsporum gypseum* should be avoided.

Sánchez *et al.* (2014) presented three clinical cases of canine dermatophytosis resolved with topical propolis treatment that involved alopecia and well-demarcated erythematous lesions. These cases were positively identified by direct observation of samples from the affected zones with 10% KOH. Each sample was cultured, leading to the isolation of *Microsporum gypseum* in one case and *Microsporum canis* in the other two cases. The animals' subsequent treatment included bathing using a commercial soap with propolis every seven days for 3 to 8 weeks, as well as the use of a self-prepared propoliscontaining ointment, which was applied to the lesions once a day for three weeks. From the second week of treatment, all cultures were negative. At the end of treatment, all cases displayed full recovery of the injuries and hair growth in these areas. In these clinical cases, treatment with propolis was effective, supporting the use of propolis as a promising natural alternative with no known collateral effects.

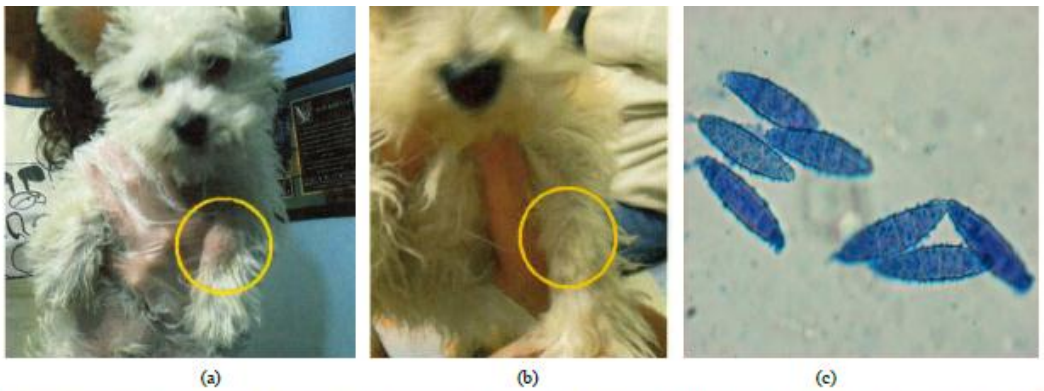
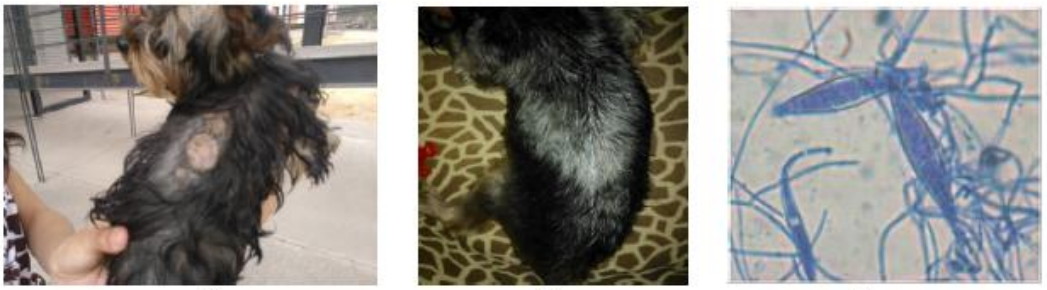


Figure 1. Case 1, Creole. (a) Alopecic lesion and erythematous left forelimb (yellow circle); (b) Left forelimb of patient without Lesion after three weeks of treatment; (c) *Microsporum gyseum* isolated from this clinical case (lactophenol cotton blue staining, 40×).

Sánchez *et al.* (2014)

(a) (b) (c)

Figure 2. Case 2, Boxer. (a) Skin lesions covering almost the entire head; (b) Patient without lesion after three weeks of treat ment; (c) *Microsporum canis* isolated from this clinical case (lactophenol cotton blue staining, 40×).



(a) (b) (c)

Figure 3. Case 3, Yorkshire. (a) Alopecic lesion and erthematous patches; (b) Patient without lesion after eight weeks of treatment; (c) *Microsporu canis* isolated from this clinical case (lactophenol cotton blue staining, 40×).

Sánchez *et al.* (2014)

Newbury *et al.* (2015) identified an endemic ***Microsporum canis*** dermatophytosis in a large, open admission, private, no-kill shelter that admitted >1200 cats per year. Fungal culture (FC) screening revealed that 166/210 (79%) and 38/99 (38%) cats in the non-public and public area were culture positive, respectively. However, pending screening FC results, the 99 cats in the public area were treated with once-weekly lime sulfur rinses and monitored with once-weekly FC. Cats in the non-public area were not treated. When FC results were available, cats were separated into low-risk (n = 61) and high-risk (n = 38) groups based upon the presence or absence of skin lesions. Low-risk cats continued to receive once-weekly topical lime sulfur and rapidly achieved culture-negative status. High-risk cats were divided into two groups based upon the number of colony-forming units/plate (low or high). All 38 cats were treated with twice-weekly lime sulfur and oral terbinafine and within 6-7 weeks only 5/38 cats were still FC-positive. These cats were moved to a separate room. Dermatophytosis was eradicated within 5 months; eradication was prolonged owing to reintroduction of disease into the remaining room of cats under treatment from three kittens returning from foster care. Continued admissions and adoptions were possible by the institution of intake procedures that specifically included careful Wood's lamp examination to identify high-risk cats and use of a 'clean break strategy'.

Moriello (2016) evaluated the antifungal efficacy of shampoo formulations of ketoconazole, miconazole or climbazole and accelerated hydrogen peroxide wash/rinse against Microsporum canis and Trichophyton species spores. Lime sulfur (1:16)-treated control, enilconazole (1:100)-treated control, accelerated hydrogen peroxide (AHP 7%) 1:20 and a 1:10 dilution of shampoo formulations of miconazole 2%, miconazole 2%/chlorhexidine gluconate 2-2.3%, ketoconazole 1%/chlorhexidine

2%, climbazole 0.5%/chlorhexidine 3% and sterile water-untreated control were tested in three experiments. In the first, a suspension of infective spores and hair/scale fragments was incubated with a 1:10, 1:5 and 1:1 dilution of spores to test solutions for 10 mins. In the second, toothbrushes containing infected cat hair in the bristles were soaked and agitated in test solutions for 10 mins, rinsed, dried and then fungal cultured (n = 12×). In the third, a 3 min contact time combined with an AHP rinse was tested (n = 10×). Good efficacy was defined as no growth. Water controls grew >300 colony-forming units/plate and all toothbrushes were culture-positive prior to testing. For the suspension tests, all test products showed good efficacy. Miconazole 2%, ketoconazole 1% and AHP showed good efficacy after a 10 min contact time. Good efficacy was achieved with a shorter contact time (3 mins) only if combined by an AHP rinse. He concluded that lime sulfur and enilconazole continued to show good efficacy. In countries or situations where these products cannot be used, shampoos containing ketoconazole, miconazole or climbazole are alternative hair coat disinfectants with a 10 min contact time or for 3 min, if combined with an AHP rinse.

Nardoni *et al.* (2015) formulated a herbal mixture composed of chemically defined essential oils (EOs) of Litsea cubeba (1%), Illicium verum, Foeniculum vulgare, and Pelargonium graveolens (0.5% each) was formulated and its antifungal activity assessed against M. canis arthrospores which represent the infective environmental stage of M. canis. Single compounds present in higher amounts in the mixture were also separately tested in vitro. Litsea cubeba and P. graveolens EOs were most effective (minimum inhibitory concentration (MIC) 0.5%), followed by EOs of I. verum (MIC 2%) and F. vulgare (MIC 2.5%). Minimum fungicidal concentrations (MFC) values were 0.75% (L. cubeba), 1.5% (P. graveolens), 2.5% (I. verum) and 3% (F. vulgare). MIC and MFC values of the mixture were 0.25% and 0.5%, respectively. The daily spray of the mixture (200 μL) directly onto infected hairs inhibited fungal growth from the fourth day onwards. The compounds present in higher amounts exhibited variable antimycotic activity, with MIC values ranging from >10% (limonene) to 0.1% (geranial and neral). Thus, the mixture showed a good antifungal activity against arthrospores present in infected hairs. These results are promising for a further application of the mixture as an alternative tool or as an adjuvant in the environmental control of feline microsporosis.

1.13. Vaccination

Several attempts have been made to develop fungal vaccines for prevention and/or therapy of dermatophytosis in cats, such as laboratory prepared fungal cell wall vaccines, an inactivated broad-spectrum dermatophyte vaccine or a liveattenuated dermatophyte vaccine. None of the investigated vaccines for cats showed sufficient protection against challenge exposure. A vaccine for prophylaxis of M. canis infection in cats and dogs is approved in Germany (Rivac Mikroderm, Riemser Arzneimittel AG, Germany). Another vaccine (Insol® Dermatophyton, Boehringer Ingelheim, Germany) is licensed for the therapeutic and prophylactic use in cats and dogs in several European countries

Reports

DeBoer and Moriello (1994) conducted an experimental infection model to assess induction of specific immunity against ***Microsporum canis*** in cats with an *M.*

canis cell wall vaccine preparation. Kittens 8–9 weeks old (*n*= 12) received five doses of either vaccine or placebo at biweekly intervals. Specific immunity was monitored via plasma anti-dermatophyte antibody titers and lymphocyte blastogenesis (LB) to dermatophyte antigens. After vaccination, cats were challenged with viable *M. canis* spores, and lesion development was monitored. Vaccinated cats developed higher anti-dermatophyte IgG, but not IgM, titers than controls, beginning after the second dose of vaccine ($P < 0.001$). During the vaccination period, specific cellular immunity as measured by LB was absent in control cats, but developed to a limited degree in vaccinated cats ($P < 0.05$). After challenge with 10^5 fungal spores per cat, both control and vaccinated cats developed active infections. The vaccine appeared to induce an antibody titer quantitatively similar to that produced by infection, but less measured cellular immunity than was seen with infection and recovery. These results suggest that induction of high titers of serum IgG or IgM antibody against *Microsporum canis* is not protective against challenge exposure.

DeBoer and Moriello (1995) evaluated a laboratory-prepared killed ***Microsporum canis*** cell-wall vaccine under conditions simulating an accidental infection of a cattery, by inoculating eight- to nine-week-old cats with the vaccine or with a placebo control. The vaccinated cats developed high titres of anti-dermatophyte IgG as measured by an ELISA, and a small cell-mediated response against M canis as measured by a lymphocyte blastogenesis assay, using a whole fungus extract. After being inoculated the cats were challenged by the introduction of an infected cat into the same room. All the vaccinated and control cats became culture-positive for M canis within four weeks of the introduction of the infected cat. Four of the six control cats and all the vaccinated cats developed lesions consistent with dermatophytosis within 16 weeks after exposure to the infected cat.

Pier *et al*. (1995) used an inactivated, broad-spectrum dermatophyte vaccine to produce an active immunity in guinea-pigs against ***Microsporum canis***. None of the vaccinates developed infection from a contact exposure challenge that produced clinical infections in 70% of the unvaccinated controls. Infection with M. canis induced antibody titres (ELISA) and delayed cutaneous hypersensitivity (DCH) reactions to itself as well as cross-reacting titres to Trichophyton equinum and T. mentagrophytes and DCH reactions to T. mentagrophytes; however vaccinated animals developed significantly higher antibody titres and DCH responses to all of these antigens than did non-vaccinated animals which had been infected or exposed. Rabbits hyperimmunized with culture filtrate antigens to single dermatophyte agents (M. canis, M. gypseum, T. equinum, and T. mentagrophytes) developed positive inter-species and inter-generic DCH cross-reactions to a battery of six skin test antigens (M. canis, M. gypseum, M. equinum, T. equinum, T. mentagrophytes var. mentagrophytes and T. verrucosum). Guinea-pigs vaccinated with a T. equinum vaccine had increased resistance to M. canis infection than did non-vaccinated controls. These findings support clinical observations which suggest establishment of a broad-based immunity in animals following infection with a single dermatophyte.

Westhoff *et al*. (2010) investigated the efficacy of an inactivated vaccine for the treatment of feline dermatophytosis in a placebo-controlled-double-blind multi-centre GCP study in Europe. Fifty-five client-owned cats with dermatophytosis caused by Trichophyton mentagrophytes or Microsporum canis, confirmed by fungal culture, were treated with either three intramuscular injections of vaccine or placebo. Treatment was applied as three intramuscular injections of vaccine or placebo every other week. Clinical symptoms were assessed at inclusion, day 14, 28 and 42. The number of lesions was counted and severity was judged based on a scoring system.

Efficacy was evaluated for the reduction of the number of lesions as well as for a combined assessment of lesion severity x number of lesions. The primary endpoint was not met for the total population of cats, but was met for cats.

Commercially available vaccines

Insol Dermatophyton inactivated vaccine developed in Boehringer Ingelheim (Switzerland), it is effective in horse, dog and cat, can be used as treatment of the disease, improving the clinical outcome. It contains strains of *T. verrucosum, T.mentagrophytes, T. sarkisovii, T. equinum,M. canis,M. canis var. distortum, M. canis var. obesum, and M. gypseum.*

- **Commercial vaccine Feo-O-Vax MC-K1** developed by Fort Dodge in USA. It is an inactivated vaccine containing the mycelium of M. canis and an adjuvant. It produces anti-dermatophyte antibody titres similar to those developed in the course of the natural infection, with a low CMI. All vaccinated cats developed the disease after a topical application of *M. canis* conidia; however, the lesions were smaller than those in the control animals. The fact that all of the animals vaccinated had lesions suggests that high titres of antibody against M. canis may not be enough for protection against the infection.

- **The inactivated vaccine Dermatovac-IV.** It contains an adjuvant and an optically standardized inactivated suspension of conidia and mycelium of the fungi *M. canis, T. equimun, M. gypseum* and *T. mentagrophytes*

- **The Ringvac bovis LTF-1301 live vaccine** is the most effective and widely used**,** marketed by Alpharma, elaborated with the LTF-130 strain of *T. verrucosum*, has a characteristic high level of immunogenicity, low virulence and great stability**,** has been used effectively in Russia and Norway**,** administered intramuscularly**,** stimulates the appropriate immune response(DHS)

- **Permavax-Tricho live vaccine** is marketed in the Czech Republic by Bioveta Ivanovice, contains an attenuated strain of *T. verrucosum*. Triggers a protective immunity status 28 days after the second inoculation, preventing the appearance of the clinical disease for 1 year after vaccination.

1.14. Decontamination

key points:

- The most important part of disinfection is the so-called ‘hard clean’ – that is, removal of debris and thorough washing with a detergent until visibly clean.
- It is important to rinse the detergent from the surface, as many disinfectants are inactivated by detergents.
- If gross debris and organic material are removed from the target surface, ready-to-use disinfectants with label claim efficacy against *Trichophyton mentagrophytes* are effective.36 it is important to apply these liberally and allow for an adequate wetting/contact time.
- Compounds containing accelerated hydrogen peroxide are recommended as an alternative to household bleach.

- Exposed soft materials can be washed in hot or cold water; bleach is optional. it is important not to overload the washer, and to use the longest wash cycle possible as agitation removes spores. if concern is high, wash the laundry twice.
- If only one or two animals are involved, it is recommended to do thorough cleaning once or twice weekly, with removal of cat hair and use of 'one step' cleaners on a daily basis in-between these cleanings.

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B. Fungal diseases of cats and dogs caused by yeasts

1. Candidosis in cats and dogs

1.1. Introduction

In dogs, *Candida spp* are ubiquitous saprophytic yeast and widely distributed in the environment and frequently colonized the skin and mucous membranes (such as the oral cavity) and genital and gastrointestinal tracts of dogs. They prefer constantly humid areas, which favour tissue maceration, as occurs in mucous membranes, mucocutaneous junctions, intertriginous areas, nail substructure inter-fingers areas, ear canals and the lateral face of the ear and genital tract membrane. Immunosuppressive states appear to preclude dogs to developing candidosis, such as iatrogenic infections associated with wound dehiscence, candidial endocarditis following prolonged immunosuppression therapy, and candidiasis associated with metastatic mast cell tumors. Clinically affected dogs present with generalized seborrheic dermatitis, alopecia, patchy erythema, and superficial erosions with histological evidence of mural folliculitis. Dogs with

systemic candidosis present with more general symptoms referable to the organs affected, but peritonitis and chronic cystitis have been reported.

Candida albicans is not a member of the normal skin flora and its presence is always the expression of a pathologic state and of its intrinsic pathogenicity. Species which are reported in dogs include:

Cutaneous candidosis

1. ***Candida parapsilosis***: Dale, 1972, Yurayart, 2013
2. ***C albicans:*** Moretti, 2004,
3. ***Candida guilliermondii***: Mueller, 2002
4. ***Candida glabrata***: Waurzyniak, 1992
5. **Candida sp** : KRAL,1960, Lee, 2011

Urinary tract, mainly cystitis

1. ***Candida albicans***: Kano,2002**,** Jin &Lin , 2005
2. ***Candida tropicalis***: Ozawa *et al*., 2005**,**Álvarez-Pérez *et al***.,** 2016**,**
3. ***Candida parapsilosis***: Kano *et al*.,2002
4. **Candida sp:** Forward *et al*.,,2002, Pressler et al.,2003, Enders *et al***.,** 2016

Peritonitis

1. ***Candida albicans***: Ong *et al*., 2009, Bradford *et al*, 2013, GLIŃSKA *et al*.. 2013, Burgess and Gaunt, 2014
2. ***Candida tropicalis***, Palmer *et al*, 1982

Oral and gastrointestinal candidosis

1. ***Candida albicans***: Refai ,1986 , Refai *et al.*, 1986 , Mancianti *et a*l., 1992 Milner *et al.*, 1997, Jadhav and Pal, 2006
2. **Candida sp** : Ochiai.2002, Biegańska *et al*., 2014

Rhinitis

1. ***Candida parapsilosis* Lamm *et al.* (2013)**

Ocular candidosis

1. ***Candida albicans***: Linek , 2004
2. **Candida sp** : Enders**,** 2016

Bronchopulmonary candidosis

1. **Candida sp** Clercx,1996

Pericarditis

1. ***Candida albicans*** Mohri . 2009.

Otitis externa

1. ***Candida albicans***.McKellar, 1990

Systemic (disseminated) candidosis

1. ***Candida albicans*:** Ruthe,1978, Ehrensaft, 1979, Holøymoen,1982, Weber,1985, Heseltine,2003, Kuwamura,2006, Recai *et al.* 2006**.** Khosravi *et al.*, 2009, Matsuda, 2009, Rogers *et al.*, 2009, Skoric *et al.*, 2011
2. ***Candida glabrata***: Schoeniger. 2002,
3. **Candida sp:** Clercx,1996, Rodríguez,1998, , Brown, 2005, Gershenson, 2011, Rogers, 2011

Healthy dogs

1. ***Candida albicans***: Edelmann *et al*,2005
2. ***Candida parapsilosis***: Brilhante *et al*,2014
3. **Candida sp** Cleff *et al*,2010, Brito *et al*, 2009

In cats, *Candida spp* are a common fungus present as a commensals on most mucous membranes including the mouth, digestive tract and vagina of cats. They also form part of the normal faecal biotome. They rarely cause primary disease, but can cause serious disease in immunocompromised or malnourished patients. The lower urinary tract is the most common site for feline *Candida spp* infection. *Candida spp* reported in cats include:

Urinary tract, mainly cystitis

1. ***Candida albicans***: Fulton *et al.***,** Jin &Lin , 2005
2. ***Candida species:*** Toll *et al.***,**2003, Pressler *et al*.,2003,

Ocular candidosis

1. **Candida sp** Gerding *et al.*, 1994

Oral candidosis

1. ***Candida albicans***: Mancianti, 1992

Rhinitis

1. ***Candida parapsilosis*** Lamm *et al*., 2013

Pyothorax

1. ***Candida albicans***: McCaw, 1984

Gastrointestinal granuloma

1. ***Candida albicans*****:** Duchaussoy *et al*., 2015

Disseminated candidosis

1. **Candida sp:** Gerding *et al*., 1994

Healthy cats

1. ***Candida albicans***: Edelmann *et al*,2005

1.2. Description of main Candida species reported in dogs cats and dogs

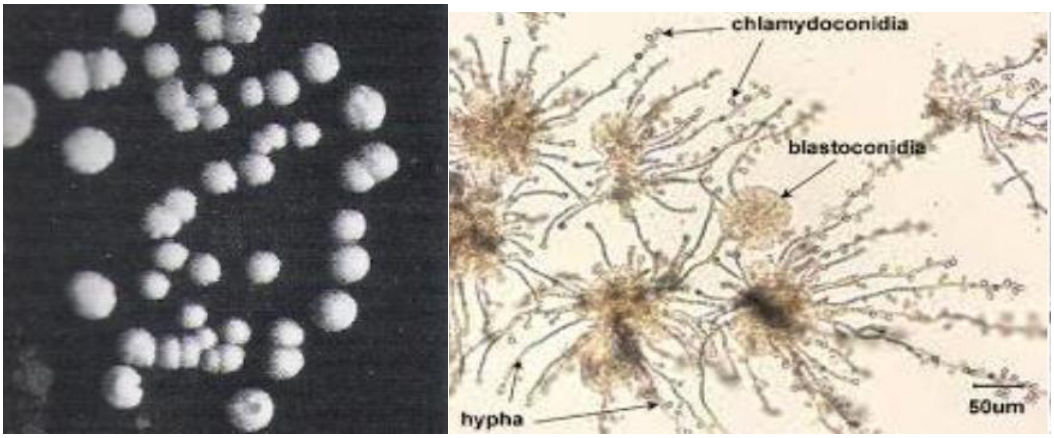
1.2.1. *Candida albicans* (Robin) Berkhout 1923

Synonyms

1. =Blastomyces albicans Brownlie: 425-431 (1920) [MB#456196]
2. =Candida biliaria Bat. & J.S. Silveira, Hospital Rio de Janeiro 56 (2): 295 (1959)
3. =Candida claussenii Lodder & Kreger, The Yeasts: a taxonomic study: 578 (1952)
4. =Candida desidiosa Cif. & Redaelli, Archiv für Mikrobiologie 6: 65 (1935) [MB#263052]
5. =Candida genitalis Bat. & J.S. Silveira, Public Instit Micol Unive do Recife 170: 11 (1962)
6. =Candida intestinalis Bat. & J.S. Silveira, Hospital Rio de Janeiro 56 (2): 293 (1959)
7. =Candida langeronii Dietrichson, Annales de Parasitol Humaine Comparée 29: 479 (1954)
8. =Candida mycotoruloidea Redaelli & Cif., Archiv für Mikrobiologie 6: 50 (1935)
9. =Candida nouvelii Saëz, Bulletin de la Société Mycologique de France 89 (1): 82 (1973)
10. =Candida truncata Vanbreus., Archives Belge de Derm et Syphil 4: 307-313 (1948)
11. =Endomyces albicans Okabe, Cblatt Bakteriol, Parasit Infek, Erste Abt: 181-187 (1929)
12. =Monilia alba Castell. & Chalm., Manual of Tropical Medicine: 1089 (1919) [MB#481761]
13. =Monilia albicans Plaut (1919) [MB#479429]

Morphology

On Sabouraud's dextrose agar colonies are white to cream coloured, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-7.0 x 3.0-8.5 um in size.



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Physiological Tests:

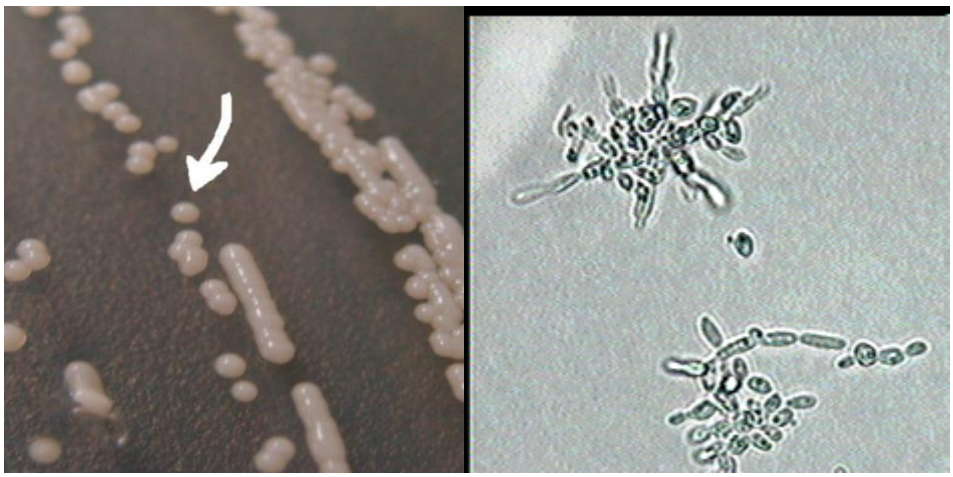
Germ Tube test + within 3 hours. Hydrolysis of Urea +,Growth on Cycloheximide medium +. Growth at 37C +, fermentation: Glucose +; Maltose +, Galactose +/-; Trehalose+/-, Sucrose (some strains +); Lactose -. **Assimilation:** Glucose +; Maltose +; Galactose +; Trehalose +; Sucrose (some negative);D-Xylose +; Soluble Starch +; D-Mannitol +; D-Glucitol (Delayed), Melezitose +/-; Glycerol +/-; Succinic acid +/-; L-Arabinose +/-; L-Sorbose +/-; D-Ribose (some positive); Citric acid +/-; DL-Lactic acid +/-. Potassium nitrate -; Lactose -; Ribito-

1.2.2. *Candida parapsilosis* (Ashford) Langeron & Talice, Annales de Parasitologie Humaine Comparée 10: 54 (1932)

≡Monilia parapsilosis Ashford, American Journal of Tropical Medicine 8: 518 (1928) ≡Candida parapsilosis var. parapsilosis , Annales de Parasitol Huma Comp (1932)
≡Mycocandida parapsilosis (Ashford) C.W. Dodge, Med mycol. 294 (1935)
≡Mycotorula parapsilopsis (Ashford) Cif. & Redaelli (1943)
≡Mycotorula parapsilosis Cif. & Redaelli, Atti dell'Istituto Bot della (1943)
=Torulopsis larvae Kawano et al.
=Monilia onychophila Pollacci & Nann., Archiv Biol.: 25-36 (1926)
=Mycotorula vesica F.C. Harrison, Trans Royal Society of Canada 22: 219 (1928)
=Blastodendrion intestinale var. epidermicum Cif. & Alfons., Zblatt Bakt (1931)
=Blastodendrion globosum Zach, Arch. Dermatol. Syph.: 99 (1933)
=Blastodendrion gracile Zach, Arch. Dermatol. Syph.: 103 (1933)
=Pseudomycoderma vesica (F.C. Harrison) C.W. Dodge, Med mycol. 236 (1935)
=Candida montrocheri M. Morelet, Bull. Soc. Sci. Nat. Arch. Toulon & Var: 6 (1968)
=Brettanomyces petrophilum I. Takeda, Iguchi, Tsuzuki & Nakano, UN: (1972
=Candida osornensis C. Ramírez & A.E. González, Mycopathol 88 (2-3): 88 (1984)

Morphology and physiology

Colonies (YPGA) are cream-coloured to yellowish, glistening and soft, mostly smooth or partly or entirely wrinkled. Pseudomycelium (RA) are present, mostly abundant, consisting of branched chains of elongate cells in more or less christmas tree-like arrangement, lateral branches gradually becoming shorter towards the hyphal apex. fermentation of glucose +, and assimilation: cellobiose, raffinose, melebiose, melezitose +, soluble starch, d-xylose +, salicin, arbutin, 5-keto-d-gluconate (but may be slowly positive), nitrate, growth at 37À¸C +, d-tryptophan (N), w/o thiamine +. Physiologically indistinguishable from Candida guilliermondii (p. 200), C. haemulonii (p. 202), C. pulcherrima (p. 217), C. tropicalis (p. 220) and some saprobic species



Candida · parapsilosis , ww.mdpi.com, plantpathology86.blogfa.com

1.2.3. *Candida glabrata* (H.W. Anderson) S.A. Mey. & Yarrow, Int J Syst Bacteriol 28: 612 (1978)

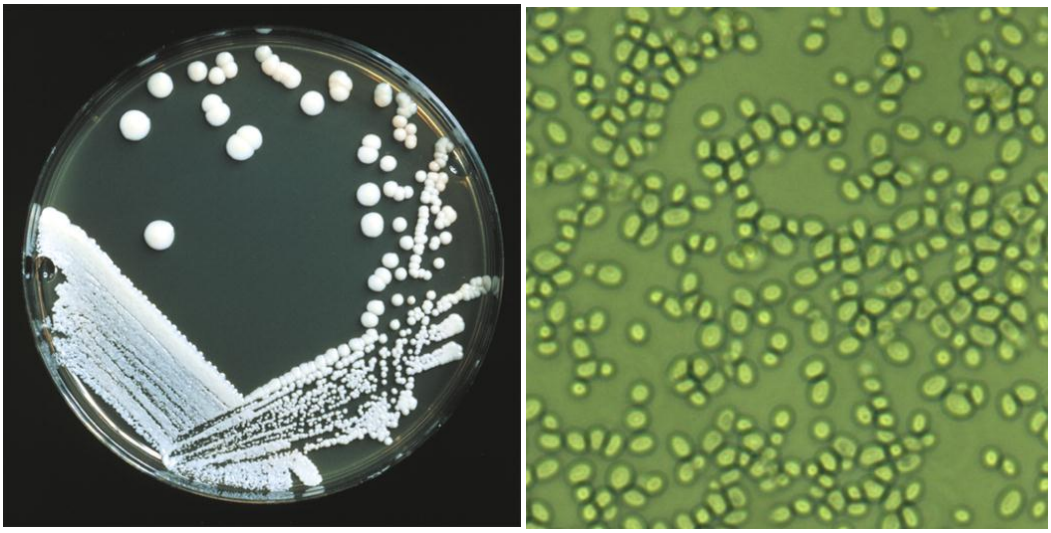
≡Cryptococcus glabratus H.W. Anderson, Journal of Infectious Diseases 21: 379 (1917) ≡Torulopsis glabrata (H.W. Anderson) Lodder & N.F. de Vries, Mycopathol 1: 102 (1938)

=Torulopsis stercoralis Uden

=Torulopsis glabrata (Anderson) Lodder & de Vries, Mycopath. Mycol. Appl. 1: 98, 1938-1939.
=Cryptococcus glabratus Anderson, J. Infect. Dis. 21: 379, 1917.

Morphology and physiology

Colonies on Glucose Peptone Agar at 25°C: after 3 days cream-coloured, smooth, dull, regular in shape, spherical, domed.Yeast-like cells are generally ovoid, single or budding 2·0-4·0 x 3·0-5·5 µm. Dalmau Plate Cultures on Corn Meal Agar: ovoid, budding cells only. No pseudomycelium (chains of elongated yeast-likecells) produced.Germ Tube Test: negative. Fermentation of Carbohydrates: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose. Assimilation of Organic Compounds: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose +Cellobiose - Inositol - Melezitose - Melibiose - Mannitol - L-Sorbose - D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol v Erythritol - Ribitol - Galactitol - D-Glucitol - Salicin - DL-Lactic Acid - Succinic Acid CitricAcid - Soluble Starch -. Assimilation of Inorganic Compounds: Nitrate -. Ability to split urea: -.



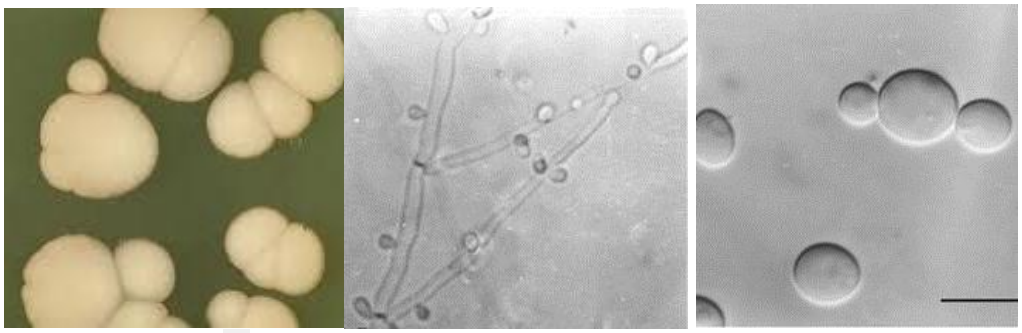
Candida glabrata, yeastcurereview.com, Wikipedia

1.2.4. *Candida tropicalis* (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)

1. Atelosaccharomyces tropicalis (Castell.) Mello, Arq de Higi e Patol Exóti 6: 263 (1918)
2. Candida albicans var. tropicalis (Castell.) Cif., Manuale de Micol Medica 2: 252 (1960)
3. Candida tropicalis var. tropicalis
4. Castellania tropicalis (Castell.) C.W. Dodge, Med mycol .: 258 (1935)
5. Endomyces tropicalis (Castell.) Castell., Centbl. Bakt. ParasitKde, Abt. 1: 236 (1911)
6. Monilia tropicalis (Castell.) Castell. & Chalm., Manual of Tropical Medicine: 1086 (1919)
7. Myceloblastanon tropicale (Castell.) M. Ota, Jap. J. Dermatol. Urol.: 178 (1927)

8. Mycotorula tropicalis (Castell.) Cif. & Redaelli, Atti dell'Isti Bota della Univ Lab (1943)
9. Oidium tropicale Castell., Philippine J Sci Sec B Medical Science 5 (2): 202 (1910)
10. Procandida tropicalis (Castell.) E.K. Novák & Zsolt, Acta Bot Acad Sci Hung (1961)

Morphology
Growth in glucose-yeast extract-peptone broth: After 3 days at 25°C, the cells are spheroidal and short-spheroidal, ellipsoidal, cylindrical, (3.2-8.0) × (4.0-8.8) µm, single, in pairs, multilateral budding. Growth on glucose-yeast extract-peptone agar: Aerobic growth is cream, smooth, butyrous, soft, glistening and convex with fringed border. Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with blastoconidia formed singly or in verticals. Septate hyphae are usually present. Formation of ascospores: Ascospores are not formed.

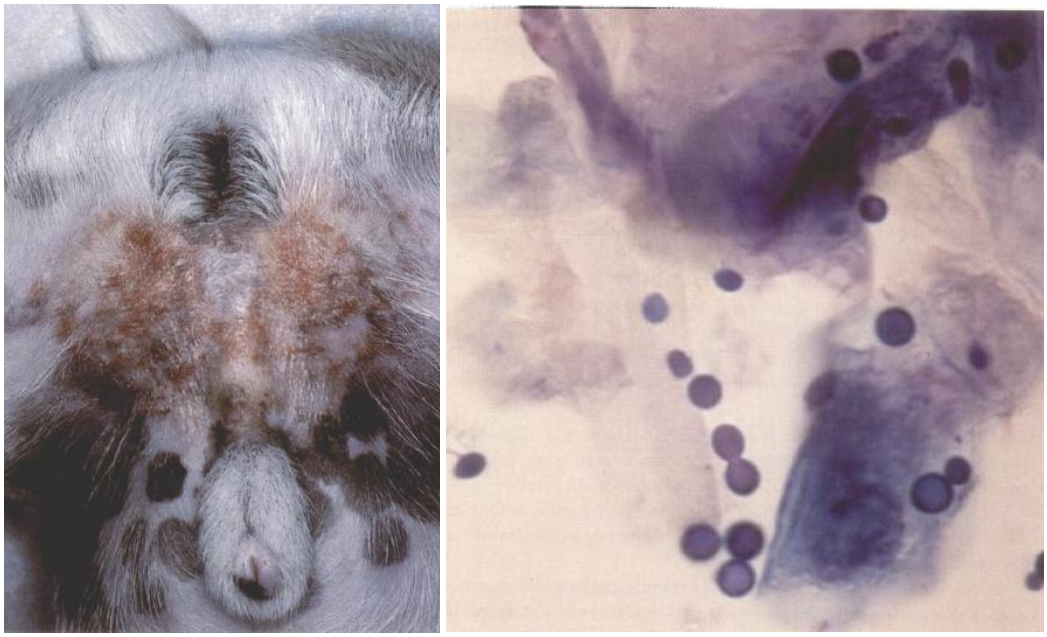


Candida tropicalis, www.healthandwellbeingnews.com, **www.bcrc.firdi.org.tw**

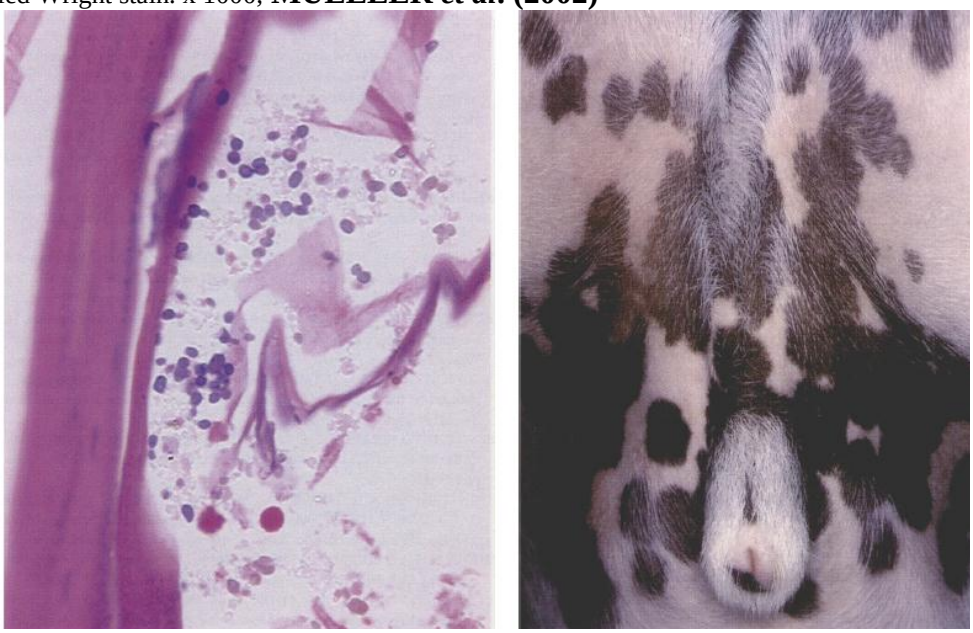
1.3. Reports on candidosis in dogs and cats

1.3.1. Cutaneous candidosis

MUELLER *et al.* (2002) reported an eight-month-old male neutered Jack Russell terrier was presented with chronic severe **dermatitis** in the inguinal area, which had developed shortly after castration. Impression smears of the affected skin revealed a small number of yeast organisms, , suspected to be Candida species. Skin biopsies and cultures were obtained. Histopathologically, a mild mononuclear superficial dermatitis was present. Epidermal hyperkeratosis was mild, but marked parakeratosis was noted with focal accumulation of neutrophils and yeast organisms in the stratum corneum. Yeasts were grown on Sabouraud dextrose agar and identified as ***Candida guilliermondii*** using ID 32C identification strip of Bio-Merieux. The dog was treated with kwtoconazole at 10 mg/kh twice daily and a shampoo containing chlorhexidine, miconazole and selenium sulphide daily, and the inguinal area improved dramatically within 10 days



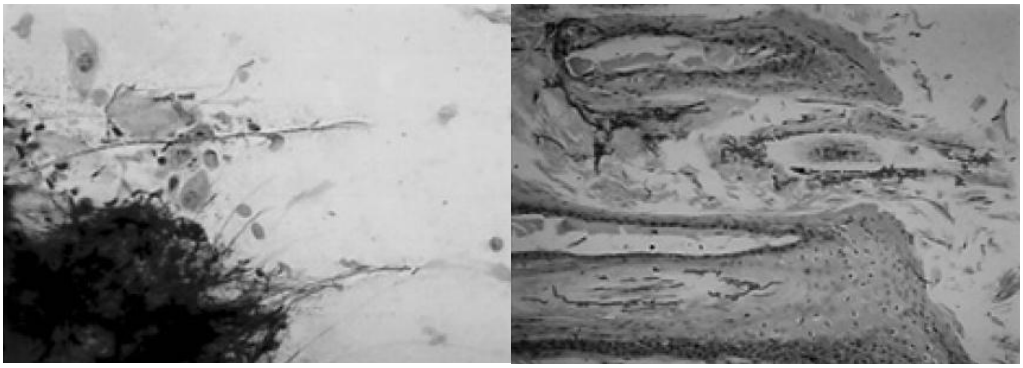
Left: Severe erythema, scaling and crusting in the inguinal area of an eight-month-old male, neutered Jack Russell terrier, between the prepuce and the scrotum bilaterally, due to Candida guilliermondii, Right: Yeast organisms found cytologically in the affected inguinal area of the Jack Russell terrier. Modified Wright stain. x 1000, **MUELLER *et al*. (2002)**



Left: Yeast organisms in the stratum corneum of the skin biopsy taken from the affected inguinal area of the Jack Russell terrier. Haematoxylin and eosin. x 1000, Right: Inguinal area of the Jack Russell terrier after systemic and topical antifungal treatment, **MUELLER *et al*. (2002)**

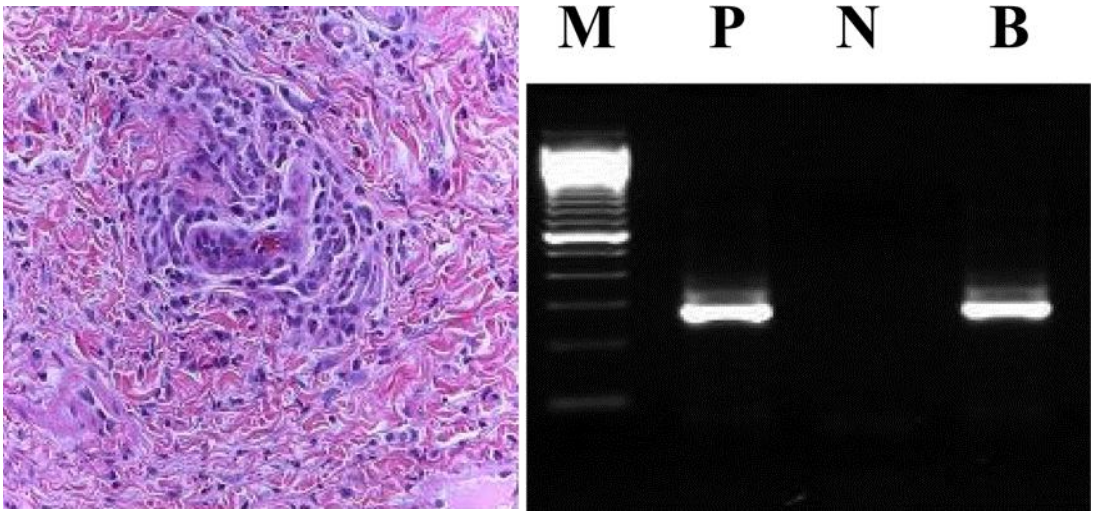
Moretti *et al.* (2004) described a clinical case of **cutaneous candidiasis** in a dog with dermatological lesions, characterized by persistent alopecia, crusts, ulcers and scales. Predisposing factors such as the use of corticosteroids, the concomitan presence of an autoimmune disease (pemphigus foliaceus) and an infection of ehrlichiosis caused by Ehrlichia canis were observed. Histopathological findings included signs of orthokeratotic hyperkeratosis, moderate follicular keratosis and light epidermic acanthosis. The reactive process included an infiltrative superficial dermatitis and a mural folliculitis with prevalent participation of macrophages and lymphocytes. They

indicated that the application **of PCR-Restriction Enzyme Analysis** (REA) method on cutaneous specimens, together with other techniques, such as mycologic, cytologic and histological examinations, allowed them to identify ***Candida albicans*** as aetiological agent in this particular case.



Left:Cytologic examination: macrophages, mononuclear cells and epithelial cells are seen in association with pseudo-hyphal structures (May-Grunwald Giemsa stain, x40). Right: Histological finding of the skin: severe fungal colonization of the follicular infundibulum (PAS stain, x25). **Moretti *et al.* (2004)**

Lee *et al.* (2011) reported one-year-old male Beagle dog with **dermatitis, alopecia and scales**. Examination of the affected dog revealed generalized alopecia, patchy erythema, and superficial erosions with histological evidence of mural folliculitis. External tests for parasites in scraped skin samples were negative. However, fungal culture tests and polymerase chain reaction revealed the existence of Candida in the lesion. These results suggest that cutaneous candidiasis may induce mural folliculitis and alopecia in dogs.



Left:Histopathological findings of a section of the skin from a dog with alopecia secondary to infection with *Candida*. Hematoxylin-eosin stain. ×200, , Right:Gel electrophoresis of DNA amplicons by *Canidida*-specific PCR. M, 100-bp DNA ladder; P, positive control; N, negative control; B, biopsied skin DNA. **Lee *et al.* (2011)**

1.3.2. Candida urinary tract infections

Fulton *et al.* (1992) diagnosed a case of ***Candida albicans*** **urocystitis** secondary to urethral stricture and administration of antibiotics was diagnosed in a cat by fungal

culturing of urine and examination of specimens. Surgical repair of the stricture and administration of 5-fluorocytosine resulted in resolution of the cystitis. Related problems included anorexia and severe weight loss, which necessitated enteral nutritional support, dehydration, renal disease, and nosocomial Pseudomonas aeruginosa urocystitis.

Kano *et al*. (2002) identified Candida species in clinical urine samples directly by the newly developed method of **PCR** analysis on 25S ribosomal DNA (rDNA). Two dogs were referred to the Animal Medical Center, Nihon University School of Veterinary Medicine, Fujisawa, Kanagawa, Japan for the examination of **chronic cystitis**. Microscopic examination of urine samples from these dogs revealed yeast cells. Urine culture on Sabouraud's dextrose agar at 27 ^{o} C for 5 days produced white to cream colored colonies. The isolates were identifical to ***Candida albicans*** and ***C. parapsilosis*** by mycological examination, respectively. The nucleotide sequences of 25S ribosomal DNA from these urine isolates showed 99% similarity to those of a reference strain of Candida albicans or C. parapsilosis. The nucleotide sequences of 25S rDNA obtained directly from urine samples were also identical to C. albicans and C. parapsilosis, respectively. Confirming the results on the isolates cultured from the same urine samples. This PCR analysis method could be available for the direct identification of Candida species in urine samples within 2 days.

Pressler *et al*. (2003) reviewed records from 20 animals (13 dogs, seven cats) with **Candida urinary tract infections**. Six ***Candida spp***. were isolated; Candida albicans was the most common isolate. Concurrent diseases or non-antifungal drugs administered within 1 month of isolation included antibiotics (n=16), corticosteroids (n=6), diabetes mellitus (n=4), non-urogenital neoplasia (n=3), and non-candidal urogenital disease (n=14). All animals had sources of local or systemic immune compromise that likely predisposed to infection. Of five animals with resolution of infection, three did not receive specific antifungal treatment. The authors conclude that correction of predisposing conditions is likely critical for management of Candida spp. urinary tract infection.

Toll *et al*. (2003) reported a 12-year-old spayed female domestic longhair cat with fungal cystitis (Candida sp). The cat had a history of chronic diabetes mellitus, hyperadrenocorticism, and bacterial cystitis caused by Escherichia coli. Antifungal agents (itraconazole and fluconazole) were administered orally without noticeable effect on the candiduria. Because of the ineffectiveness of these treatments, intravesicular administration of 1% clotrimazole solution was performed weekly for 3 treatments. Complete resolution of urinary candidiasis was detected after the third infusion. Intravesicular administration of clotrimazole solution appears to be a safe and effective treatment of fungal cystitis in cats.

Jin and Lin (2005) studied 35 animals (23 dogs, 12 cats) with fungal **urinary tract infections** (UTIs) were retrospectively. Dysuria, hematuria, increased frequency of micturition, anorexia, depression, and pyrexia were the most common clinical signs noted. Seven species of fungi were identified in the affected animals. ***Candida albicans*** was the most common isolate. Most animals diagnosed with fungal UTI also had other concurrent urinary tract or medical problems. Lower urinary tract diseases, diabetes mellitus, neoplasia, and renal failure were the most common concurrent or preceding diseases identified. Resolution of fungal UTI occurred in 12 animals that received specific antifungal treatment.

Ozawa ***et al.*** **(2005)** identified an isolate from urine of a dog with **cystitis** molecularly as ***Candida tropicalis*** and its minimum inhibitory concentration (MIC) was determined by a microdilution method. The 25S ribosomal DNA sequence analysis indicated that the clinical isolate was essentially identical to that of C. tropicalis and distinct from other Candida species. The MIC(50) and the MIC(90) of fluconazole (FLZ) for the clinical isolate of C. tropicalis was 6.25 and 25 microg/ml, respectively, indicating that susceptibility of the clinical isolate of C. tropicalis to FLZ was less than for other strains of C. tropicalis as well as C. albicans. The molecular analysis as presented in this study assisted the diagnosis of candidiasis by identifying the yeasts in urine samples within 2 days. The patient dog, a 10-year-old male Shih Tzu dog (7.0 kg) referred for examination of cystitis was successfully treated with itraconazole.

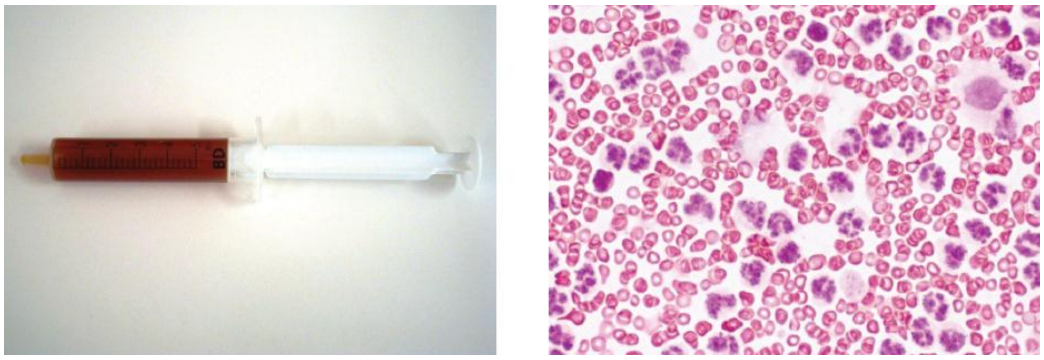
Enders *et al.* (2016) described the clinical presentation, diagnosis, histologic lesions, and outcome of endogenous mycotic endophthalmitis secondary to **candiduria** in a three-year-old female spayed Dachshund. The dog was being treated for Evans syndrome for one month prior to being diagnosed with candiduria and fibrinous uveitis OS. The left eye was enucleated due to secondary glaucoma, and the fungal urinary tract infection was treated successfully. Uveitis developed in the contralateral eye with relapse of the urinary tract infection in the following weeks. The right eye was medically managed until secondary glaucoma developed and was subsequently enucleated. Histopathology of both eyes showed evidence of endophthalmitis with intralesional fungal organisms, consistent with Candida spp. Ocular candidiasis is rare in dogs.

1.3.3. Candida peritonitis

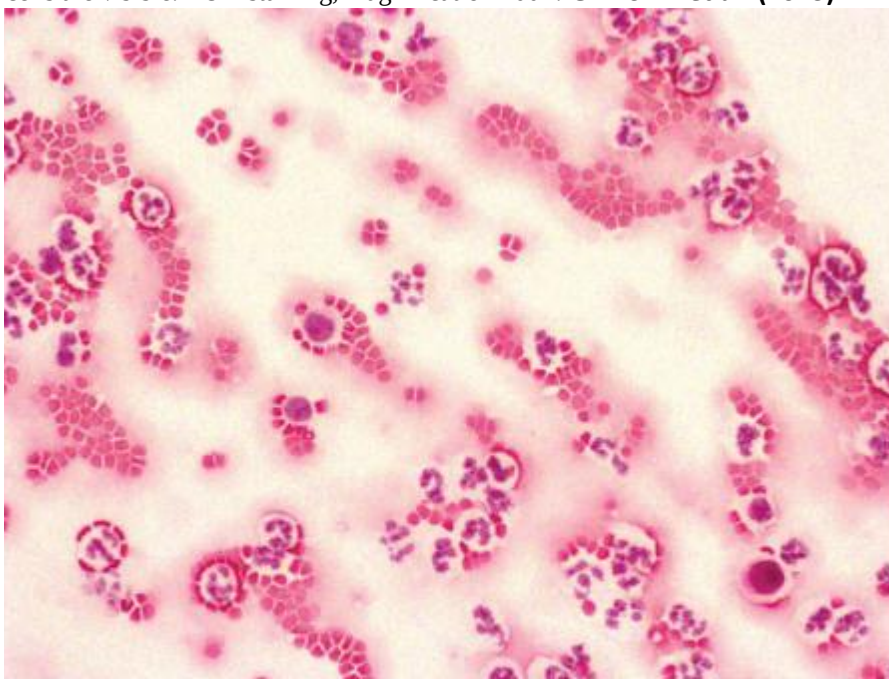
Ong *et al.* (2010) reported a 15-week-old Papillon that developed **peritonitis** secondary to enterectomy site dehiscence. A pure growth of ***Candida albicans*** was obtained from the abdominal fluid. Surgical repair of the dehiscence was performed and antifungal therapy instituted with fluconazole postoperatively. A marked exudative process was noted postoperatively with production of large volumes of fluid from the abdominal drain. Fresh frozen plasma and pentastarch were provided for oncotic support. Recovery was complicated by megaesophagus, however, the patient gradually improved and was discharged 11 days after surgery.

Bradford *et al.* (2013) described 5 cases of dogs with **peritonitis** complicated by Candida spp; 3 dogs with ***C. albicans***, one dog with C. albicans and ***C. glabrata***, and one dog with C. glabrata only. The 3 dogs with C. albicans peritonitis presented with duodenal perforation due to NSAID therapy, intestinal resection and anastomosis following postspay-surgery dehiscence, and intestinal foreign body removal. The 2 dogs with C/ glabrata peritonitis had undergone cholecystectomy due to gall bladder rupture and dehiscence of intestinal biopsy removal sites following exploratory laparatomy. In all cases, initial diagnosis of fungal peritonitis was made via cytologic examination of peritoneal effusions, which revealed marked pyogranulomatous inflammation with numerous 3-8 μm oval, deeply basophilic yeast organisms with thin clear capsules noted within phagocytes and extracellularly. In addition, germ tube formation, hyphae, and pseudohyphae were rarely seen in some of the cases with pure C. albicans. Identity of the organisms was determined by culture in all cases and confirmed by PCR in 3 cases.

GLIŃSKA *et al.* (2013) reported a male dog of Pointer breed, 10 years of age. The clinical examination revealed severe tenderness and a tension in the abdominal walls. The ultrasound examination detected fluid in the peritoneal cavity. The fluid collected from the peritoneal cavity displayed typical features of exudative fluid. In the cytological examination of the fluid, numerous stimulated endothelial cells, lymphocytes, and neutrophils were found. In the microbiological examination of the peritoneal fluid, ***Candida albicans*** was cultured. On the basis of the clinical signs, laboratory test results of the peritoneal fluid, and microbiological culture, the diagnosis of fungal **peritonitis** was made.

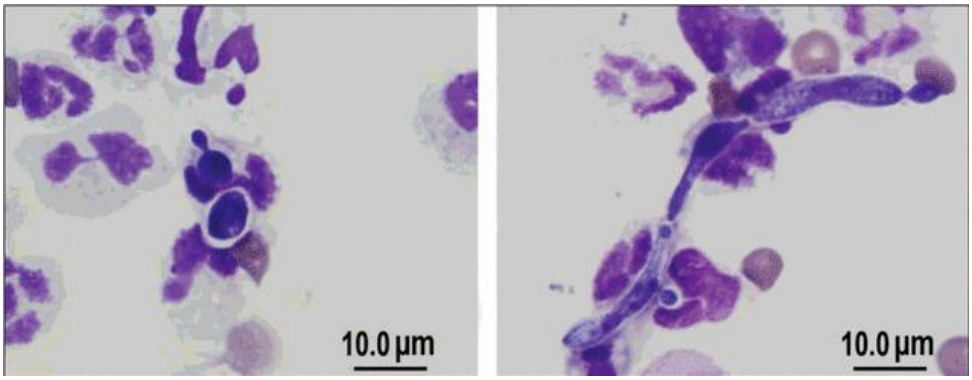


Left: Brownish blood-red fluid collected from the peritoneal cavity of the dog with fungal peritonitis. Right: Smear of peritoneal fluid sediment. Numerous erythrocytes, neutrophils, and individual peritoneal cells are visible. H&E staining, magnification 400×. **GLIŃSKA *et al.* (2013)**



Smear of peritoneal fluid sediment. Erythrocytes, neutrophils, and individual lymphocytes are visible. H&E staining, magnification 200×. **GLIŃSKA *et al.* (2013)**

Burgess and Gaunt (2014) reported a case of peritonitis in a 9-month-old spayed female Dachshund, which was subjected to surgical removal of a gossypiboma that had adhered to the hehunum. During reevaluation, ultrasonography revealed abdominal fluid accumulation, in which intracellular small, ovoid to large segmentally constricted, rarely branching or budding structures with thin wall were seen. The organism was identified as ***Candida albicans***.

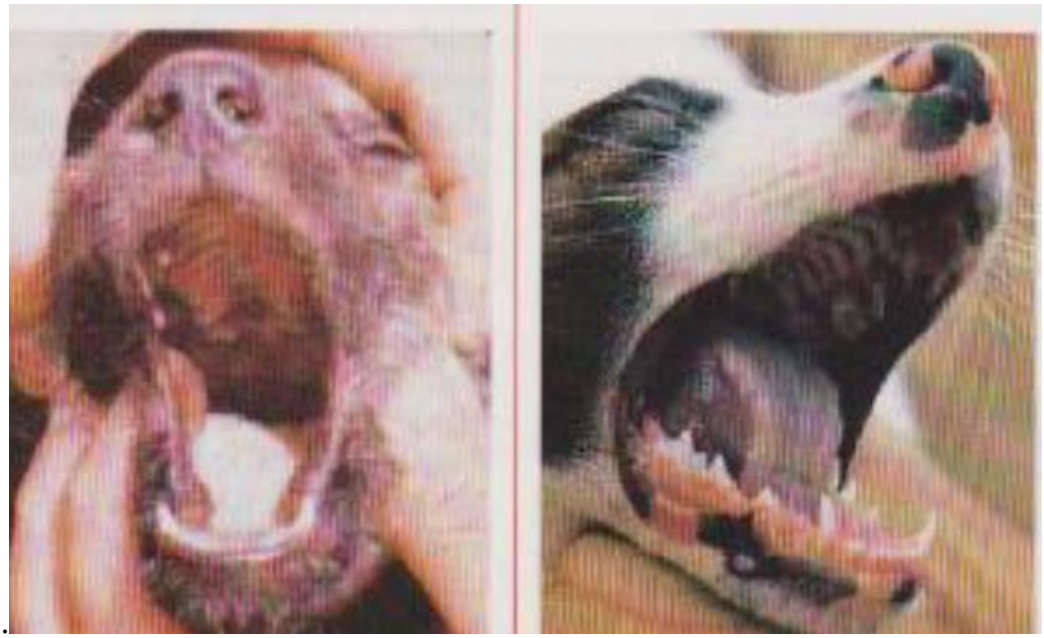


,budding, thin-walled extracellular structures **Burgess and** Gaunt (2014)

1.3.4. Oral candidosis

Refai (1986) reported 9 puppies, 1-3 months old with oral inflammation and white coating of the whole tongue following treatment with antibiotics. Puppies, which were not treated with antibiotics showed only inflammation at the edges of the tongue. Cultures from both cases yielded pure colonies of *Candida albicans*. All puppies responded to treatment with nystatin suspension 4 times daily for 12-13 days.

Refai *et al.* (1986) performed an experimental infection of 6 puppies with ***Candida albicans.*** The puppies were divided into 3 groups, each of two animals. The puppies in group 1were injected i.m. with 10 mg per kg tetracyclin hydrochloride daily for 5 successive days, then the puppies together with those of group 2 were streaked on their tongues and cheeks with a pure culture of Candida albicans. The puppies in group 3 were left untreated as contact control. All animals were kept under observation for one month. Oral lesions appeared only in group 1 seven days post infection in the form of glossitis and the tongue was covered with a thick creamy white pseudomembrane, which was easily removed leaving an inflamed surface. Candida albicans was reisolated from the oral lesions.



extensive white coating of the tongue of 2-month-old puppy, following treatment with chloramphenicol and tetracyclin, Inflammation of the tongue edges through Candida albicans in a 3-month-old puppy, which was not treated with antibiotics

Mancianti *et al.* (1992) examined 35 FIV-seropositive cats and 55 FIV-seronegative matched cats were examined for yeasts (**oropharyngeal swabs**) and dermatophytes (hair brushings). The frequency of isolation of ***Candida albicans*** and Cryptococcus neoformans was significantly higher in the former group. The only dermatophyte isolated was Microsporum canis. Its prevalence was three times higher among FIV-infected cats than among control animals.

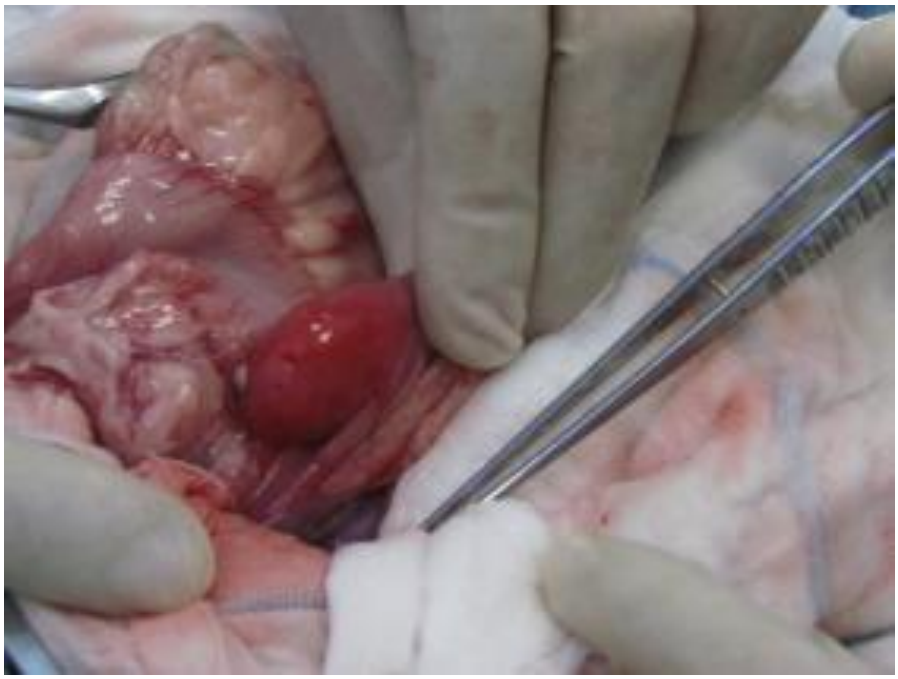
Milner *et al.* (1997) presented a 3-year-old German shepherd dog with a history of lifelong episodic diarrhoea. An adverse reaction to food was considered the most likely cause of the diarrhoea. The dog had received prolonged antibiotic therapy for most of its life as well as receiving probiotics containing the yeast *Saccharomyces cerevisiae* (syn. S. boulardi) for a year before referral. The probiotic was discontinued 2 months before to referral. Examination and culture of faecal samples identified yeast-like organisms, S. cerevisiae and ***Candida famata.*** S. cerevisiae has been isolated from humans in association with predisposing conditions such as prolonged sojourns in hospital, immunosuppression, broad-spectrum antibiotic therapy and prosthetic devices, but is regarded as non-pathogenic in humans and is rarely associated with disease in animals. C. famata has been isolated from animals, humans and the environment, but is regarded as a very rare pathogen. No evidence of immunosuppression was found in the dog. The presence of yeasts in the faecal isolates and the history of prolonged use of antibiotics and probiotics with a concurrent adverse reaction to food, suggest that conditions may have occurred within the bowel that made it possible for the yeasts to colonize parts of it. This has apparently not been reported before.

Jadhav and Pal (2006) cultured oral swabs from 34 dogs showing symptoms of **stomatitis** or **gingivitis** such as anorexia, halitosis, bleeding within the oral cavity, dysphagia, ptyalism (salivation) and submandibular lymphadenopathy for isolation of the causative agent. ***Candida albicans*** was isolated from four (11.8%) dogs. The isolates were sensitive to clotrimazole, fluconazole and amphotericin-B but were resistant to nystatin. The routine application of Pal's sunflower seed medium and Narayan stain in microbiological laboratories is highly emphasized. It was recommended that the role of C. albicans, as the etiologic agent of canine stomatitis, should be carefully investigated in various clinical related disorders of dogs as well as in other animals.

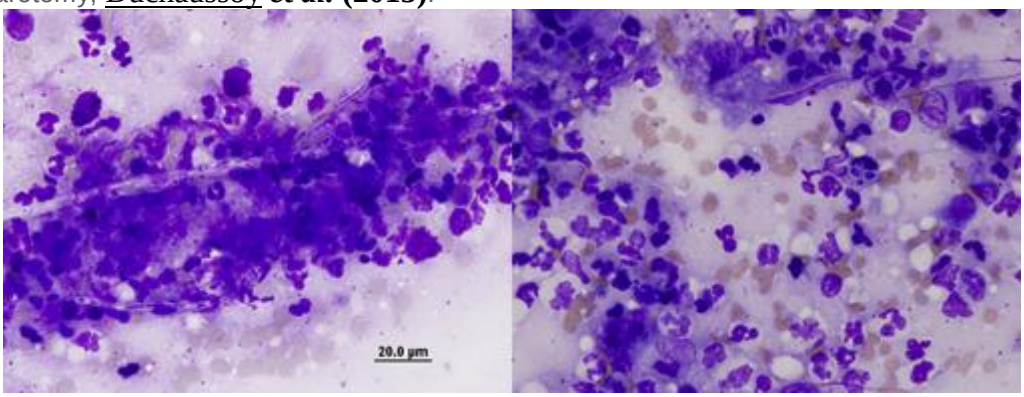
Biegańska *et al.* (2014) reviewed the literature data referring to opportunistic mycoses in pet dogs and cats suffering from other concurrent diseases, comparable to human medical disorders with high risk of secondary mycoses. The incidence of opportunistic mycoses is higher in such individuals, mostly because of their impaired immunity. The main risk factors are primary and secondary types of immunodeficiency connected with anti-cancer treatment or neoplastic disease itself. Moreover, literature data and the results of our investigations show that **Candida** yeasts are prevalent among diabetic animals and indicate that these fungi are the main etiological agents of secondary infections of the **oral cavity, GI** and urogenital tracts. Other important conditions possibly favoring the development of mycoses are concurrent infections of cats with FeLV and FIV viruses. Thus, in all cases of the mentioned underlying diseases, animals should be carefully monitored by repeated mycological examination, together with inspection of other parameters. Also,

the prophylaxis of opportunistic mycoses should be carefully considered alike other factors influencing the prognosis and the outcome of primary diseases.

Duchaussoy ***et al.*** **(2015)** reported a 3.5 year-old cat suffering from chronic vomiting. Abdominal ultrasonography revealed a focal, circumferential thickening of the wall of the duodenum extending from the pylorus aborally for 3 cm, and an enlarged gastric lymph node. Cytology of fine-needle aspirates of the intestinal mass and lymph node revealed an eosinophilic inflammatory infiltrate and numerous extracellular septate acute angle branching fungal-type hyphae. Occasional hyphae had globose terminal ends, as well as round to oval blastospores and germ tubes. ***Candida albicans*** was cultured from a surgical biopsy of the duodenal mass. No underlying host immunodeficiencies were identified. Passage of an abrasive intestinal foreign body was suspected to have caused intestinal mucosal damage resulting in **focal intestinal candidiasis**. The cat was treated with a short course of oral itraconazole and all clinical signs resolved.



Focal circumferential lesion in the proximal duodenum visualised during the exploratory laparotomy, Duchaussoy ***et al.*** **(2015)**.



Cytological preparation of the duodenal mass, modified Wright–Giemsa stain. Note the presence of visible hyphae (A) and round to oval blastospores and germ tubes of 2–3 µm (B), Duchaussoy ***et al.*** **(2015)**

1.3.5. Ocular candidiosis

Gerding *et al.* (1994) diagnosed a case of **ocular** and systemic candidiasis in an immunosuppressed and diabetic 12-year-old cat that initially was examined because of polyuria, polydipsia, and urinary tract disease. Bilateral recurrent corneal erosions and chorioretinitis, urinary tract infections attributable to bacteria or Candida sp, and renal dysfunction developed during the next 2 months. Examination of corneal scrapings revealed spherical to oval, budding, yeast-like cells. The cat's condition progressively deteriorated, and it was euthanatized. Toxoplasmosis was diagnosed by fecal flotation and from serum titers, and pituitary-dependent hyperadrenocorticism was detected at postmortem histologic evaluation. Candida budding yeasts and pseudohyphae with blastospores were detected in the corneas, vitreous bodies, retinas, CNS, pharynx, trachea, esophagus, kidneys, and urinary bladder at postmortem examination.

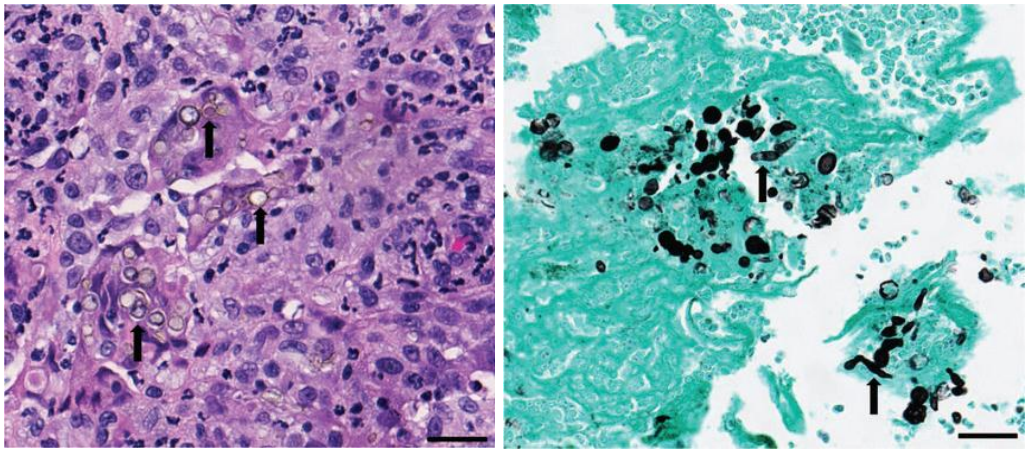
Linek (2004) presented a case of **mycotic endophthalmitis** in the dog caused by Candida albicans. The 3-year-old dog had a history of bloody diarrhea 3 months previously. The dog presented with acute signs of unilateral panuveitis. Aqueocentesis, vitreocentesis, and routine blood tests were performed but did not contribute to the diagnosis. The posterior segment could not be visualized because of flare and fibrin. On day 7 ultrasonography showed retinal separation which progressed to vitreous compartmentalization and abscessation by day 14. Three weeks after onset, glaucoma developed and enucleation was performed. Histology revealed the yeast **Candida** to be the causative agent. Post-enucleation serum Candida antibody titer was 1 : 640 (human threshold 1 : 120), as determined by agglutination test. A relapse of enteric signs 3 months later led to the diagnosis of chronic lymphocytic enteritis. A hematogenous route of infection was suspected.

Enders *et al.* (2016) described the clinical presentation, diagnosis, histologic lesions, and outcome of endogenous mycotic **endophthalmitis** secondary to candiduria in a three-year-old female spayed Dachshund. The dog was being treated for Evans syndrome for one month prior to being diagnosed with candiduria and fibrinous uveitis OS. The left eye was enucleated due to secondary glaucoma, and the fungal urinary tract infection was treated successfully. Uveitis developed in the contralateral eye with relapse of the urinary tract infection in the following weeks. The right eye was medically managed until secondary glaucoma developed and was subsequently enucleated. Histopathology of both eyes showed evidence of endophthalmitis with intralesional fungal organisms, consistent with Candida spp. Ocular candidiasis is rare in dogs. To the authors' knowledge, this is the first report of endogenous mycotic endophthalmitis with concurrent candiduria in a dog.

1.3.6. Candida rhinitis

Lamm *et al.* (2013) reported a 9-year-old female spayed Domestic Medium Hair cat with a 2-week history of sneezing, which progressed to swelling over the nasal planum. The cat had been under veterinary care for inflammatory bowel disease and had been treated with 1.25 mg/kg prednisolone once a day for approximately 1 year. On physical examination, an approximately 2-3 mm diameter, round polypoid pink soft-tissue mass was protruding slightly from the right nostril. Through histologic examination of representative sections from the mass, there was a severe

diffuse infiltrate of epithelioid macrophages and neutrophils that surrounded frequent 15-20 µm yeast organisms.It was diagnosed as Granulomatous rhinitis. A Grocott methenamine silver stain revealed the presence of pseudohyphae in addition to the previously noted yeast forms. Real-time polymerase chain reaction (PCR) for Cryptococcus neoformans, Ajellomyces dermatitidis (syn. Blastomyces dermatitidis), Coccidioides immitis, Ajellomyces capsulatus (syn. Histoplasma capsulatum), Malassezia spp., and Candida spp. was performed on the paraffin-embedded sample. The **PCR for Candida spp**. was positive; the product was then sequenced and was determined to be consistent with ***Candida parapsilosis***. Following the PCR diagnosis and prior to treatment of the infection, C. parapsilosis was cultured from a nasal swab. The infection in the cat in the current report was considered opportunistic and secondary to immunosuppression, following treatment for the inflammatory bowel disease.



Left:Fungal rhinitis in a cat due to *Candida parapsilosis* from a rostral nasal mass, marked infiltrate of macrophages and neutrophils that surround variably pigmented yeast organisms (arrows). Hematoxylin and eosin. Right:Yeast and pseudohypheal forms (arrows) are highlighted with a Grocott methenamine silver **Lamm *et al.* (2013)**

1.3.7. Candida pericarditis

Mohri *et al.* (2009) reported a 5-year-old castrated mongrel dog with anorexia and vomiting. Laboratory testing revealed immune-mediated hemolytic anemia (IMHA), and so treatment was initiated with multiple immune-suppressing drugs, achieving partial remission from IMHA. However, cardiac tamponade due to purulent **pericarditis** was identified as a secondary disease. Culture of pericardial fluid yielded numerous ***Candida albicans*** and multidrug-resistant Acinetobacter sp. Pericardiocentesis was performed, and the condition of the dog improved. However, the dog died the next day

1.3.8. Bronchopulmonary candidosis

Clercx *et al.* (1996) reported a seven-year-old, female golden retriever with a paroxysmal, chronic cough and dyspnea, dysphagia, facial pruritus, anterior uveitis, and deteriorating general condition. A severe, mixed interstitial and alveolar pattern, with poorly defined amorphous lesions, was seen on thoracic radiographs. Multiple, whitish nodules disseminated on the hyperemic respiratory mucosa were noted on bronchoscopy. Escherichia coli and Aspergillus fumigatus were cultured from the bronchoalveolar lavage. Granulomatous lesions in numerous organs were identified

during necropsy, and Aspergillus fumigatus and Candida spp. were cultured from **lung and kidney tissues**. Microscopic granulomatous lesions were compatible with mycotic infection; however fungal organisms were not observed.

1.3.9. Otitis externa

McKellar *et al.* (1990) reported otitis externa in a foxhound pack associated with ***Candida albicans.***

1.3.10. Systemic (disseminated) candidosis

Ehrensaft *et al.* (1979) injected 14 dogs intravenously with 10^7 *Candida albicans.* Seven of these animals were rendered leukopenic with cyclophosphamide. Both groups cleared organisms from the circulation. Normal dogs remained well and showed no gross or microscopic evidence of candidiasis at autopsy. In contrast, leukopenic animals died 1-6 days after receiving ***C. albicans*** and demonstrated a consistent picture of disseminated candidiasis. Features of this model similar to human infection include regular candiduria but only occasional candidemia despite severe tissue involvement. The reproducibility of this model system provides a basis for *in vivo* investigation of systemic fungal disease in the compromised host.

Holøymoen *et al.* (1982) reported a case of systemic candidiasis (***Candida albicans***) in a 1 1/2 year old dog is reported. Clinically, the first manifestation was enlargement of a superficial inguinal lymph node. Later several peripheral lymph nodes were affected and a fistulous opening appeared, communicating with an inflammatory process in the right humerus. Necropsy revealed gross lesions in the kidneys, pancreas and multiple lymph nodes. In addition, microscopic lesions were observed in the myocardium and the bone marrow of the right humerus. The lesions, which contained large fungal colonies, were mainly granulomatous with numerous multinuclear giant and epitheloid cells, but necrosis and suppuration were also evident. The site of invasion is not known. However, a previous perianal and abdominal dermatitis, which was treated locally with antibiotics and corticoids, could possibly have been a mycotic infection.

Gerding *et al.* (1994) diagnosed a case of ocular and **systemic candidiasis** in an immunosuppressed and diabetic 12-year-old cat that initially was examined because of polyuria, polydipsia, and urinary tract disease. Bilateral recurrent corneal erosions and chorioretinitis, urinary tract infections attributable to bacteria or Candida sp, and renal dysfunction developed during the next 2 months. Examination of corneal scrapings revealed spherical to oval, budding, yeast-like cells. The cat's condition progressively deteriorated, and it was euthanatized. Toxoplasmosis was diagnosed by fecal flotation and from serum titers, and pituitary-dependent hyperadrenocorticism was detected at postmortem histologic evaluation. Candida budding yeasts and pseudohyphae with blastospores were detected in the corneas, vitreous bodies, retinas, CNS, pharynx, trachea, esophagus, kidneys, and urinary bladder at postmortem examination.

Clercx *et al.* (1996) reported a seven-year-old, female golden retriever with a paroxysmal, chronic cough and dyspnea, dysphagia, facial pruritus, anterior uveitis, and deteriorating general condition. A severe, mixed interstitial and alveolar pattern,

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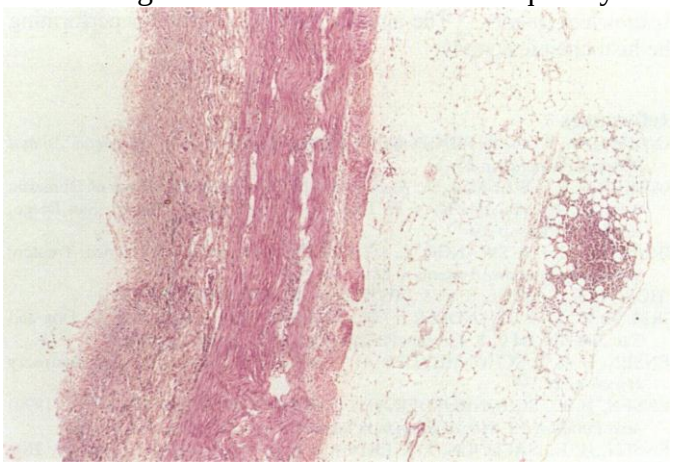
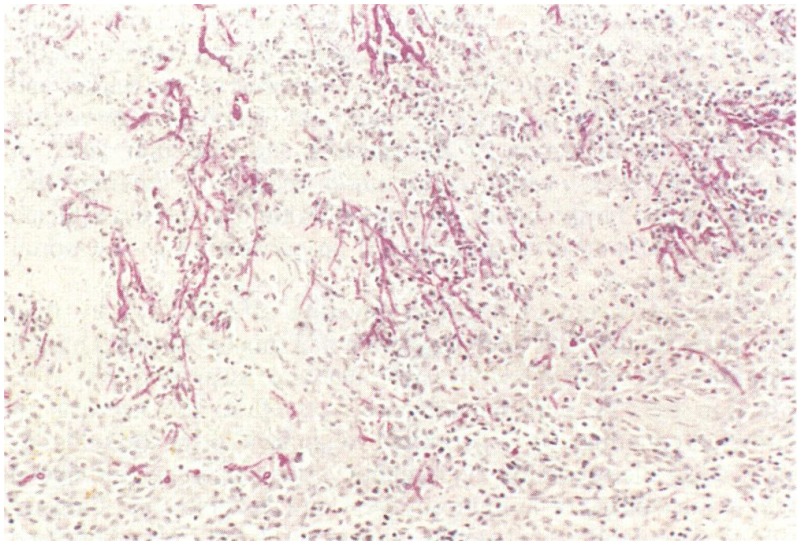
Rodriguez *et al.* (1998) described a case of intestinal and **systemic candidiasis** in a rottweiler puppy which had been infected with canine parvovirus (cpv). A two-and-a-half-month-old female rottweiler showed depression, anorexia and lethargy followed by an acute onset of fever (41°C), watery vomitus and bloody diarrhoea. faecal haemoagglutination test for cpv was positive. Ten days after the onset of clinical signs the dog died showing intense dehydration and cachexia. Gross lesions were most pronounced in the duodenum, jejunum and ileum. Tissues from the gastrointestinal tract, liver, kidney, spleen, mesenteric lymph nodes, heart, lung, brain and urinary bladder were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and Grocott' s methanamine silver (GMS). Branching septate hyphae and pseudohyphae with small blastospores invading the epithelial lining and lamina propria extended irregularly to the submucosa and muscular layers. Numerous granulomas were observed in the peritoneum surrounding the small intestine, liver, spleen andkidneys. All PAS- and GMS-positive elements within lesions were unequivocally identified as **Candida species** as a strong and uniform reactivity was obtained only with a heterologously absorbed Candida-specific antibody. Pyogranulomatous lymphadenitis, splenitis, hepatitis, nephritis, pneumonia and myocarditis were characterised by nonencapsulated foci in which centres of necrotic cellular debris were surrounded by a moderate infiltration of neutrophils, macrophages, lymphocytes and some multinucleated cells. The central areas of the granulomas contained numerous PAS- and GMS-positive oval to round blastospores mixed with pseudohyphae comprising chains of elongated yeast-like cells, or with tubular septated hyphae growing in a radiating pattern and morphologically identical to the organisms described in the intestine. Invasion of blood vessels and consequent development of thrombosing vasculitis were observed frequently.



Small intestine. Necrosis of the mucosa and a focus of granulomatous peritonitis. H&Eand eosin x 25,
Rodriguez *et al.* (1998)



Spleen. Necrosis with mycotic proliferation in a lymphoid follicle. PAS, **Rodriguez *et al.* (1998)**

Schoeniger (2002) reported a6-year-old, male German shepherd dog suffering from diarrhoea, vomiting, weight loss, polyuria and polydypsia, and persistent leukopenia. Reported pertinent clinicopathological data included a CBC consistent with pancytopenia characterized by neutropenia, monocytopenia, lymphopenia, thrombo-cytopenia and anemia, and a bone marrow aspirate revealing marked myeloid hypoplasia and mild erythroid and megakaryocytic hypoplasia. Per clinical history, the dog was icteric, had elevated liver enzymes (ALT, ALKP and GGT) and a prolonged activated partial thromboplastin time (PPT). Gross Findings: The carcass was emaciated, icteric and had multiple petechiae, ecchymotic and/or effusive hemorrhages within the subcutis, diaphragm, intercostal muscles, lungs, liver, mesenteric lymph nodes, kidney, urinary bladder, gastric and intestinal mucosa, and serosa. The gingival mucosa had multiple ulcers measuring 1.0 x 0.5 cm in greatest dimension. Pleural and abdominal cavities both contained serosanguinous effusions admixed with fibrin strands. Fibrinous strands covered serosal surfaces of the diaphragm, lungs, liver, stomach and intestine and caused adherence between the diaphragm and liver and between intestinal loops. The liver was diffusely yellow-green, diffusely enlarged and friable with multiple perivascular necrotic foci which measured 0.3 cm in greatest dimension and were rimmed by hemorrhage. Mesenteric lymph nodes were diffusely enlarged, dark-red and bulged on cut surface. Renal cortices were olive green and papillae had orange discoloration. Kidneys contained multiple acute and subacute, 0.3 cm in diameter cortical infarcts characterized by wedge-shaped cortical foci which were either red and slightly raised or tan, slightly depressed and rimmed by hemorrhage. The bone marrow of femur, humerus, several vertebrae and ribs was diffusely yellow and fatty. Histopathologic findings: Primary hepatic lesions were multifocal, periportal and centrilobular necrotizing hepatitis and necrotizing vasculitis. There were numerous intralesional 4-7 µm pseudohyphae and 3-5 µm blastospores. Necrotic foci with similar intralesional pseudohyphae and blastospores were present within mesenteric lymph nodes. Renal lesions included multiple septic cortical infarcts characterized by coagulation and liquefactive necrosis, hemorrhage, infiltration with viable and degenerated neutrophils and numerous intralesional small, Gram positive, coccoid bacteria. The hypocellular bone marrow contained primarily adipose connective tissue and hemosiderin-laden macrophages. There was marked depletion of myeloid precursor cells and mild

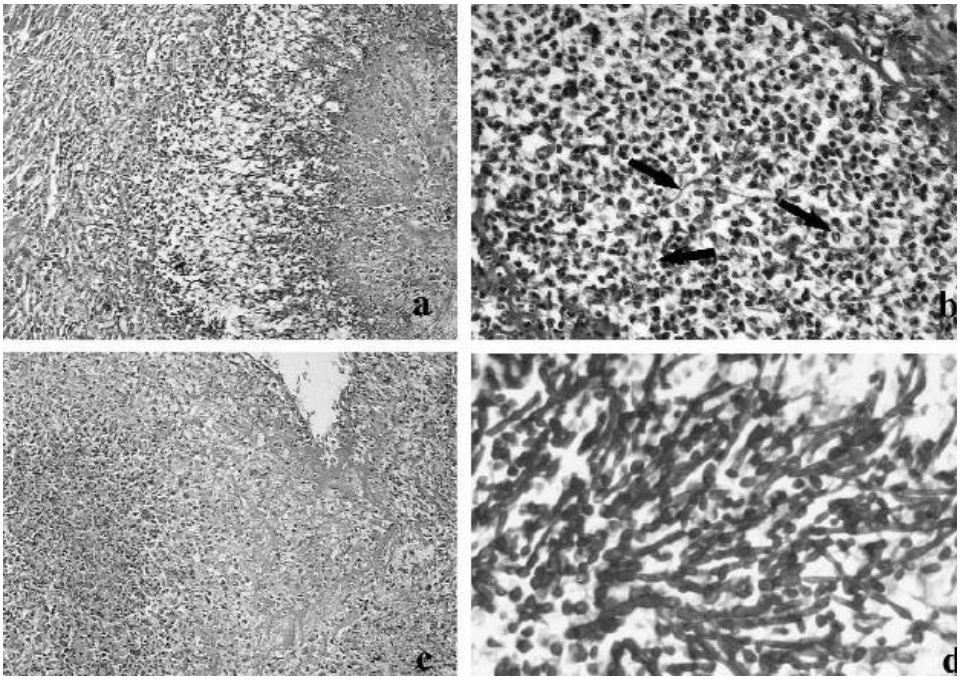
depletion of erythroid precursor cells and megakaryocytes. *Enterococcus spp*. was isolated from liver, kidney and spleen. ***Candida* (*Torulopsis) glabrata*** was isolated from liver tissue.

Heseltine *et al.* (2003) described an 11-year-old spayed female Scottish Terrier with **systemic candidiasis**. The diagnosis was made on the basis of results of microbiologic culture of specimens from urine and venous catheters and histologic examination of tissues obtained post mortem. Factors that predisposed the dog of this report to systemic candidiasis included diabetes mellitus, corticosteroid and broad-spectrum antimicrobial administration, venous and urinary catheterization, and administration of nutrition parenterally. The development of pyrexia and leukocytosis in dogs with risk factors that predispose to Candida species infections warrants evaluation via microbial culture of specimens from urine and vascular catheters used in those dogs.

Brown *et al*. (2005) described a **systemic Candida spp. infection** in a dog with no obvious underlying deficiency in host resistance. Cytopathology, histopathology, transmission electron microscopy, and immune-histochemical staining were used to determine the etiology of the causative agent.

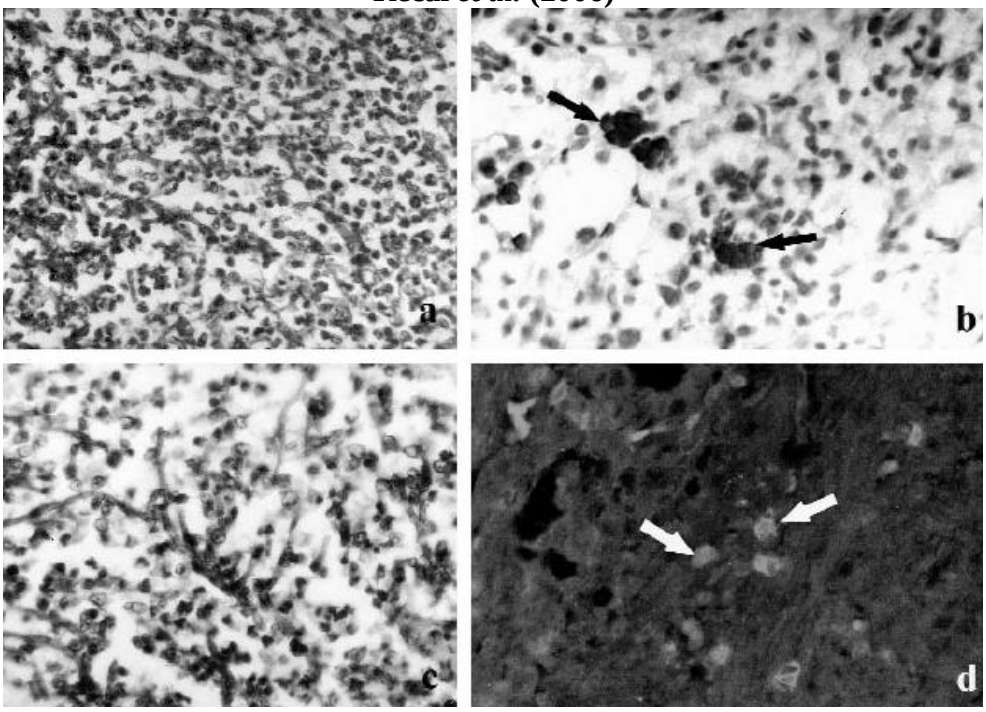
Kuwamura *et al.* (2006) reported **a** 4-year-old male Shiba dog initially presented with pain of an undetermined origin and hypersensitivity to touch. Seven days later, the dog developed ataxia, hind-leg weakness and knuckling. The dog died on 20 days after presentation. Postmortem examination revealed a mass in the body of thoracic vertebra. Histopathologically, the mass consisted of granulomatous inflammation, including fungal organisms that were immunohistochemically positive for ***Candida albicans***. Similar granulomatous lesions were observed in the systemic lymph nodes, kidneys, pancreas, spleen, prostate gland, thyroid glands and heart. This case was diagnosed as **systemic candidiasis** with spondylitis.

Recai *et al.* (2006) described the clinical course, cultural and pathological findings of **systemic candidiasis** in 3 dogs. Pathologically, pyogranulomatous lesions in various organs were present in all dogs. Blastospores, pseudohyphae, and true hyphae of ***Candida albicans*** were observed with periodic acid Schiff, Gomori's methenamine silver, and immunohistochemistry. Administrations of broad-spectrum antibiotics and corticosteroids and, in one dog, parvoviral infection were thought to be predisposing factors leading to opportunistic infection. The combined effect of immunosuppressants and antibiotics might have led to Candida colonization and dissemination in these dogs



a: Epicardial surface of the myocardium. There is intense pyogranulomatous inflammation with widespread coagulation necrosis and many small developing *Candida* cells invading from the epicardium into the myocardium. Dog 1. H&E. x90. b: Pyogranulomatous pleuritis containing many inflammatory cells and small developmental forms of *Candida* cells. Dog 2. H&E. x180. c: Serosal surface of the jejunum. Pyogranulomatous lesion extending from the serosa to the omentum. Dog 3. H&E. x120. d: Pericardium showing PAS positive blastospores, long filamentous pseudohyphae, and hyphae. Dog 1. PAS. x360.

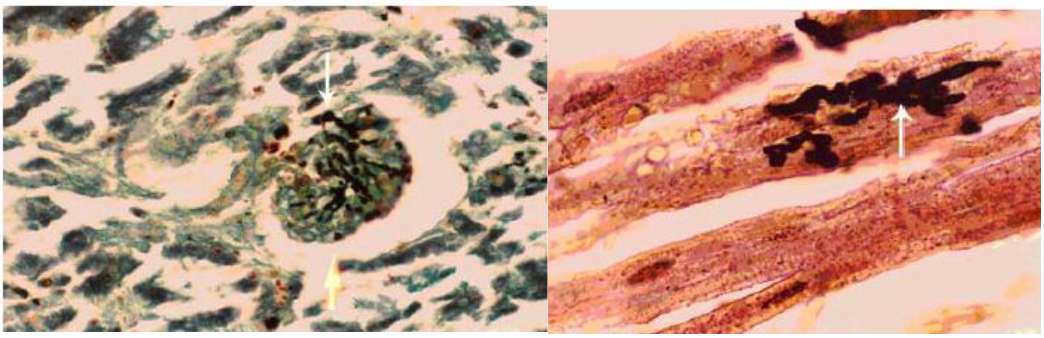
Recai *et al.* (2006)



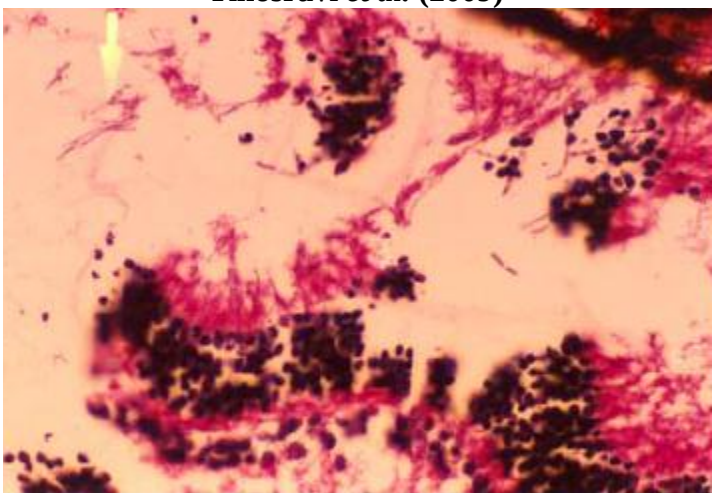
a: Immunohistochemical localizations of *Candida albicans* developmental forms located in the center o pyogranulomatous lesion on the pericardium. Dog 1. IP x360. b: Immunohistochemical demonstratior *Candida* cells invading from the epicardium into the myocardium. Dog 1. IP x360. Inset: blastospores ir the spleen with no recognizable inflammatory lesion. Dog 1. IP x360. c: Fungal developmental form and inflammatory cells on the peritoneal surface of the liver. Dog 2. IP x360. d: Weak positive reactior for parvoviral antigens in macrophages in the lamina propria of the small intestine. Fluorescein antibody technique. Dog 3. x420.

Recai *et al.* (2006)

Khosravi *et al.* (2009) reported the mycological and histopathological findings of **experimental disseminated candidiasis** in dogs. The dogs were immunosuppressed by intravenous administration of cyclophosphamide and after 5 days, they were challenged with 1 × 105 blastospores of ***C. albicans*** by intravenous injection. Both mycological and histopathological examinations were performed for detection of *Candida* in various tissues. The results showed that the highest counts of *C. albicans* were recovered from the lungs, followed by the kidneys, heart and liver on day 2 after challenge. The presence of yeast mixed with hyphal forms of *C. albicans* was confirmed in all tissues. In most tissues, the yeast cells of *Candida* were predominant, whereas hyphal forms, particularly true hyphae, were mostly found in the brain and eyes.



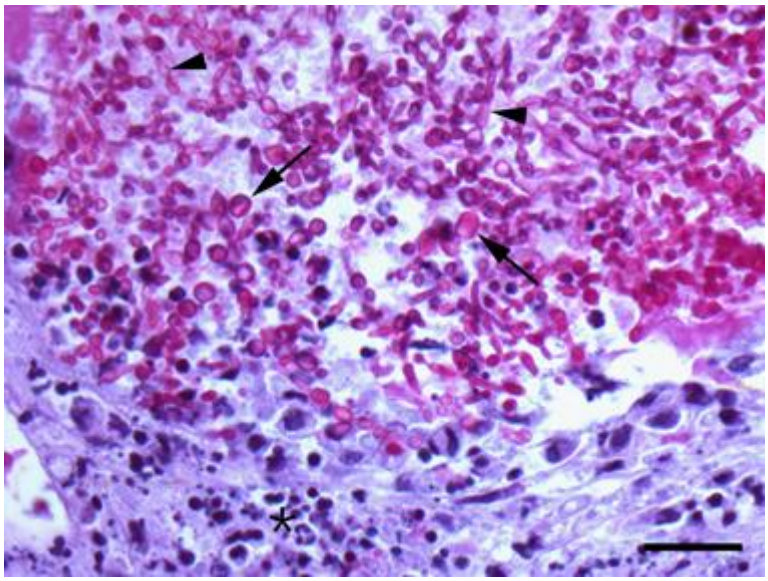
Left:Colonization of numerous yeast cells along with pseudohyphae in glomerular tufts of kidney. Right: yeast cells along with pseudohyphae in muscular fibers of the myocardium, **Khosravi *et al.* (2009)**



Numerous true hyphae of C. albicans in retina of the eye, **Khosravi *et al.* (2009)**

Matsuda *et al*. (2009) reported a 5-year-old female miniature dachshund presenting with persistent vomiting and diarrhea. The dog had two concurrent rare pathological conditions: **systemic candidiasis** and mesenteric mast cell tumor with multiorgan metastases. Neoplastic mast cells formed mass in the mesentery of the cecal-colonic region and were also found in the liver, spleen, kidneys, lungs, adrenal grands, ovaries, bone marrow and other tissues. The cells had intracytoplasmic granules with metachromasia and were immunohistochemically positive for c-kit and histamine. Granulomatous lesions with fungal organisms were present in the heart, lungs, kidneys, pancreas, subserosal and surrounding adipose tissue of the duodenum, thyroid glands and mesenteric mass, and phagocytosed organisms were detected in the liver and bone marrow. Bacteriologically and immunohistochemically, the fungi were consistent with ***Candida albicans***.

Rogers *et al*. (2009) identified fungal sepsis was in a 2-year-old dog following intestinal dehiscence 4 days after abdominal surgery. Septic peritonitis was identified at admission and evidence of dehiscence at the previous enterotomy site was found during an exploratory laparotomy. Both Gram-positive cocci and ***Candida albicans*** were cultured from the abdominal cavity. Candida sp. was also subsequently cultured from a central venous catheter. Euthanasia was performed due to failure to respond to therapy. Fungal organisms, morphologically consistent with Candida spp., were found in the lungs and kidney on postmortem histopathologic examination indicating **disseminated candidiasis**.



A section of kidney showing PAS-positive oval, 5–6 µm, budding yeast-like cells (blastoconidia) (arrows) and segmentally constricted pseudohyphae (arrowheads) within the renal papilla. There are multifocal regions of necrosis (*) (400 × magnification, scale bar=40 µm), **Rogers *et al*. (2009)**.

Gershenson *et al*. (2011) presented a 2 yr old spayed female German shepherd with a chief complaint of acute onset paraparesis and weight loss. At presentation, the dog was pyrexic, nonambulatory, and had generalized muscle wasting. Neurolocalization was consistent with a thoracolumbar spinal cord lesion. An abdominal ultrasound was performed and revealed a focal dilation (4 cm) of the terminal aorta with evidence of blood stasis consistent with an aortic aneurysm. The dog was euthanized shortly after admission to the hospital and a post mortem examination was performed. Fungal organisms were identified in the aortic aneurysm as well as from the thoracic vertebrae, mesenteric lymph nodes, axillary lymph nodes, spleen, kidneys, liver,

lungs, and heart. Although the morphology was consistent with **Candida spp**., immunohistochemistry and **PCR** could not definitively identify the causative organism.

Skoric *et al.* (2011) reported ***Candida albicans*** as the aetiological agent of multi-systemic infections in dogs. A two year-old female Hovawart dog was presented with marked alteration in its health condition characterised by weakness, fever, anorexia, abdominal pain, cachexy and generalized lymphadenopathy. A radiograph of the abdominal cavity showed several non-specific nodular lesions in the mesentery, ranging in size up to 10 cm in diameter. At necropsy, extensive enlargement of lymph nodes and the presence of numerous whitish to grey nodules of different sizes in several organs were evident. Histopathological examination revealed pyogranulomatous inflammation characterized by large areas of necrosis surrounded by neutrophilic granulocytes, macrophages, multinucleated giant cells, and a variable admixture of lymphocytes and fungi-like organisms in in all affected organs. Numerous branching hyphae, subsequently identified by mycological cultivation as Candida albicans, were observed. A periodic acid Schiff (PAS) reaction to prove the presence of fungi in tissues was positive. Examination of tissue samples of affected organs using polymerase chain reaction (**quantitative Real-Time PCR**) and cultivation was negative for the presence of all members of the Mycobacterium tuberculosis complex

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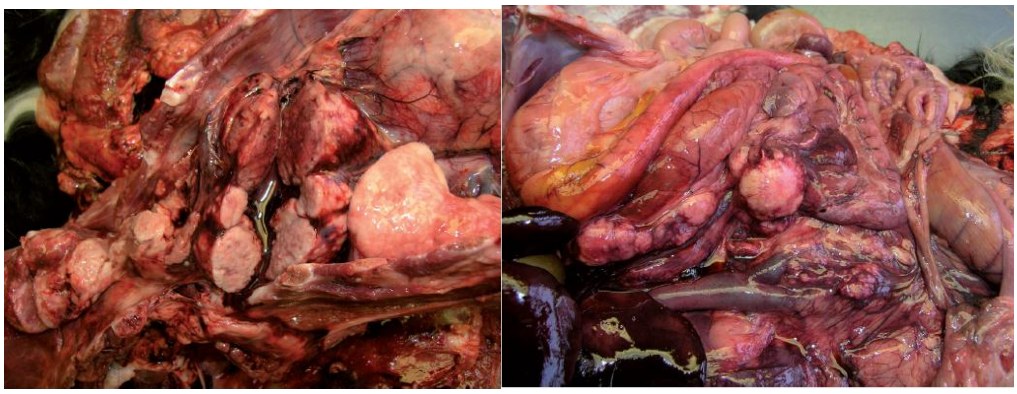
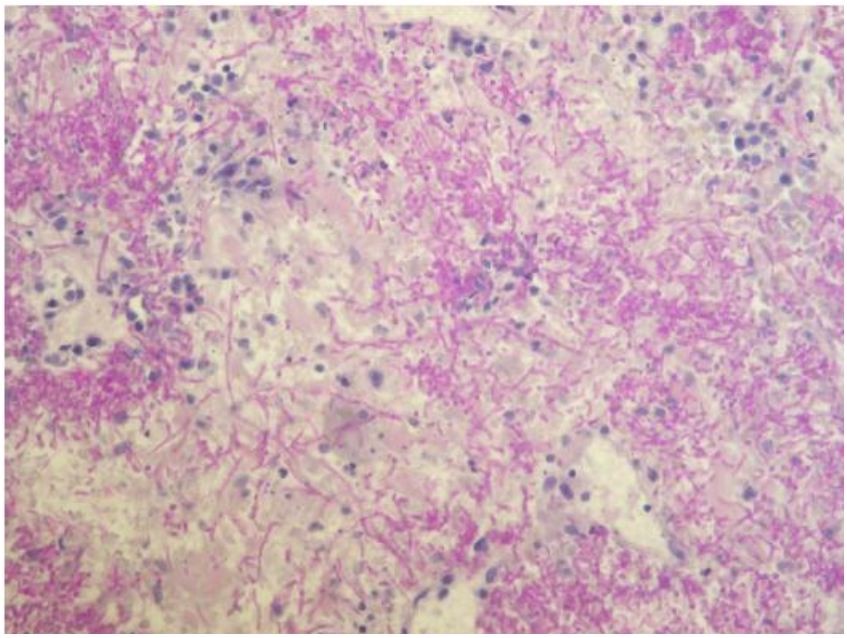


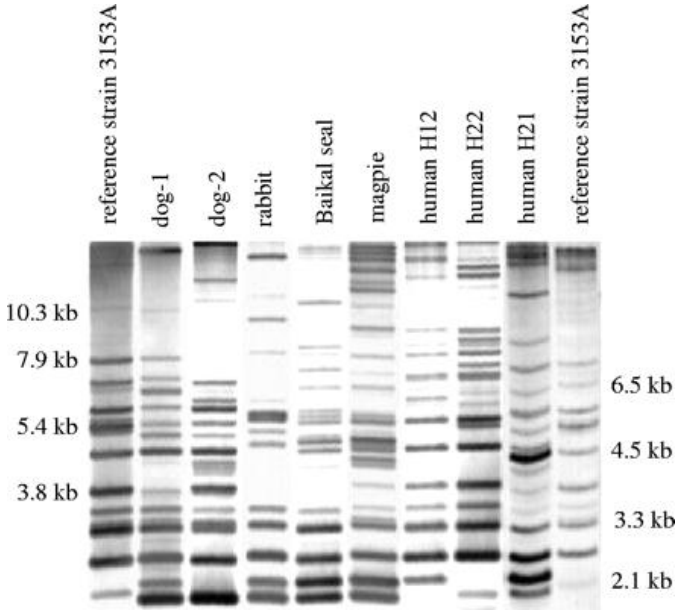
Left:Markedly enlarged mediastinal lymph nodes with necrotic focci on the cut section, Right: Multiple whitish pyogranulomas in the mesentry and lymph nodes, **Skoric *et al.* (2011)**



PAS positive hyphae of C. albicans in central necrosis of the pyogranuloma, **Skoric *et al.* (2011)**

1.4. Reports on molecular studies of Canida species isolated from dogs and cats

Edelmann *et al*. (2005) analyzed ***Candida albicans*** isolates from different human and animal individuals by **Ca3 fingerprinting**. They obtained no evidence for host-specific genotypes and for the existence of species-specific lineages, even though a certain degree of separation between human and animal isolates was found. Therefore, animals could potentially serve as reservoirs for human Candida infection..



Ca3 fingerprints of *Candia albicans* isolates. EcoRI-digested genomic DNA from eight animal and human *C. albicans* isolates was subjected to Southern blot analysis applying a digoxigenin-labeled Ca3

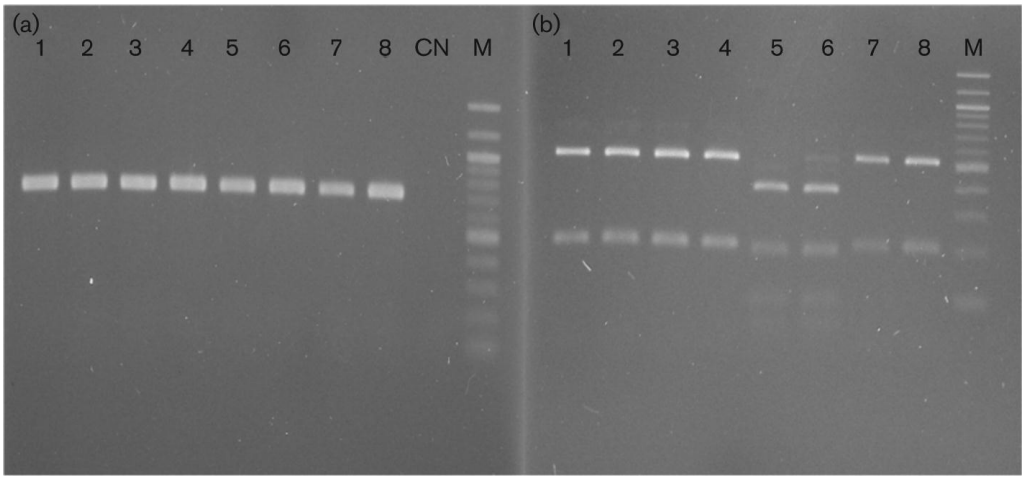
sequence. DNA from *C. albicans* strain 3153A (13) was included as a molecular weight and band intensity standard. **Edelmann *et al*. (2005)**

Ozawa *et al.* (2005) identified an isolate from urine of 10-year-old male Shih Tzu dog (7.0 kg) with cystitis molecularly as ***Candida tropicalis*** and determined its minimum inhibitory concentration (MIC) by a microdilution method. The 25S **ribosomal DNA sequence analysis** indicated that the clinical isolate was essentially identical to that of C. tropicalis and distinct from other Candida species. The MIC(50) and the MIC(90) of fluconazole **(**FLZ) for the clinical isolate of C. tropicalis was 6.25 and 25 microg/ml, respectively, indicating that susceptibility of the clinical isolate of C. tropicalis to FLZ was less than for other strains of C. tropicalis as well as C. albicans. The molecular analysis as presented in this study assisted the diagnosis of candidiasis by identifying the yeasts in urine samples within 2 days. The patient dog, was successfully treated with itraconazole.

Brito *et al.* (2009) used PCR amplification followed by agarose gel electrophoresis (**PCR-AGE**) and the manual method (morphological characteristics, biochemical profiles and culturing on CHROMagar-Candida) and VITEK 2 automated method to test a total of 30 fungal strains from dog sources. The strains were obtained from cases of dermatitis, otitis externa and from the ears, oral mucosa, vaginal mucosa, prepuce and perianal region of clinically normal dogs. After identification as Candida yeasts by the manual method, the strains were analyzed using both VITEK and PCR-AGE methods. Isolates of C. parapsilosis ATCC 22019, C. krusei ATCC 6258 and C. albicans ATCC 10231 were included as controls. The universal primers ITS1, ITS3 and ITS4 were used in two independent PCR reactions. Of 30 yeast isolates, 3 isolates (Saccharomyces cerevisiae, C. rugosa and C. parapsilosis) that were incompletely identified by the manual method were identified with the PCR-AGE and VITEK methods. The results revealed a 96.7% and 86.7% concurrent identification between the PCR-AGE and VITEK methods versus the manual method, respectively. PCR-AGE showed a greater level of concordance with the manual method, besides being faster and more sensitive than the other methods examined, and is therefore indicated for routine diagnostic testing of Candida spp. strains from veterinary sources.

Brilhante *et al.* (2014) performed a study to identify strains of the ***Candida parapsilosis*** complex isolated from animals. They used 28 isolates of C. parapsilosis sensu lato recovered from clinically healthy animals (15 dogs, 10 psittacines (granivorous/frugivorous birds), two raptors (carnivorous birds) and one *Macrobrachium amazonicum* prawn). The strains were characterized phenotypically, followed by **molecular identification** of the species through PCR-restriction enzyme analysis. Molecular identification of the *C. parapsilosis* strains was performed according to the protocol defined by Tavanti *et al.* (2005), using primers S1F (5′-GTTGATGCTGTTGGATTGT-3′) and S1R (5′-CAATGCCAAATCTCCCAA-3′) for amplification of the partial sequence (716 nt) of the gene that encodes the secondary alcohol dehydrogenase (SADH). The amplified DNA was subsequently digested for 90 min with the *Ban*I enzyme (New England Biolabs). The digestion products were submitted to electrophoresis on 2% (w/v) TAE/agarose gel containing ethidium bromide (0.05 μg ml $^{-1}$) and were then visualized with a transilluminator. The results obtained were compared with the digestion patterns of the control strains *C. parapsilosis* ATCC 22019, *C. orthopsilosis* ATCC 96139 and *C. metapsilosis* ATCC

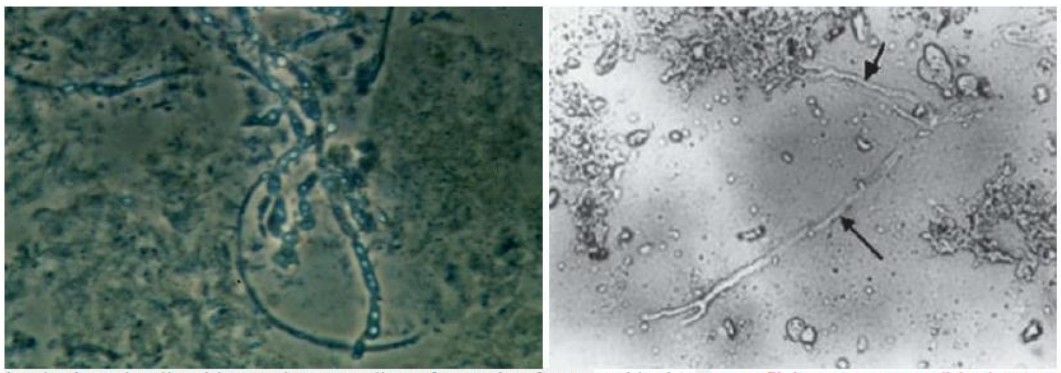
96143. Molecular analysis showed 13 C. parapsilosis sensu stricto, 10 Candida orthopsilosis and five Candida metapsilosis strains. In vitro resistance to fluconazole was observed in three strains of C. parapsilosis sensu stricto and two C. metapsilosis.



Classification of isolates using PCR and restriction enzyme analysis. (a) Representative gel confirming the identity of the strains as part of the *C. parapsilosis* complex (strains 1–8). (b) Digestion with *Ban*I allowing species classification: *C. parapsilosis sensu stricto*, 550–600 and 200 nt (strains 1, 2, 3, 4, 7 and 8); *C. metapsilosis*, 400 and 200 nt (strains 5 and 6). CN, negative control; M, molecular marker. **Brilhante *et al.* (2014)**

1.5. Diagnosis of candidosis

Direct microscopic examination of 10-40% KOH preparation or stained films by Gram, Giemsa, Parker's ink, calcofluor or methylene blue etc.



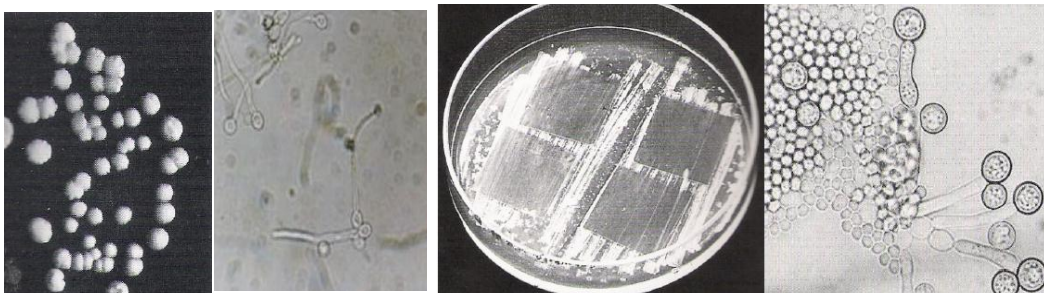
Lactophenol cotton blue wet preparation of scraping from oral lesion..www.flickr.com, www.slideshare.net

Isolation and identification

Samples are inoculated on Kimmig , Sabouraud dextrose , blood , Czapek Dox agar or chromogenic agar , incubated at 370C and 25oC for 2-3 days.

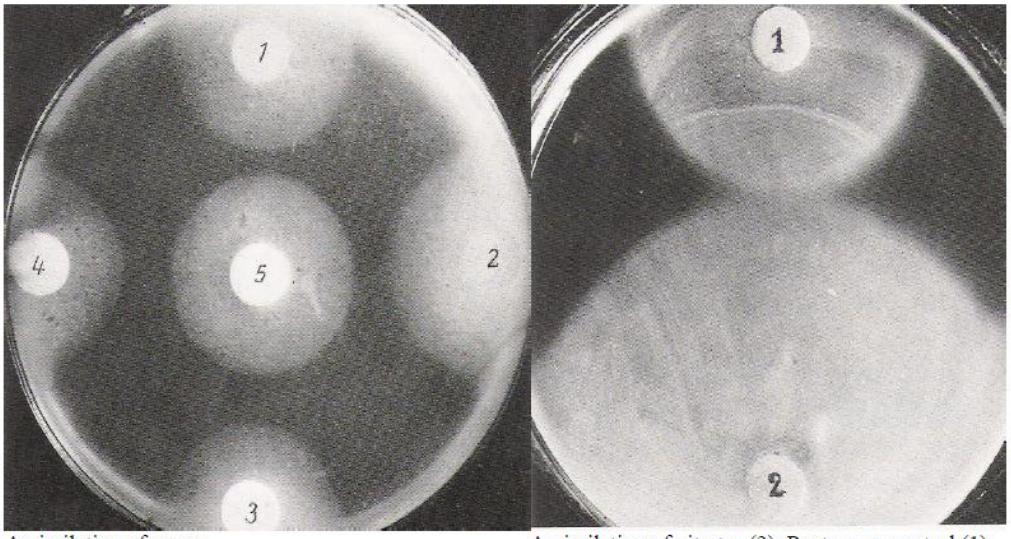
On Sabouraud dextrose agar, *C. albicans* develops within 24-48 h raised, creamy, opaque colonies of 1-2 mm in diameter. After several days of incubation, the colonies

show radiating outgrowths penetrating the medium. *C. albicans* is capable of producing yeast cells, pseudohyphae, true hyphae and chlamydospores. For germ tube testing a suspension of the suspected colony is made in 0.5 ml serum, incubated for 2-4 h at 370C and examined microscopically for the development of germ tubes, which extend from the cell without septum or constriction. After 72 h at 250C the inoculated plates are examined for pseudohyphae and chlamydoconidia.



C. albicans colonies Germ tubes Rice agar Pseudohyphae, chlamydospores

Fermentation and assimilation of sugars are done by the conventional methods or by the use of commercial kits



Assimilation of sugars Assimilation of nitrates (2). Peptone as control (1)

Serological diagnosis of candidosis

Many serological tests using soluble cytoplasmic antigen of Candida albicans cells are used for detection of antibodies in the serum of patients suspected to be infected with *Candida albicans*. Because of the ubiquitous nature of Candida species , these serologic tests are limited in discriminating between normal and disease levels of

antibodies. More specific tests are used for detection of circulating C. albicans surface antigens and cytoplasmic proteins. Serological tests commonly

1.6. Antifungal sensitivity and Treatment

Chan and Balish (1978) assessed the effect of Gram-negative sepsis (Escherichia coli) on the capacity of polymorphonuclear leucocytes (PMN) to phagocytize and kill ***Candida albicans***. The PMN's from septic dogs phagocytized C. albicans as well as PMN's from non-septic dogs. The PMN's from septic dogs that phagocytized C. albicans underwent a spontaneous lysis at a much higher rate than PMN's from non-septic dogs. A functional difference in PMN's from normal and septic dogs was indicated.

Ruthe *et al.* (1978) designed an experimental canine model was designed to evaluate the effect of granulocyte transfusions on systemic infection with ***Candida albicans*** in the granulocytopenic host. Each of a pair of dogs was rendered granulocytopenic with a single intravenous (i.v.) dose of cyclophosphamide (50 mg/kg body weight) and challenged with 10(6) Candida albicans organisms administered i.v. when granulocyte counts were less than or equal to 500/mm3. Granulocytes procured by leukofiltration were infused into six experimental dogs 1, 24, 48, and 72 hr after challenge with Candida. An average of 13 +/- 1.3 X 10(9) granulocytes were administered per infusion, producing an average 1-hr increment of 588 +/- 146 granulocytes/mm3 over the pretransfusion granulocyte count. Experimental and control dogs were killed 96 hr after challenge and organs examined grossly and by quantitative culture techniques to measure the extent of infection. All animals receiving granulocyte transfusions had significantly less tissue infection than nontransfused controls (p less than 0.05). It was concluded that granulocyte transfusions are effective in reducing the severity of infection by Candida albicans during periods of leukopenia.

Weber *et al.* (1985) evaluated the activity of **ketoconazole** in neutropenic dogs with **systemic candidiasis**. Five dog pairs were made neutropenic by intravenous cyclophosphamide (50 mg/kg) and challenged with either 10(6) or 10(7) colony-forming units (CFU) of ***Candida albicans***. Half of the dogs received ketoconazole (10 mg/kg) daily beginning 24 h after challenge. All were killed at 96 h and liver, spleen, and kidney were cultured. Of four dogs given 10(6) CFU, two untreated dogs had 9 X 10(3) to 1 X 10(5) CFU/g wet tissue, compared to 0 CFU in ketoconazole-treated dogs. With inoculum increased to 10(7) CFU, three untreated dogs had 2 X 10(4) to 3 X 10(5) CFU/g wet tissue, while three ketoconazole dogs had 0-5 X 10(3) CFU/g wet tissue. The effect of ketoconazole on autologous marrow reconstitution in dogs with systemic candidiasis was examined by infusing autologous cryopreserved marrow into four dogs one day after lethal whole body irradiation (800 rad). Once neutropenic, they were challenged with 10(7) CFU of C. albicans. Two dogs received no ketoconazole and died of disseminated candidiasis, without marrow reconstitution. Two dogs received ketoconazole for 25 days. Prompt marrow recovery occurred and they remained healthy. There was no evidence of infection at death. These studies quantitatively demonstrated the in vivo effectiveness of ketoconazole in reducing tissue infection with C. albicans in neutropenic dogs.

Fulton *et al.* (1992) diagnosed a case of ***Candida albicans*** urocystitis secondary to urethral stricture and administration of antibiotics was diagnosed in a cat by fungal

culturing of urine and examination of specimens. Surgical repair of the stricture and administration of **5-fluorocytosine** resulted in resolution of the cystitis. Related problems included anorexia and severe weight loss, which necessitated enteral nutritional support, dehydration, renal disease, and nosocomial Pseudomonas aeruginosa urocystitis.

Forward *et al.* (2002) used intermittent bladder infusion with clotrimazole for treatment of candiduria in a dog

Ozawa *et al.* (2005) identified an isolate from urine of 10-year-old male Shih Tzu dog (7.0 kg) with cystitis molecularly as ***Candida tropicalis*** and determined its minimum inhibitory concentration (MIC) by a microdilution method. The 25S **ribosomal DNA sequence analysis** indicated that the clinical isolate was essentially identical to that of C. tropicalis and distinct from other Candida species. The MIC(50) and the MIC(90) of **fluconazole (**FLZ) for the clinical isolate of C. tropicalis was 6.25 and 25 microg/ml, respectively, indicating that susceptibility of the clinical isolate of C. tropicalis to FLZ was less than for other strains of C. tropicalis as well as C. albicans. The molecular analysis as presented in this study assisted the diagnosis of candidiasis by identifying the yeasts in urine samples within 2 days. The patient dog, was successfully treated with itraconazole.

Cleff *et al.* (2011) evaluated the in vitro activity of the essential oil extracted from Origanum vulgare against sixteen Candida species isolates. Standard strains tested comprised C. albicans (ATCC strains 44858, 4053, 18804 and 3691), C. parapsilosis (ATCC 22019), C. krusei (ATCC 34135), C. lusitaniae (ATCC 34449) and C. dubliniensis (ATCC MY646). Six Candida albicans isolates from the vaginal mucous membrane of female dogs, one isolate from the cutaneous tegument of a dog and one isolate of a capuchin monkey were tested in parallel. A broth microdilution technique (CLSI) was used, and the inoculum concentration was adjusted to 5 x 10(6) CFU mL(-1). The essential oil was obtained by hydro-distillation in a Clevenger apparatus and analyzed by gas chromatography. Susceptibility was expressed as Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC). All isolates tested in vitro were sensitive to O. vulgare essential oil. The chromatographic analysis revealed that the main compounds present in the essential oil were 4-terpineol (47.95%), carvacrol (9.42%), thymol (8.42%) and terpineol (7.57%). C. albicans isolates obtained from animal mucous membranes exhibited MIC and MFC values of 2.72 μL mL(-1) and 5 μL mL(-1), respectively. MIC and MFC values for C. albicans standard strains were 2.97 μL mL(-1) and 3.54 μL mL(-1), respectively. The MIC and MFC for non-albicans species were 2.10 μL mL(-1) and 2.97 μL mL(-1), respectively. The antifungal activity of O. vulgare essential oil against Candida spp. observed in vitro suggests its administration may represent an alternative treatment for candidiasis.

Yurayart *et al.* (2013) determined and compared the susceptibility levels of yeasts isolated from dogs with and without seborrheic dermatitis (SD) using the disk diffusion method (DD) for itraconazole (ITZ), ketoconazole (KTZ), nystatin (NYS), terbinafine (TERB) and 5-fluorocytosine (5-FC) and the broth microdilution method (BMD) for ITZ and KTZ. The reliability between the methods was assessed using an agreement analysis and linear regression. 28 ***C. parapsilosis*** isolates were identified based on physiological characteristics and an approved molecular analysis. Only 46 - 60% of the tested C. parapsilosis isolates were susceptible to KTZ, TERB and 5-FC, but ITZ and NYS were effective against all. The frequency of KTZ- and ITZ-resistant

C. parapsilosis was 29% and 7%, and the MIC90 values were 1 µg/ml and 0.5-1 µg/ml, respectively. Regarding the agreement analysis 0.2-1% of very major errors occurred among C. parapsilosis. There were no significant differences in the yeast resistance rates between dogs with and without SD and a high rate of KTZ resistant was reported in C. parapsilosis.

Brilhante *et al.* (2014) performed a study to assess their in vitro antifungal susceptibility profile and in vitro production of virulence attributes. They used 28 isolates of C. parapsilosis sensu lato recovered from clinically healthy animals (15 dogs, 10 psittacines (granivorous/frugivorous birds), two raptors (carnivorous birds) and one *Macrobrachium amazonicum* prawn). The strains were characterized phenotypically, followed by molecular identification of the species through PCR-restriction enzyme analysis. The susceptibility of the strains to amphotericin B, itraconazole, voriconazole, fluconazole and caspofungin was assessed through broth microdilution. The results of the susceptibility tests indicated that the MIC range was 0.125–1 µg ml^{-1} for AMB, 0.03125–0.5 µg ml^{-1} for ITC, 0.03125–0.125 µg ml^{-1} for VRC, 0.5–16 µg ml^{-1} for FLC and 0.0625–2 µg ml^{-1} for CAS. Resistance to fluconazole was observed against three strains of *C. parapsilosis sensu stricto* and two of *C. metapsilosis*, whilst high MICs (2 µg ml^{-1}) were observed for caspofungin against one strain of *C. parapsilosis sensu stricto* and five of *C. orthopsilosis*.

Álvarez-Pérez *et al.* (2016) reported multi-azole resistance acquisition by ***Candida tropicalis*** after prolonged antifungal therapy in a dog with **urinary candidiasis**. Pre- and post-azole treatment isolates were clonally related and had identical silent mutations in the ERG11 gene, but the latter displayed increased azole minimum inhibitory concentrations. A novel frameshift mutation in ERG3 was found in some isolates recovered after resistance development, so it appears unlikely that this mutation is responsible for multi-azole resistance.

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2. Cryptococcosis in cats and dogs

2.1. Introduction

- Cryptococcosis is the most common systemic fungal disease in cats worldwide. Infections with *Cryptococcus* species may also occur in several other mammalian species, including dogs.
- Cryptococcosis in cats and dogs is caused by *Cryptococcus neoformans* and *Cryptococcus gattii*.
- **The environmental reservoir** of *C neoformans* is usually related to bird faeces, particularly pigeon droppings. However, this yeast has also been found in decaying trees, wood and plant debris, waterways and soil, all usually contaminated with bird excrement.
- **The epidemiology** of clinical disease depends largely on the species of infecting organism. *Cryptococcus neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D) are globally distributed and infect predominantly immunocompromised hosts. *Cryptococcus gattii* (serotypes B and C) has recently been recognized as a species distinct from *C. neoformans* based on molecular and mating type characteristics .
- **The primary route of infection** in cats and dogs is the nasal cavity, although, more rarely, transmission can also occur via cutaneous inoculation of fungal forms. The incubation period varies from months to years, with the source of infection often remaining unknown.

- **The most frequent clinical manifestation** of cats and dogs cryptococcosis is associated with the nasal form, but the disease can occur in several other distinct clinical forms, with involvement of the central nervous system (CNS), ocular, cutaneous, lymph nodes, and even pulmonary, abdominal and periarticular connective tissues.[3,4] Ocular lesions are a common manifestation of systemic cryptococcosis (observed in about one-third of clinical cases), primarily manifesting as multifocal chorioretinitis.
- **A definitive diagnosis** of cryptococcosis can be established using cytological examination, serology for the detection of antibodies (cryptococcal antigen latex agglutination test), fungal culture, histopathology and PCR. allows identification of the implicated species and genotype.
- **The treatment of cryptococcosis** in cats and dogs usually combines surgical excision of localised granulomas and administration of antifungal azole drugs, such as fluconazole, itraconazole and ketoconazole. However, cats with CNS infection and/or systemic disease often need treatment with amphotericin B plus flucytosine. Therapy should be maintained until at least 2–4 months after the resolution of clinical signs.. The prognosis for feline cryptococosis is good to excellent when the disease is diagnosed in the early stages. Nevertheless, CNS involvement negatively affects prognosis.

2.2. Cryptococcosis in cats

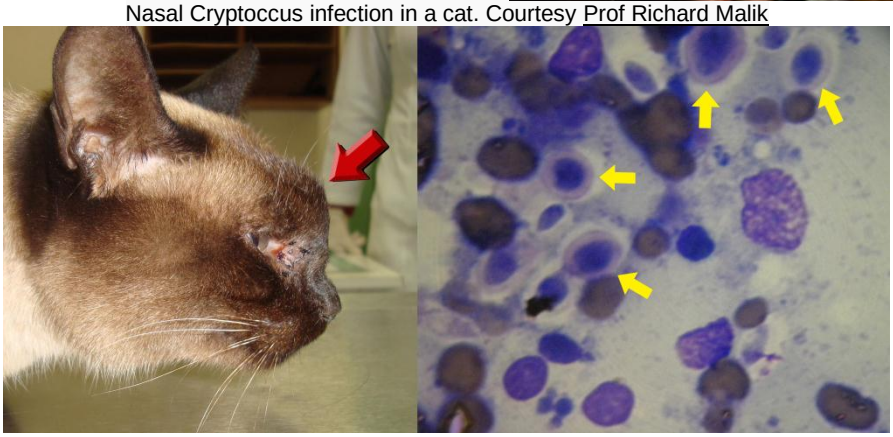
In cats, cryptococcosis can be either focal or disseminated, affecting a single organ system or many.

- Can begin insidiously, and may gradually become more severe over weeks or months.
- Fever may be absent, and if present, is often mild.
- Other nonspecific signs can include lethargy, anorexia and weight loss.
- Cats with localized infections, including those in the nasal cavity, do not necessarily have constitutional signs.

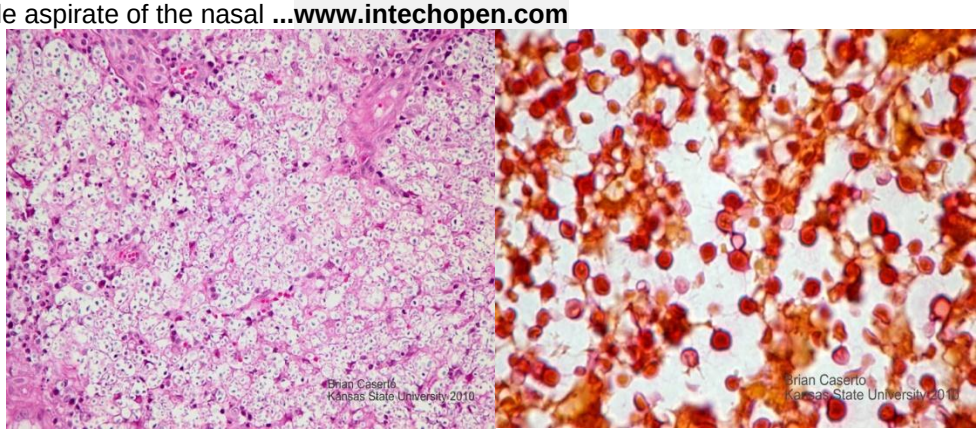
The clinical signs

- **Nasal cryptococcosis**
  - Frequently seen clinical signs include sneezing, snoring or snorting, dyspnea, nasal deformities and/ or a mucopurulent, serous or sero-sanguineous nasal discharge.
  - Polyp-like masses sometimes protrude from one or both nostrils

Nasal Cryptoccus infection in a cat. Courtesy Prof Richard Malik



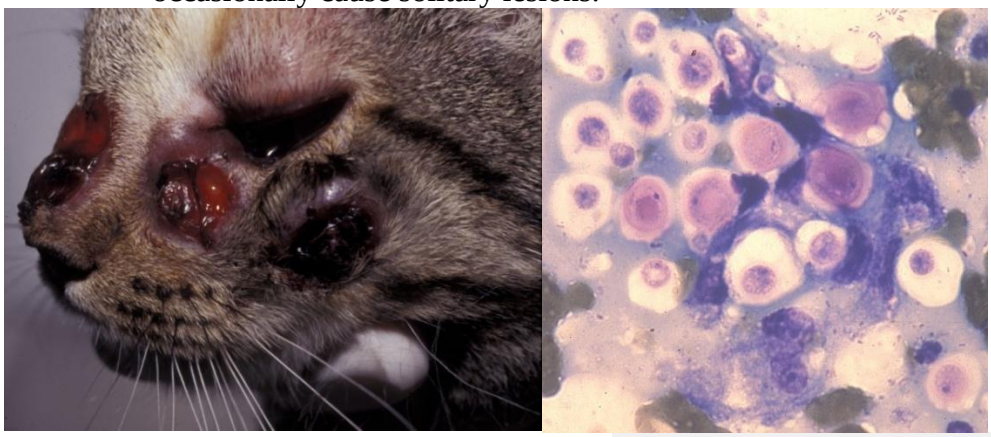
Feline cryptococcosis. left: a cat presenting a nasal masse (red arrow). right: Cytology by fine needle aspirate of the nasal **...www.intechopen.com**



Left: Nasal turbinate: The submucosa contains large numbers of fungal yeasts with a large clear capsule and a faintly basophilic nucleus. Right: Mucicarmine stain of feline nasal turbinates with Cryptococcus neoformans: The cell walls stain red and the capsule is clear. **Vet.Path.Forum**

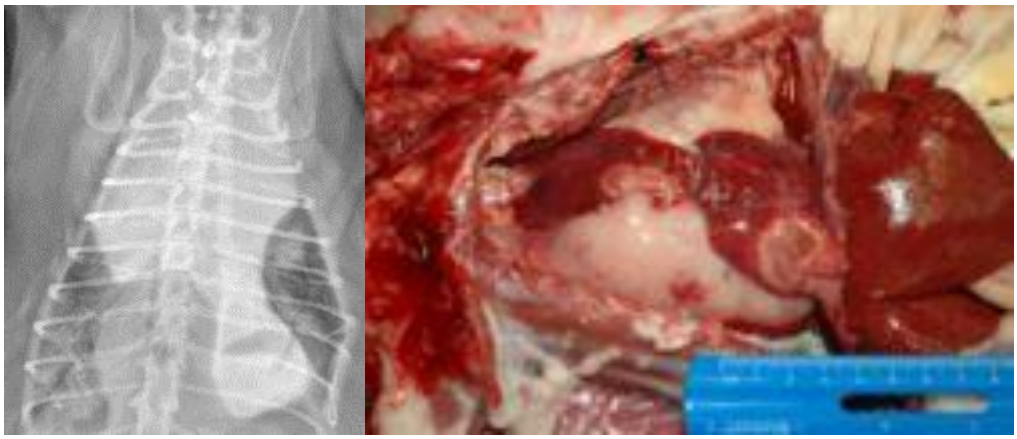
- **Cutaneous or subcutaneous swellings and nodules**
  - May be seen on the face, particularly the bridge of the nose, side of the face, upper lip or nostril.
  - Some of these lesions may ulcerate. In addition, the submandibular lymph nodes are often enlarged. With time, infections involving the nasal cavity can spread to adjacent structures.
  - Ulcerative or proliferative lesions may develop on **the tongue, gingiva or palate**. Extension to the **ear** can result in otitis media and vestibular signs.
  - Cutaneous involvement usually appears as fluctuant or firm papules and nodules. Some skin lesions may ulcerate, but there is little or no

pruritus. Generalized skin disease suggests disseminated cryptococcosis. Direct inoculation of organisms into the skin can occasionally cause solitary lesions.



Cat cryptococcosis. multiple foci of ulcerative dermatitis; aspirate from a cutaneous lesion contains numerous Cryptococcus neoformans yeast organisms surrounded by a nonstaining capsulewww.vetnext.com

- **Lower respiratory cryptococcosis**
  - can also occur in cats, although it is less common than upper respiratory lesions.
  - Syndromes may include pneumonia, pleuritis and mediastinal masses.

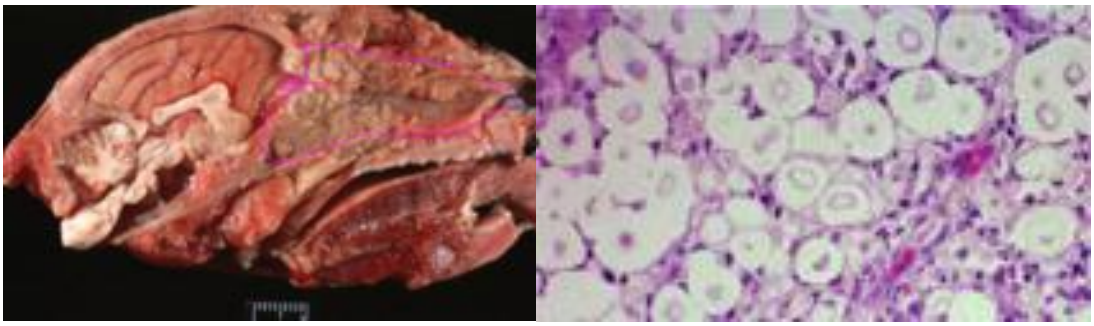


Lateral thoracic radiograph of a 12-year-old Siamese with pulmonary cryptococcus. There is a soft tissue mass in the cranial mediastinum as well as in the dorsal portion of the mid thorax causing ventral and caudal displacement of the tracheal carina. There is also severe atelectasis of all lung lobes. Right:Post-mortem of a 12-year-oldSiamese with pulmonary cryptococcus. Prof Allison Zwingenberger

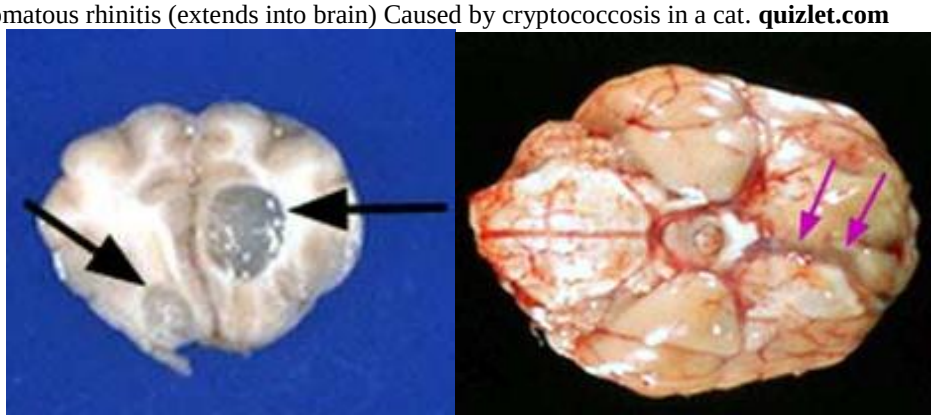
- **CNS involvement cryptococcosis**
  - Both focal mass lesions (cryptococcomas) and cryptococcoal meningitis may be seen.
  - The neurological signs can be mild to severe, with various presentations such as a change in temperament or behavior,

depression, disorientation, vestibular signs (e.g., head tilt, circling, nystagmus), head pressing, ataxia, paresis or paralysis, tremors, seizures, abnormal pupillary responses and blindness.

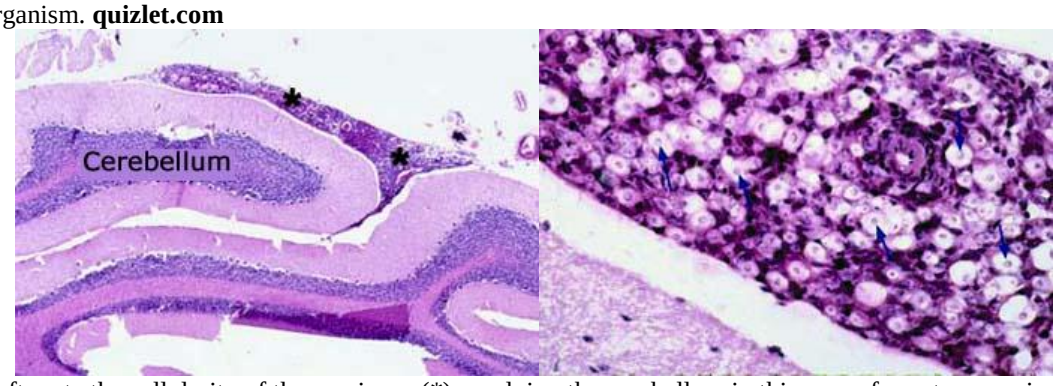
- Meningitis may appear as pain over the thoracolumbar spine or pelvic limbs, but hyperesthesia and nuchal rigidity are uncommon. Deficits of cranial nerves 5 to 12 are often found.
- The CNS is sometimes involved even if there are few or no obvious neurological signs. In one case, the only sign was unusual sleepiness.



Granulomatous rhinitis (extends into brain) Caused by cryptococcosis in a cat. **quizlet.com**



Note the two circumscribed areas in this cat brain that are very gelatinous in appearance. This gelatinous appearance is typical for *Cryptococcus* and is due to the mucinous capsule surrounding each organism. **quizlet.com**



Left, note the cellularity of the meninges (*) overlying the cerebellum in this case of cryptococcosis. Right: a higher magnification of the cellular infiltration.which is granulomatous to pyogranulomatous, **quizlet.com**.

- **Ocular cryptococcosis**
  - Chorioretinitis, optic neuritis, panophthalmitis, retinal hemorrhages and iridocyclitis have been reported. T

- Small transparent focal retinal detachments with a minimal inflammatory response may be seen.
- Ocular lesions often accompany other syndromes, specially CNS disease. Some cats can become blind.

- **Other organs** which can also be affected by **cryptococcosis** include:
  - the bone (osteomyelitis),
  - mediastinum,
  - heart,
  - thyroid gland,
  - spleen,
  - liver
  - urinary tract.

2.3. Cryptococcosis in dogs

- Compared to cats, dogs are more prone to develop disseminated cryptococcosis
- Cryptococcus neoformans can affect the eyes and central nervous system (CNS) and cause optic neuritis, granulomatous chorioretinitis and meningoencephalitis.
- About 50 percent of dogs diagnosed to have lesions in their respiratory tract, which is often the lungs, and most dogs have a granuloma in more than one system. Lesions may develop in the nasal cavity.
- Cryptococcus neoformans quite often attacks the kidneys, lymph nodes, spleen and liver of dogs, heart valves, thyroid, adrenal, muscle, pancreas, gastrointestinal tract, bone, myocardium, and prostate.
- When Cryptococcosis cause lesions in dogs, they can be different from the kind of granuloma mass jelly made by many organisms (often with very little inflammation).
- Cryptococosis lesions in dogs usually consists of an aggregate of encapsulated organisms covered by reticular connective tissue.
- The average age of infected dogs is 3.5 years and, unlike cats, there is no gender predisposition.
- Overrepresented dog breeds include American Cocker Spaniels and Labrador Retrievers in North America, and Doberman Pinschers and Great Danes in Australia.
- Cryptococcosis affects the same four organ systems as with cats, but the CNS and eyes are more commonly involved in dogs than in cats. The clinical signs are similar to those found in cats except that fever (103-105° F) is seen more often in affected dogs (25% of cases).

Clinical signs

- Frequently affected sites in the dog include both the respiratory tract and CNS.

- Signs primarily of upper respiratory tract involvement have been documented in some dogs, especially those infected with C. gattii; however,
- Concurrent involvement of the lower respiratory tract or CNS is common in this species.

- **Disseminated cryptococcosis** is reported to be more common in dogs than cats.abnormal pupillary responses and blindness.
- **Meningitis** may appear as pain over the thoracolumbar spine or pelvic limbs, but hyperesthesia and nuchal rigidity are uncommon. Deficits of cranial nerves 5 to 12 are often found.
- **The CNS** is sometimes involved even if there are few or no obvious neurological signs..
- **Ocular lesions** reported cases are
  - chorioretinitis,
  - optic neuritis,
  - panophthalmitis,
  - retinal hemorrhages and
  - iridocyclitis
  - small transparent focal retinal detachments with a minimal inflammatory response. Ocular lesions often accompany other syndromes, especially CNS disease. Some dogs can become blind.
- **Cutaneous involvement**
  - Usually appears as fluctuant or firm papules and nodules.
  - Some skin lesions may ulcerate, but there is little or no pruritus.
  - Generalized skin disease suggests disseminated cryptococcosis.
  - Direct inoculation of organisms into the skin can occasionally cause solitary lesions.

Other organs which can also be affected.including

- the bone (osteomyelitis),
- mediastinum,
- heart,
- thyroid gland,
- spleen,
- liver and
- urinary tract

2.4. Reported cases.

Sutton (1981) described the main features of *Cryptococcus neoformans* infection in 6 dogs, all of which were of large breed, were **central nervous system** involvement in all cases and diversity of the initial presenting signs. The respiratory tract was affected

in one case, but the bronchopneumonia did not appear to be of cryptococcal origin. Immunosuppressive factors and other diseases which are believed to increase the susceptibility of man and animals to cryptococcal infection did not appear to be of importance in these cases.

Medleau *et al*. (1985) diagnosed **cutaneous cryptococcosis** in 3 cats. No other organ involvement was found. One cat has remained healthy after surgical excision of the cryptococcal skin lesion. One cat was euthanatized after diagnosis. The third cat was treated successfully with a 5-month course of ketoconazole.

Malik *et al.* (1992) evaluated 29 cats with naturally occurring cryptococcosis prior to commencing oral **fluconazole therapy** (25–100 mg every 12 h). Affected cats ranged from 2 to 15 years-of-age. Male cats (19; 66%) and Siamese cats (5; 21%) appeared to be over-represented in comparison to the hospital's cat population. Mycotic rhinitis was observed in 24 (83%) of the cases, although nasal cavity involvement was subtle in four animals. Disease of the skin and subcutaneous tissues was present in 15 cases (52%) and amongst these the nasal plane (seven cats) and bridge of the nose (seven cats) were most commonly involved. Primary infection of the central nervous system was not encountered, although one cat developed meningoencephalitis and optic neuritis as a sequel to longstanding nasal cavity disease. Antibodies against the feline immunodeficiency virus (FIV) were detected in eight cats (28%), and these cats tended to have advanced and/or disseminated disease. There was a tendency for cats to develop cryptococcosis during the Australian summer. Organisms were cultured from 27 cases. ***Cryptococcus neoformans* var. *neoformans*** was isolated from 21 cats, while ***Cryptococcus gattii*** was identified in the remaining six. The response to oral fluconazole was excellent in this series, which included many cats with advanced, longstanding or disseminated disease. The fungal infection resolved in all but one advanced case which died after only 4 days of therapy. A dose of 50 mg per cat, given every 12 h, produced a consistently good response without side effects. Lower doses were effective in some cases, while 100 mg every 12 h was required to control the infection in one cat. Serum fluconazole levels obtained during chronic dosing (50 ± 18 mg l^{-1}, mean ± SD; 50 mg per cat every 12 h) were highly variable (range 15–80 mg l^{-1}). Concurrent FIV infection did not impart an unfavourable prognosis, although affected cats often required prolonged courses of therapy.

Malik *et al.* (1995) analysed the clinical and mycological findings in 20 consecutive cases of cryptococcosis evaluated between 1981 and 1995 retrospectively. Typically, young adult dogs (median age 2 years) of either sex were affected. Dobermann Pinschers and Great Danes were significantly over-represented in relation to other breeds and crossbred dogs, and there was no trend for cryptococcosis to be acquired at a particular time of year. ***Cryptococcus neoformans*** was cultured from 18 dogs, with 16 isolates further characterized. Of these, ***C. neoformans var. neoformans*** was isolated from 12 cases, while the remaining four strains were ***C. gattii***. Dogs with *C. gattii* infections resided in rural (two cases) or suburban (two cases) environments. Ten dogs were presented as a result of infection of structures inside, adjacent to, or contiguous with the nasal cavity. Seven dogs were presented primarily for signs of central nervous system disease, of which at least three also had cryptococcal rhinosinusitis. One dog had **cryptococcal pneumonia** and also possible mycotic **rhinitis**, another had **disseminated** disease with lymph node and skin involvement,

while the last dog was presented for vomiting referable to **cryptococcal mesenteric lymphadenitis**. Treatment consisting of surgery and/or antifungal drug therapy was successful in the majority of animals in which it was attempted, including two of three cases with **meningo-encephalitis**.

Medleau *et al*. (1995) used **Itraconazole** in 35 cats with cryptococcosis. Treatment response was determined by comparing clinical signs before, during, and after treatment. It could not be evaluated in 7 cats because they died during treatment from causes unrelated to cryptococcosis. Of the remaining 28 cats, treatment response was classified as success in 16 cats (57%), as improvement in 8 cats (29%), and as a failure in 4 (14%). The failures were due to death or euthanasia from drug toxicity (1 cat), progressive fungal disease (2 cats), and relapse 1 year after treatment (1 cat). The cats that improved did not undergo a 1 -year posttreatment evaluation because they were lost to follow-up (3 cats), died or were euthanatized for other reasons (4 cats), or had a noncompliant owner (1 cat). For the 16 cats in which treatment was successful, the median itraconazole dose was 13.8 mg/kg body weight daily (range, 10.9 to 26.7 mg/kg/d), and the median duration of treatment was 8.5 months (range, 4 to 16 months). Five of these cats had previously been treated unsuccessfully with ketoconazole.

Gerds-Grogan and Dayrell-Hart **(1997)** reported 19 cats with cryptococcosis at the Veterinary Hospital of the University of Pennsylvania between April 1986 and May 1995. Compared to other studies, these 19 cases showed increased **neurological and ophthalmological** involvement. Males were affected more often than females. Season and environment appeared to influence time of onset or presentation to the hospital. Clinical pathology did not show typical changes. It is possible that the organism was present frequently in the urine but was mistaken for fat droplets. Treatment with ketoconazole was unrewarding in cases with central nervous system (CNS) involvement.

Jacobs *et al.* (1997 evaluated the relationship between treatment outcome and location of cryptococcal infection, gender, magnitude of pretreatment, cryptococcal antigen titers, results of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) serology, and serial changes in antigen titers during and after treatment in a prospective and nonrandomized study of 35 cats with cryptococcosis. A commercial **cryptococcal latex agglutination kit** (CALAS; Meridian Diagnostic Inc, Cincinnati, OH) was used to detect cryptococcal antigen in sera. All cats were treated with **itraconazole** (Sporanox; Janssen Pharmaceutica Inc, Titusville, NJ). Pretreatment mean log titers for serum cryptococcal antigen were not influenced by location of the infection. Treatment outcome was not influenced by gender, location of the infection, or magnitude of pretreatment serum antigen titer. Treatment outcome was influenced by FeLV and FIV status; cats seropositive for FeLV or FIV had a higher likelihood oftreatment failure ($P = .008$). The cryptococcal antigen titers of cats successfully treated decreased with significant linearity over time during treatment ($r = -.64$, $P < .000001$), whereas the corresponding titers for cats not treated successfully did not decrease with significant linearity ($r = -.03$, $P > .9$). For cats in which treatment was successful, antigen titers decreased significantly from pretreatment values by 1.3 orders of magnitude at 2 months after initiation of treatment. By 10 months after initiating treatment, log titers decreased by at least 2 orders of magnitude in all cats successfully treated, and 9 of 16 cats had undetectable titers. In contrast, in 5 of 6 cats

in which treatment failed, antigen titers were unchanged or increased in magnitude even after at least 6 months of treatment.

Malik *et al.* (1997) collected nasal washings from a random source of dogs and cats, concentrated them by centrifugation and plated then onto bird seed agar containing antibiotics. ***Cryptococcus neoformans* var. *neoformans*** was isolated from eight of 56 dogs (14%) and three of 45 cats (7%). More than 100 colonies of *C. neoformans* were present on the plates from seven of the 11 positive animals. Absence of cryptococcal antigen in the serum of these animals, and failure to demonstrate yeast-like organisms or significant pathology in nasal biopsies, suggested that the nasal cavity of these animals was not infected by *C. neoformans* but rather that blastoconidia and/or basidiospores were carried asymptomatically.

BArrs *et al.* (2000) presented a 12-year-old, FIV-positive, domestic longhair cat with a history of sneezing and coughing during the previous seven months. On thoracic radiographs, a prominent bronchial pattern and three focal, opacified nodules were seen. Cytology of bronchoalveolar lavage fluid demonstrated spherical, capsulate, narrow-necked, budding yeasts within macrophages. Culture of the fluid yielded a heavy growth of ***Cryptococcus neoformans* var *neoformans***. The serum latex cryptococcal antigen agglutination test titre was 158. The cat was treated with itraconazole and the cough resolved over a 5-month period but then recurred. Repeat thoracic radiographs showed resolution of the pulmonary nodules but a persistent bronchial pattern. Adult nematodes and ova with morphology characteristic of *Capillaria aerophila* were seen in bronchoalveolar lavage fluid and no yeasts were cultured from the fluid. The cryptococcal titre was zero. The lungworm infection was treated successfully with abamectin and the cough resolved. Immunosuppression related to FIV infection may have predisposed this cat to sequential respiratory tract infections.

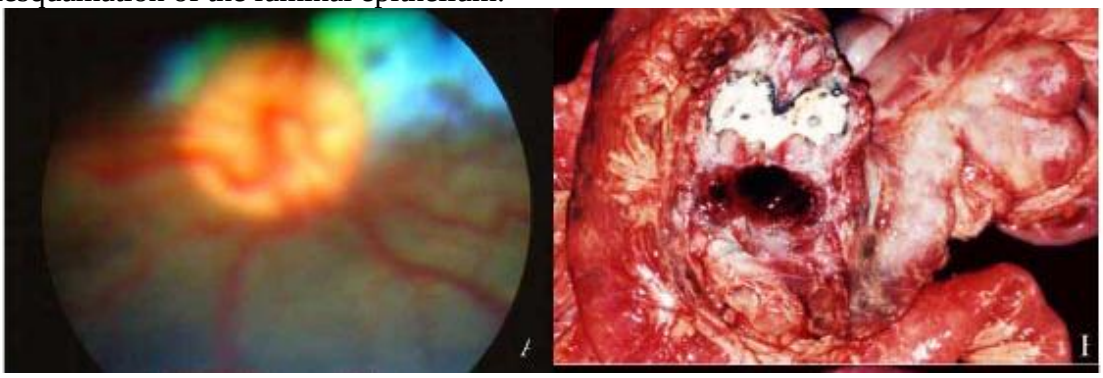
Beatty *et al* (2000) presented 3 cats with cryptococcosis. In two cats, ***Cryptococcus neoformans* var *neoformans*** was isolated from the tympanic bulla. In the remaining cat, otitis media/interna was considered to be secondary to occlusion of the auditory tube by a nasopharyngeal granuloma associated with a ***Cryptococcus gattii*** infection. This report emphasises the importance of maintaining an index of suspicion for a fungal aetiology in cats with signs of otitis media/interna, particularly in countries with a high prevalence of cryptococcosis. The presence of *C neoformans* may be overlooked with potentially fatal consequences where only standard methods for bacterial isolation are used to examine samples obtained from the middle ear

Honsho *et al*. (2003) reported a male Boxer dog aged 2 years and 11 months with a history of a gastrointestinal disorder of two months duration, with apathy, hyporexia, progressive weight loss and visual deficit. Ataxia and vocalization were observed during hospitalization. The animal had been treated previously with antibiotics and immunosuppressive doses of corticoids to control chronic inflammatory bowel disease. The dog died five days later. Gross and microscopic observations indicated systemic cryptococcosis. The alimentary tract, eyes, brain, kidneys, pancreas and lymph nodes were involved. Ophthalmic examination revealed a reduced response to light, intraocular pressure of 3 mmHg for both eyes, discrete episcleral congestion with moderate conjunctival hyperemia, and discrete corneal edema. Mild *rubeosis*

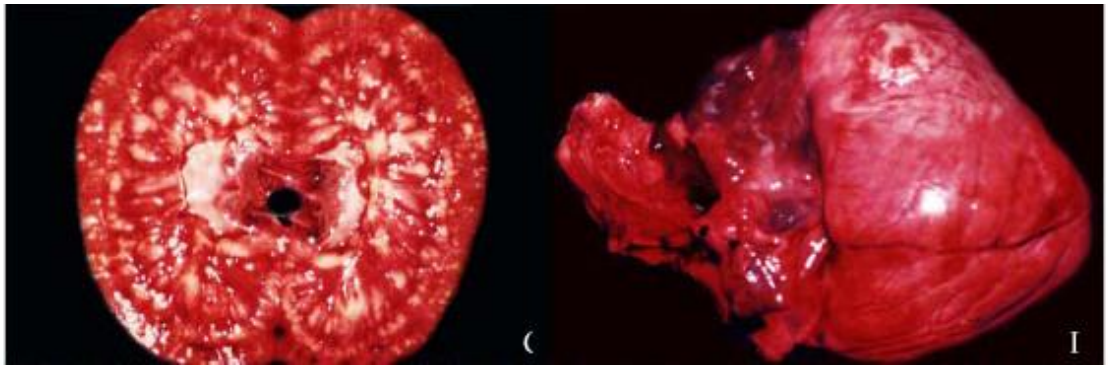
iridis and the presence of a white color granuloma measuring approximately 1mm in diameter were also observed in the iris of the right eye. In the posterior segment there was diffuse vitreal exudation in the right eye, as well as peripapillary hemorrhage, retinal elevation in various areas suggestive of granulomatous chorioretinitis, and the presence of small pigmented nodules scattered in the tapetal transition of the left eye.

Necroscopic examination showed that the gastric mucosa had countless ulcers with raised borders ranging in diameter from 2 to 5mm, and that the mucosa of the small intestine had ulcers as large as 1 cm in size. The rectal mucosa presented countless nodules about 1.5cm in diameter with bloody content. The mesentery was thickened and all mesenteric lymph nodes were enlarged. The pancreas was also enlarged. The liver was dark brown in color with a rugose aspect and granulated upon palpation. Several other whitish nodules were detected in the kidney - 0.5-1.0cm in diameter - and brain (1-3mm in diameter). Examination of the thoracic cavity showed pulmonary hepatization and in the heart a whitish region about 2cm in diameter in the right ventricle epicardium and infiltrating to the myocardium. Fragments of these organs were collected and fixed in 10% formalin and later processed for histology by paraffin embedding.

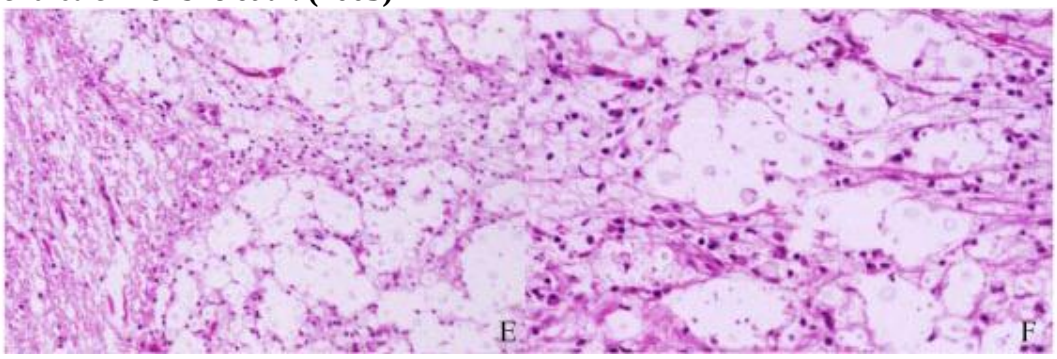
Histopathological examination of the kidneys showed thickening of the Bowman capsules with edema, glomerular degeneration and a mild mononuclear inflammatory infiltrate. The lungs were emphysematous, with thickening of the alveolar walls which were infiltrated by large numbers of encapsulated microorganisms, many of them of small size and with a budding aspect, characteristic of *C. neoformans.* These features were clearly demonstrated by PAS staining and GMS. Other organs such as intestine, liver, stomach, pancreas, lymph nodes, brain, cerebellum and spinal cord presented the same type of lesion, i.e., the massive presence of fungi and a slight mononuclear infiltrate. In the intestine there was necrosis and the presence of large amounts of fungi. Fungi were also observed free in the intestinal lumen due to desquamation of the luminal epithelium.



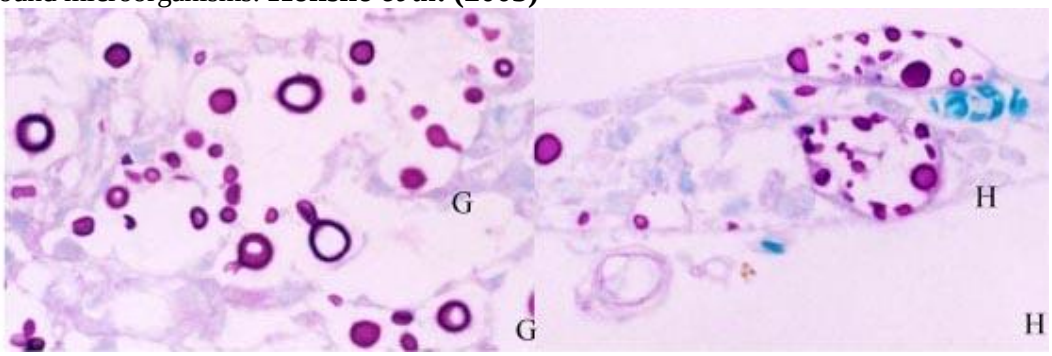
Organs of a dog with cryptococcosis. A) Fundus tapetal with discrete pigmented spots, papilloma, vitreous exudation and granulomatous lesions. B) Mesenteric lymph node showing a necrosis area. **Honsho *et al*. (2003)**



C) Kidney with several small whitish areas D) Heart showing whitish area in the right ventricule. **Honsho *et al*. (2003)**



E) Microscopic appearance of kidney necrotic area with small round microorganisms. F) Microscopic appearance of intestine showing necrotic area in the epithelium with small round microorganisms. **Honsho *et al*. (2003)**



G) Brain showing C. neoformans cells H) Meninge showing C. neoformans cells, **Honsho *et al*. (2003)**

Lester *et al*. (2004) determined clinical and pathologic findings associated with an outbreak of cryptococcosis in an unusual geographic location (British Columbia, Canada) in 20 cats, and 15 dogs. A presumptive diagnosis of cryptococcosis was made on the basis of serologic, histopathologic, or cytologic findings, and a definitive diagnosis was made on the basis of culture or immunohistochemical staining. No breed or sex predilections were detected in affected dogs or cats. Eleven cats had neurologic signs, 7 had skin lesions, and 5 had respiratory tract signs. None of 17 cats tested serologically for FeLV yielded positive results; 1 of 17 cats yielded positive results for FIV (western blot). Nine of 15 dogs had neurologic signs, 2 had periorbital swellings, and only 3 had respiratory tract signs initially. Microbiologic culture in 15 cases yielded 2 isolates of *Cryptococcus neoformans* var *grubii*(serotype A) and 13 isolates of *C gattii* (serotype B); all organisms were susceptible to amphotericin B and ketoconazole. Serologic testing had sensitivity of 92% and specificity of 98%.It was concluded that serologic titers were beneficial in identifying infection in animals with

nonspecific signs, but routine serum biochemical or hematologic parameters were of little value in diagnosis. Most animals had nonspecific CNS signs and represented a diagnostic challenge. Animals that travel to or live in this region and have nonspecific malaise or unusual neurologic signs should be evaluated for cryptococcosis.

Duncan *et al.* (2005a) evaluated deep and superficial nasal fungal cultures of 280 dogs and 94 cats. They identified four (4.3%) cats and three (1.1%) dogs with *C. gattii* serotype B in their nasal cavity. Serum samples collected from 266 dogs and 84 cats identified six (7.1%) cats and two (0.8%) dogs with a positive cryptococcal antigen titer. Overall cats were 4.4 times more likely than dogs to be positive on one or both tests. Identification of sub-clinical infection and nasal colonization is an important step in the characterization of the outbreak of clinical cryptococcosis on Vancouver Island.

Duncan *et al.* (2005b) reported the follow-up data on a cohort of seven cats and five dogs identified in a previous study as sub-clinically infected with Cryptococcus spp. or colonized by ***C. gattii***. Two cats progressed to clinical disease within four to six months of initial detection of antigenemia and nasal cavity colonization. The ten other animals remained asymptomatic but many were repeatedly positive on cryptococcal antigen testing or nasal fungal culture suggesting protracted infection or colonization. The results indicate that asymptomatically infected animals may clear the organism, remain sub-clinically infected or progress to clinical disease. Factors influencing the transition from exposure to disease require further investigation.

Duncan *et al.* (2006a) mentioned that, **Cryptococcus gattii** has emerged since 1999 as an important pathogen of humans and animals in southwestern British Columbia. Historically thought to be restricted to the tropics and subtropics, C. gattii has posed new diagnostic and treatment challenges to veterinary practitioners working within the recently identified endemic region. Clinical reports of canine and feline cryptococcosis caused by C. gattii diagnosed between January 1999 and December 2003 were included in this case series. The most common manifestations of disease were respiratory and central nervous system signs. Multivariate survival analysis revealed that the only significant predictor of mortality was the presence of central nervous system signs upon presentation or during therapy. Case fatality rates in both species were high. Further investigation into effective treatment regime is warranted.

Duncan *et al.* (2006b) conducted a study to determine the risk factors associated with ***Cryptococcus gattii*** infection in dogs and cats residing on Vancouver Island in British Columbia, Canada. In this study. 20 dogs and 29 cats with C gattii infection and matched controls were involved. Dogs and cats with a confirmed or probable diagnosis of cryptococcosis resulting from infection with C gattii were enrolled by veterinarians, and owners completed a questionnaire designed to obtain information pertaining to potential risk factors for the disease. Owners of matched control animals were also interviewed. Odds ratios and 95% confidence intervals or paired t tests were calculated to determine significant associations. Animals were enrolled during 2 noncontiguous periods in August 2001 to February 2002 (8 dogs and 9 cats enrolled) and May to December 2003 (12 dogs and 20 cats enrolled). Risk factors significantly associated with development of cryptococcosis included residing within 10 km of a logging site or other area of commercial soil disturbance, above-average level of

activity of the animal, travelling of the animal on Vancouver Island, hunting by the animal, and owners hiking or visiting a botanic garden. Results indicated that dogs and cats that were active or that lived near a site of commercial environmental disturbance had a significantly increased risk of developing C gattii infection. Veterinarians should communicate these risks to owners in context because cryptococcosis was an uncommon disease in this population.

Duncan *et al.* (2006c) collected. nasal swabs and serum samples from dogs and cats residing within the Coastal Douglas Fir biogeoclimatic zone on Vancouver Island, where clinical cases have been reported. Deep and superficial nasal fungal cultures of 280 dogs and 94 cats identified four (4.3%) cats and three (1.1%) dogs with ***C. gattii*** serotype B in their nasal cavity. Serum samples collected from 266 dogs and 84 cats identified six (7.1%) cats and two (0.8%) dogs with a positive cryptococcal antigen titer. Overall cats were 4.4 times more likely than dogs to be positive on one or both tests. Identification of sub-clinical infection and nasal colonization was an important step in the characterization of the outbreak of clinical cryptococcosis on Vancouver Island.

Kano *et al.* (2008) reported a case of systemic infection caused by ***Cryptococcus albidus*** in a cat. The patient had a history of paralysis of the hind legs and had been treated with prednisone for 1 month. Microscopic examination of a fine needle biopsy specimen from a right popliteal lymph node showed granulomatous inflammation with many encapsulated yeast cells. Moreover, microscopic examination of Indian ink preparations of the cerebrospinal fluid revealed encapsulated ovoid yeast cells. Thus this case was diagnosed to be cryptococcosis. However, the cat died after treatment for three days with **voriconazole**. Isolates recovered from samples of the cerebrospinal fluid, liver and spleen were identified as *C. albidus* by molecular analysis, as well as through morphologic and biochemical studies. Therefore, this case indicates that *C. albidus* should be considered as a potential feline pathogen.

Chapman and Kirk (2008) diagnosed a **cryptococcal urinary tract infection** (UTI) in a male domestic shorthaired cat presented for evaluation of stranguria and pollakiuria The (UTI) was diagnosed cytologically and via fungal culture. No evidence of systemic involvement was found. Chronic renal failure was a concurrent disease in this cat. Treatment consisted of oral fluconazole. Clinical signs resolved after 2 weeks of therapy, and fluconazole was discontinued after 6 months when negative urine culture results indicated resolution of the infection. This case demonstrated that correct identification of cryptococcal UTI allowed for administration of therapy that can be associated with resolution of clinical signs.

Bowles *et al.* (2009) presented two young, large-breed female dogs with an acute onset of sneezing and nasal discharge. One patient had concurrent epistaxis and facial deformity. Decreased airflow was noted through the left nostril in Case 1, while Case 2 showed facial deformity. Nasal radiographs from Case 1 showed a soft tissue opacity in the left nasal cavity and frontal sinus. Rhinoscopy revealed roughened, erythematous nasal turbinates in both patients, and a mass in the left caudal nasal cavity of Case 1. Cryptococcus spp. were demonstrated histopathologically on a nasal biopsy. Tissue culture and serum antigen titres were positive for Cryptococcus spp. The diagnosis was chronic rhinitis secondary to ***Cryptococcus gattii*** infection in Case 1, and ***Cryptococcu neoformans*** infection in Case 2.

Byrnes *et al.* (2009) isolated ***Cryptococcus gattii*** from a 1.5-year-old dog with systemic cryptococcosis in Oregon. The dog had no link to Vancouver Island or British Columbia, Canada. The 2 isolates were both the VGIIa Vancouver Island major genotype. Findings are consistent with expansion of the Vancouver Island outbreak onto the mainland Pacific Northwest region of the United States.

McGill *et al*. (2009) conducted a retrospective study of cryptococcosis in domestic animals residing in Western Australia over an 11-year-period (from 1995 to 2006) by searching the data base of Murdoch University Veterinary Teaching hospital and the largest private clinical pathology laboratory in Perth. Cryptococcosis was identified in 155 animals: 72 cats, 57 dogs, 20 horses, three alpacas, two ferrets and a sheep. There was no seasonal trend apparent from the dates of diagnosis. Taking into account the commonness of accessions to Murdoch University, cats were five to six times more likely to develop this disease than dogs, and three times more likely than horses, while horses were almost twice as likely as dogs to become infected. Amongst the feline cohort, Ragdoll and Birman breeds were over-represented, while in dogs several pedigree breeds were similarly overrepresented. Dogs and horses tended to develop disease at an early age (one to five years), while cats were presented over a much wider range of ages. In cats and dogs the upper respiratory tract was the most common primary site of infection, while horses and alpacas tended to have lower respiratory involvement. The most striking finding of the study was the high frequency with which ***C. gattii*** was identified, with infections attributable to this species comprising 5/9 cats, 11/22 dogs, 9/9 horses and 1/1 alpaca, where appropriate testing was conducted. Preliminary molecular genotyping suggested that most of the *C. gattii* infections in domestic animals (9/9 cases) were of the VGII genotype. This contrasts the situation on the eastern seaboard of Australia, where disease attributable to *C. gattii* is less common and mainly due to the VGI genotype. *C. gattii* therefore appears to be an important cause of cryptococcosis in Western Australia.

Poth *et al.* (2010) described an uncommon case of cryptococcosis in an apparently immunocompetent cat caused by ***Cryptococcus magnus***. An amputation of the complete left foreleg and excision of the ipsilateral cervical lymph node were performed in a young-adult male Domestic Shorthair cat due to suspicion of a tumor. Granulomatous dermatitis, panniculitis, myositis, and lymphadenitis were diagnosed histologically. Intralesional, numerous round-to-ovoid yeast cells showing no capsule were detected within macrophages using special staining methods. The tissue material cultured on Sabouraud's glucose agar at 26°C yielded abundant growth of yeast colonies. Morphological, physiological, and molecular analyses of the yeasts demonstrated that the fungus was *C. magnus*. Response to treatment with fluconazole was fast and effective, and one year after the end of the therapy no further clinical signs of infection were observed.

Trivedi *et al.* (2010) reviewed medical records of cats and dogs with cryptococcosis. Information collected included geographic location, species, signalment, and tissues or organs involved. Cryptococcosis was confirmed via serology, cytology, histology, or microbial culture, and molecular typing was performed. Odds ratios and 95%

confidence intervals were calculated to determine significant associations among variables. Other comparisons were evaluated via χ^2 or unpaired *t* tests. American Cocker Spaniels were overrepresented, compared with other dog breeds. Serum cryptococcal antigen test results were positive in 51 of 53 cats and 15 of 18 dogs tested. ***Cryptococcus gattii*** was more commonly detected in cats (7/9 for which species identification was performed), and ***Cryptococcus neoformans*** was more commonly detected in dogs (6/8). Six of 7 *C gattii* isolates from cats were molecular type VGIII. Distribution of involved tissues was different between cats and dogs in California and between populations of the present study and those of the previously reported Australian study. They concluded that strains of *Cryptococcus* spp appeared to have host specificity in dogs and cats. Differences in lesion distribution between geographic locations may reflect strain differences or referral bias. Antigen assays alone may not be sufficient for diagnosis of cryptococcosis in cats and dogs

Trivedi *et al.* (2011) wrote a **review** in which they drew attention to literature relating to epidemiology, CNS involvement and advanced diagnostic imaging to update clinicians regarding research findings relevant to clinical practice. They mentioned mentioned that Cryptococcosis, principally caused by *Cryptococcus neoformans* and *Cryptococcus gattii*, is the most common systemic mycosis of cats worldwide. Cats may be infected following inhalation of spores from the environment, with the nasal cavity suspected as being the initial site of colonization and subsequent infection. Other sites of infection in cats are the skin, lungs, lymph nodes, central nervous system (CNS), eyes and, occasionally, periarticular connective tissue. Cryptococcosis can be diagnosed using serology (antigen testing), cytologic examination of smears, histopathology or culture. Treatment of localized disease is generally successful using azole antifungal drugs; however, cats with CNS involvement or disseminated disease require additional treatment with amphotericin B, with or without flucytosine. The prognosis is variable, depending on host and pathogen factors. Some cats require long-term (>1 year) treatment or indefinite therapy. Cats of any breed, gender and age may be affected. Retroviral status does not appear to be a risk factor for developing cryptococcosis and indoor cats are not protected from disease. Feline cryptococcosis occurs worldwide, but is most frequently reported in Australia, western Canada and the western United States. Species and molecular type vary in different geographical regions and may affect clinical presentation and antifungal susceptibility patterns. Serologic tests that detect cryptococcal antigen in serum are sensitive and specific, but false negatives can occur in cats with localized disease. Long-term drug therapy can be expensive and has the potential for toxicity. The extent to which the pathogenicity and antifungal susceptibility is affected by molecular type is currently under study.

Cardoso *et al.* (2013) described a case of a **nasal granuloma** in a cat due to C. *gattii*. The confirmation of the specie ***Cryptococcus gattii*** and its molecular type were performed using the PCR-RFLP molecular techniques. The isolated strain was identified as *C. gattii* type VGII and was susceptible to all antifungal drugs tested. The characterization and molecular investigation of this microorganism are relevant because they could help better understand the epidemiology of the infection and to guide us to treat properly the disease.

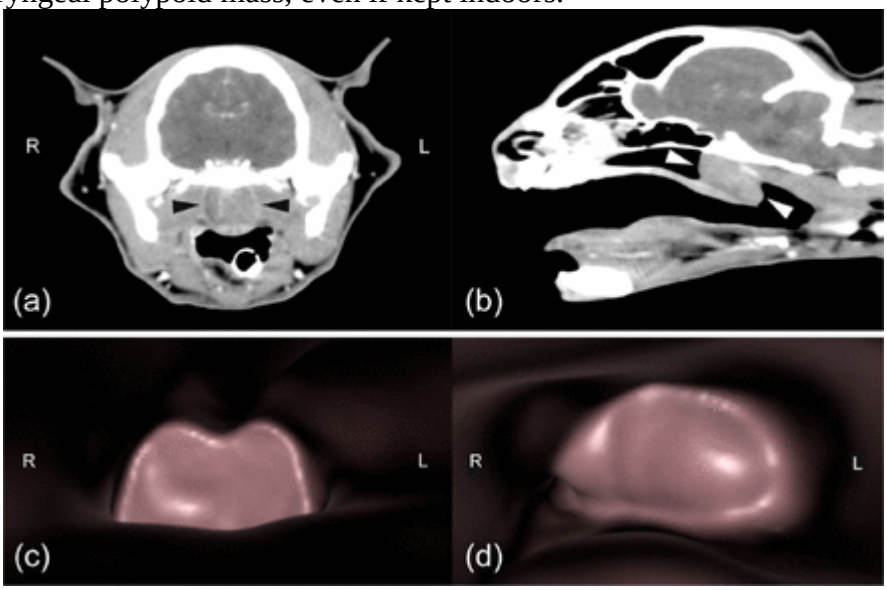
Pennisi *et al.* (2013) mentioned that Cryptococcosis is worldwide the most common systemic fungal disease in cats; it is caused by the *Cryptococcus neoformans–Cryptococcus gattii* species complex, which includes eight genotypes and some subtypes (strains) with varying geographical distribution, pathogenicity and antimicrobial susceptibility. Cats acquire the infection from a contaminated environment. The prognosis is favourable in most cases, provided a diagnosis is obtained sufficiently early and prolonged treatment is maintained. Basidiospores are the infectious propagules of *Cryptococcus* species as they penetrate the respiratory system and induce primary infection. Asymptomatic colonisation of the respiratory tract is more common than clinical disease. Avian guanos, particularly pigeon droppings, offer favourable conditions for the reproduction of *C neoformans*. Both *Cryptococcus* species are associated with decaying vegetation. Cryptococcosis caused by *C neoformans* or *C gattii* is indistinguishable clinically. The disease can present in nasal, central nervous system (which can derive from the nasal form or occur independently), cutaneous and systemic forms. An easy and reliable test for cryptococcosis diagnosis is antigen detection in body fluids. Only isolation and polymerase chain reaction allow identification of the species genotype. Amphotericin B, ketoconazole, fluconazole and itraconazole have all been used to treat cats. Surgical excision of any nodules in the skin, nasal or oral mucosa assists recovery. Continued treatment is recommended until the antigen test is negative. Efficient preventive measures have not been demonstrated. Vaccines are not available.

Danesi *et al.* (2014) sampled cats from 162 urban and rural feral cat colonies over 3 years. Of 766 cats from which nasal swabs were obtained, *Cryptococcus* spp. were recovered from 95 (12.6%), including 37 *C. magnus* (4.8%), 16 *C. albidus* (2.0%), 15 *C. carnescens* (1.9%), 12 *C. neoformans* (1.6%), as well as *C. oeirensis* (*n* = 3), *C. victoriae* (*n* = 3), *C. albidosimilis* (*n* = 2),*Filobasidium globisporum* (*n* = 2), *C. adeliensis* (*n* = 1), *C. flavescens*(*n* = 1), *C. dimnae* (*n* = 1), *C. saitoi* (*n* = 1), and *C. wieringae* (*n* = 1) with prevalence <1%. Thirteen *Cryptococcus* species were identified by polymerase chain reaction and sequencing of internal transcribed spacer amplicons. Statistical analysis did not identify any predisposing factors that contributed to nasal colonization (eg, sex, age, season, or habitat). Results suggest that asymptomatic feral cats may carry *C. neoformans* and other *Cryptococcus* species in their sinonasal cavity. Genotyping of the specific cryptococcal isolates provides a better understanding of the epidemiology of these yeasts.

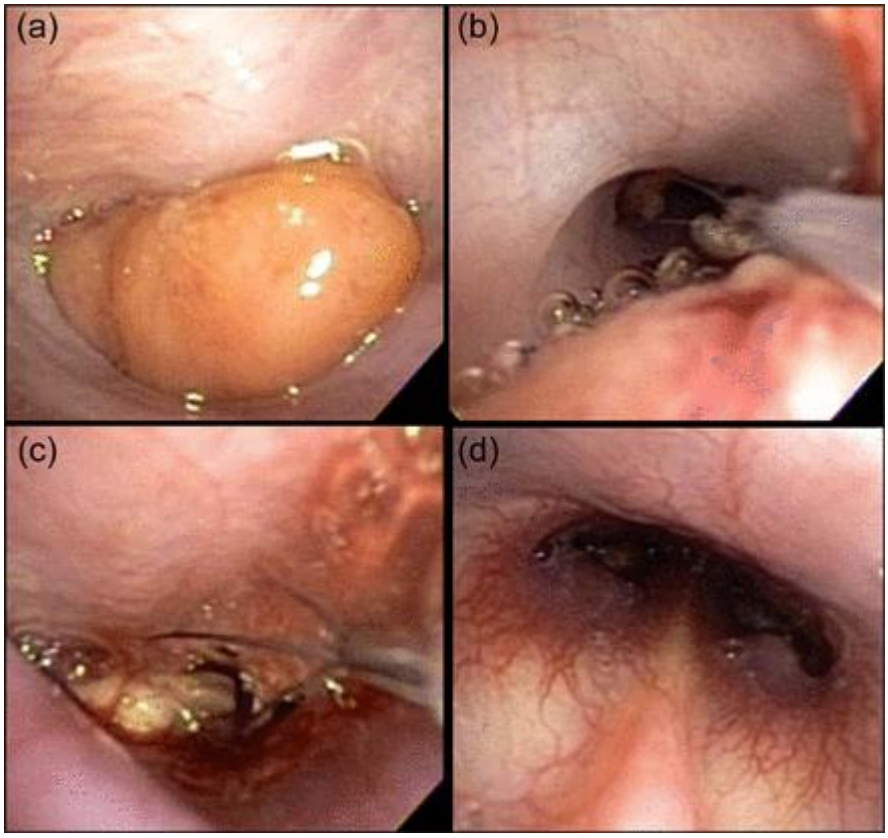
O'BRIEN *et al.* (2004) carried ou a retrospective study of 155 cats and 40 dogs diagnosed with cryptococcosis between 1981 and 2001. Age, sex, breed, clinical findings, feline immunodeficiency virus and feline leukaemia virus status (in cats), species of *Cryptococcus* causing disease and region of domicile were recorded. Associations between variables were tested. Male and female cats were affected equally. Age ranged from 1 to 16 years, with a preponderance of cats aged between 2 and 3 years. Siamese, Himalayan and Ragdoll breeds were over-represented. Rural cats were more frequently infected with *Cryptococcus gattii*. Retroviral infection was not identified as a predisposing condition and was not correlated with either species of *Cryptococcus* or physical findings. Most cats had signs of nasal cavity infection, which was typically localised for a substantial period before invasion of adjacent structures or dissemination. Male and female dogs were affected equally. A marked preponderance of young, large breed dogs was noted. Border Collies, Boxers, Dalmatians, Dobermann Pinschers, Great Danes and German Shepherds were over-

represented.*Cryptococcus* species involved was not affected by place of domicile. Although nasal cavity involvement was important, the canine cohort had a greater propensity to develop secondary central nervous system involvement and disseminated disease than feline cases. There were no clinical findings in either cats or dogs which could be reliably used to distinguish disease caused by *Cryptococcus neoformans* variety *grubii* from disease caused by *Cryptococcus gattii*. Both *Cryptococcus* species appear to be primary pathogens of cats and dogs, with the upper respiratory tract presumed to be the predominant primary site of inoculation in most but not all cases.

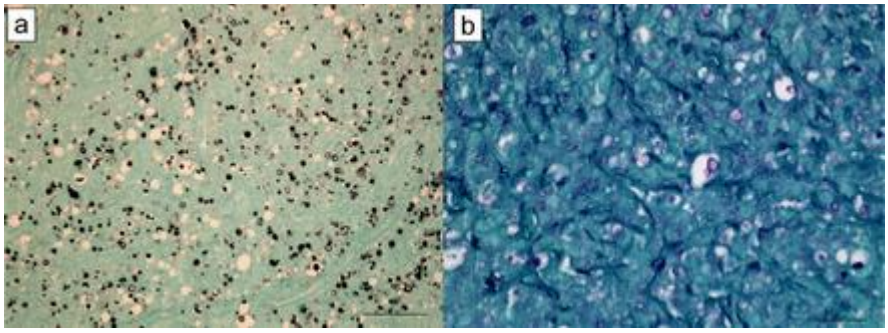
Livet *et al*. (2015) reported an indoor 9-year-old castrated male domestic cat with a 4 month history of increased upper airway noise. Computed tomography revealed a nasopharyngeal polypoid mass, which was removed endoscopically with basket forceps. Histopathology was compatible with a polypoid granulomatous pharyngitis with Cryptococcus-like organisms. This was supported by a positive serum latex cryptococcal antigen agglutination test (LCAT). Minimal inflammation of the nasal tissue was noted on histopathology, with no evidence of fungus. Following endoscopic removal of the mass, the patient was treated with systemic antifungal medication (itraconazole). One year after diagnosis, the LCAT titer was negative and the cat remained free of clinical signs. Relevance and novel information. This case report emphasized the importance of considering Cryptococcus species as a potential etiology in cats presented with signs of nasopharyngeal obstruction with an isolated nasopharyngeal polypoid mass, even if kept indoors.



(a) Transverse postcontrast computed tomography (CT) image of the nasopharynx showing the rim-enhancing polypoid mass completely occupying the nasopharyngeal lumen (black arrowheads). (b) Sagittal reformatted postcontrast CT image showing the rim-enhancing polypoid mass completely occupying the nasopharyngeal lumen (white arrowheads). (c) Three-dimensional (3D) CT image in a rostrocaudal (antegrade) direction showing the polypoid mass. (d) 3D CT image in a caudorostral (retrograde) direction showing the polypoid mass protruding slightly beyond the caudal margin of the soft palate, **Livet *et al*. (2015)**



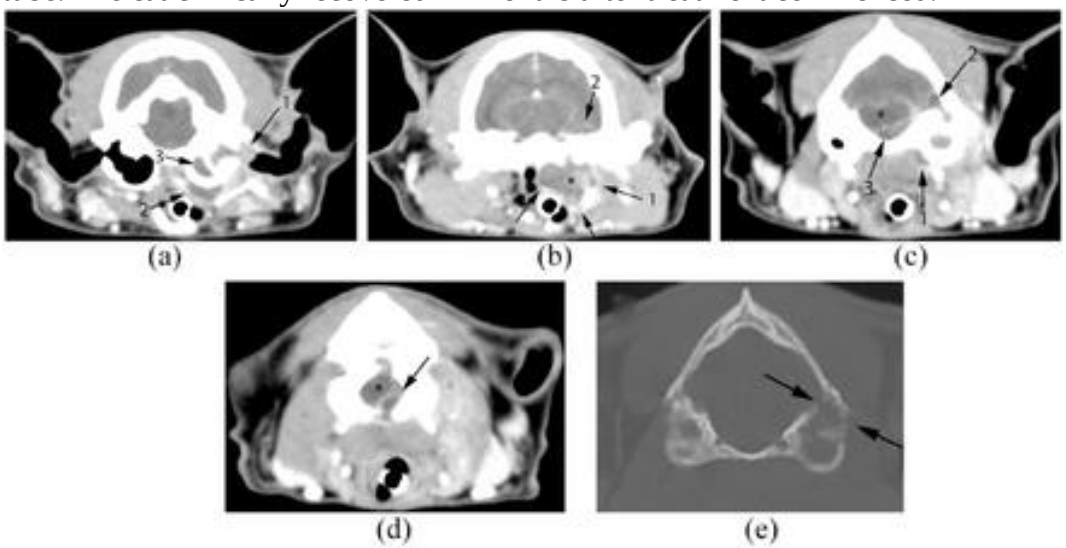
Nasopharyngoscopy in a cat with a nasopharyngeal polypoid mass. (a) Note the well-defined appearance of the mass completely filling the nasopharynx prior to withdrawal. (b) A grasping basket passed through the channel of the endoscope is advanced cranial to the mass. (c) The basket is opened and then pulled caudally in order to grasp the entire mass. (d) Severely inflamed nasal choanae are visible following mass removal, **Livet *et al*. (2015)**



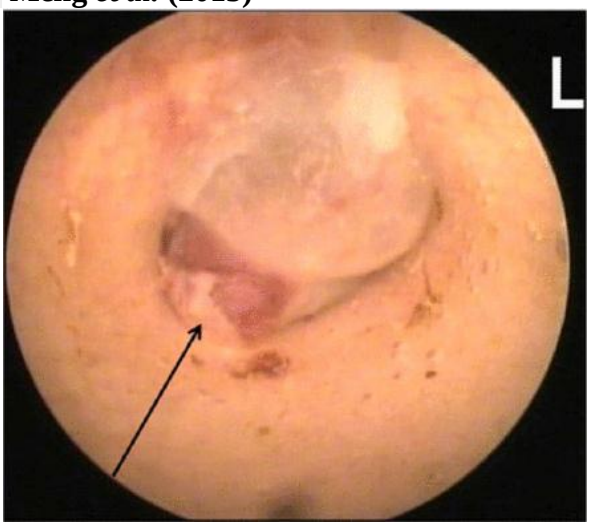
Histopathology of the polypoid granulomatous pharyngitis. (a) Gomori methenamine silver stain. The polypoid mass contains numerous yeast measuring 4–8 μm in diameter. (b) Periodic acid–Schiff stain. The yeast are surrounded by a clear zone corresponding to the capsule. Narrow-based budding, highly suggestive of *Cryptococcus*, is present, **Livet *et al*. (2015)**

Meng *et al.* (2015) reported a 7-year-old spayed domestic longhair cat from Perth, Western Australia, with left-sided head tilt, dysphonia, head shaking, inappetence and weight loss. A polypoid lesion had previously been removed from the external ear canal. Otitis media with extension into the external ear canal was suspected and investigated using video-otoscopy and computed tomography examination. Invasive disease with extension from the middle ear to the base of the skull, and intracranial extension into the caudal fossa and cranial cervical vertebral canal was detected. Cytology of external ear canal exudate showed capsulated budding yeasts and*Cryptococcus gattii* VGII was cultured. Treatment with amphotericin B infusions

and oral fluconazole was prescribed, with nutritional support via oesophagostomy tube. The cat clinically recovered 12 months after treatment commenced.



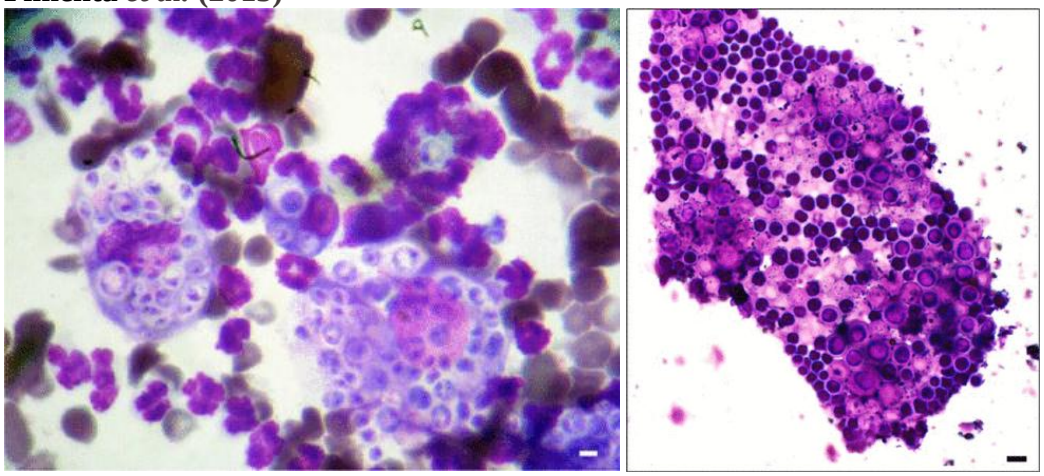
(a) The medial portion of the left external ear canal is filled with strongly and homogeneously contrast-enhancing soft tissue attenuating material (1). The ear canal lining is moderately contrast enhancing (compared with the right). The left tympanic bulla is filled with mildly and homogeneously contrast-enhancing material (3). Similar moderately enhancing material is seen extending ventromedially from the left bulla tympanica. A rim of strong contrast enhancement outlines this region (2). (b) The material seen ventromedial to the tympanic bulla continues caudally and forms a 1.6 cm (width) × 1.3 cm (height) × 2.5 cm (length) soft tissue structure, poorly enhancing centrally but strongly enhancing peripherally (1). This mass (*) distorts local tissue architecture such that the nasopharyngeal lumen is narrowed >50%, and the hyoid bones are displaced laterally. Additionally, at this level, there is a rim of contrast enhancement seen in the ventral aspect of the left temporal lobe of the cerebrum (2). (c) At the level of the caudal aspect of the left tympanic bulla, the soft tissue lesion can still be seen ventromedial to the bulla (1). Additionally, there is a rim of contrast enhancement outlining a hypoattenuating area in the left lateral cerebellum (2). There is poorly contrast-enhancing soft tissue attenuating material in the caudal fossa that is displacing the brainstem dorsally and to the right (3). Additionally, there is strong meningeal contrast enhancement in this region. (d) The poorly contrast-enhancing material in the cranial cavity can be followed further caudally, to the level of the atlas. This image, at the level of the foramen magnum, demonstrates abnormal tissue (arrow) displacing and compressing the cervical spinal cord (*) dorsally and to the right. (e) Bone window computed tomography image at the most caudal aspect of the bulla. Note the discontinuity of the temporal bone (black arrows), indicative of bony lysis, **Meng *et al.* (2015)**



Video-otoscopic photograph of the left tympanic membrane, which appears to have been breached by abnormal inflammatory tissue (arrows), which extends into the tympanic bulla

Pimenta *et al.* (2015) reported a clinical case of blepharitis due to *Cryptococcus neoformans* yeasts in a 2-year-old stray cat from northern Portugal (Vila Real) without concurrent naso-ocular signs. Ophthalmological examination revealed mucopurulent discharge from an open wound in the right upper and lower lids. Slit-lamp biomicroscopy showed a normal anterior segment, and intraocular pressure was within the normal reference interval. No fundoscopic alterations were detected in either eye by direct and indirect ophthalmoscopic examination. Cytological examination of an appositional smear showed numerous polymorphic neutrophils and macrophages, together with spherical yeast cells compatible with *Cryptococcus* species. Molecular analysis by means of PCR and restriction fragment length polymorphism identified *C neoformans* genotype VNI. The cat was treated with itraconazole, and amoxicillin and clavulanic acid, combined with a commercial ear ointment and an imidacloprid/moxidectin spot-on application for bilateral parasitic otitis caused by *Otodectes cynotis*. One month after treatment, the clinical signs were completely resolved. Localised cutaneous lesions, as in the present case, probably result from contamination of cat-scratch injuries with viable encapsulated yeasts.

Mucopurulent discharge from an open wound in the right upper and lower lids, after treatment, **Pimenta *et al.* (2015)**



Appositional smear showing numerous polymorphic neutrophils and spherical yeast cells with a prominent unstained capsule compatible with *Cryptococcus*species (Diff-Quik; scale bar = 20 µm)
Microscopical examination of fungal culture: cells of encapsulated yeasts compatible with *Cryptococcus* species (Hiss staining; scale bar = 20 µm), **Pimenta *et al.* (2015)**

2.5. Aetiology of cryptococcosis in cats and dogs

2.4.1. *Cryptococcus neoformans* (San Felice) Vuillemin, 1901

Synonyms:

1. *Saccharomyces neoformans San Felice, Annali Ig. Sperim.: 241 (1895)*
2. *Torula neoformans* (San Felice) J.D. Weis, Journal of Medical Research 7 (1902)
3. *Blastomyces neoformans* (Vuill.) Arzt, Archiv Dermatolo und Syphilis 145: 311 (1924)
4. *Debaryomyces neoformans* (San Felice) Redaelli, Cif. & Giordano, Boll. Sez. Ital. Soc. Int. Microbiol.: 24 (1937)
5. *Lipomyces neoformans* (San Felice) Cif., Manuale de Micologica Medica 2: 214 (1960)
6. *Torulopsis neoformans var. sheppei* A. Giord. [MB#456608]
7. *Cryptococcus hominis* Vuill., Revue Générale des Sci. Pures et Appl, 12: 735 (1901)
8. *Torula histolytica* J.L. Stoddart & Cutler, Studies from the Rockefeller Institute for Medical Research (1916)
9. *Cryptococcus hominis var. hondurianus* Castell., J.Trop, Med. Hyg. 36: 297-321 (1933)
10. *Cryptococcus meningitidis* C.W. Dodge, Medical mycology.: 333 (1935)
11. *Cryptococcus neoformans var. grubii* Franzot et al., J. Clin. Microbiol. 37: 839 (1999)

Morphology Colonies of *Cryptococcus neoformans* are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in color. The growth rate is somewhat slower than *Candida* and usually takes 48 to 72 h. It grows well at 25°C as well as 37°C. Ability to grow at 37°C is one of the features that differentiates *Cryptococcus neoformans* from other *Cryptococcus* spp. However, temperature-sensitive mutants that fail to grow at 37°C in vitro may also be observed . At 39-40°C, the growth of *Cryptococcus neoformans* starts to slow down.

Micromorphology On cornmeal tween 80 agar, *Cryptococcus neoformans* produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations. The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced.

2.4.1. *Cryptococcus gattii* (Vanbreusghem & Takashio) Kwon-Chung & Boekhout, Taxon 51 (4): 806 (2002)

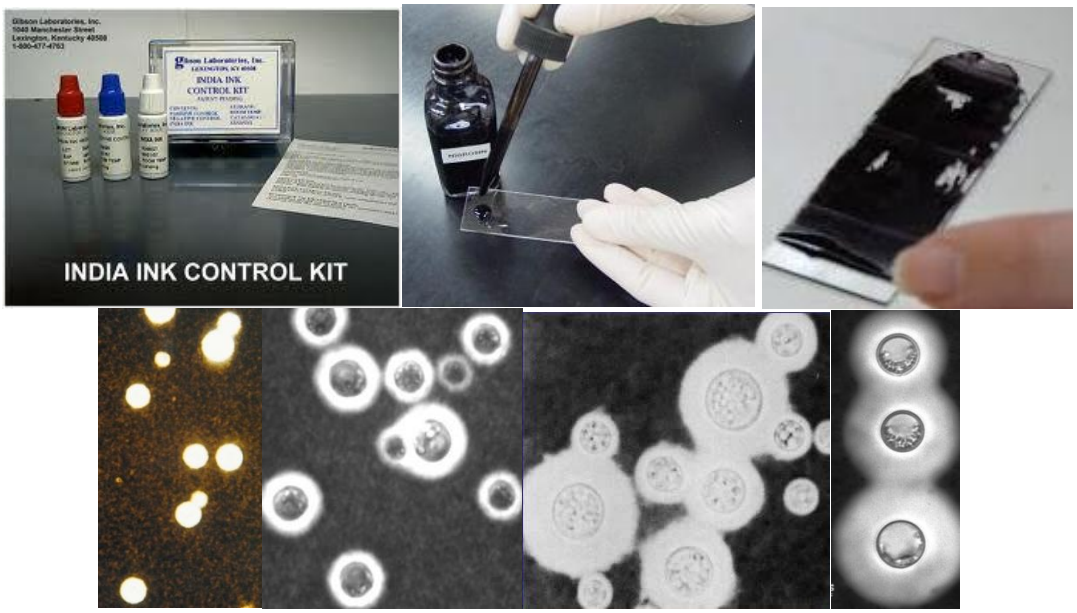
Synonyms:

1. *Cryptococcus neoformans var. gattii* Vanbreuseghem& Takashio, Annal. de la Soci. Belge de Méd.Trop. 50 (6): 701 (1970)
2. *Cryptococcus neoformans var. gattii* Vanbreuseghem & Takashio ex De Vroey & Gatti, Mycoses 32 (12): 675 (1989)
3. *Cryptococcus bacillisporus* Kwon-Chung & J.E. Benn., Intern.J.Syste. Bacteriol.28: 618 (1978)
4. *Cryptococcus neoformans var. shanghaiensis* W.Q. Liao et al., Chinese Med. J.: 287 (1983)

2.5. Diagnosis of cryptococcosis

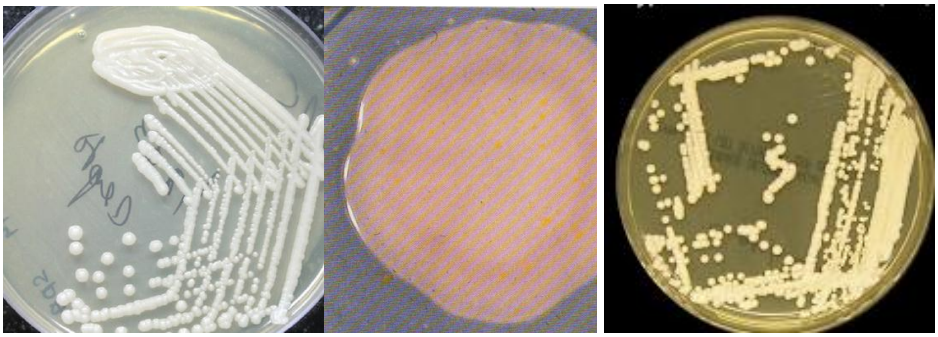
Direct microscopic examination of India ink preparation

Preliminary diagnosis of cryptococcal infection is made by direct microscopic examination of india ink preparations of samples.



Isolation and identification

Definitive diagnosis is confirmed by the culture of specimens, often the cerebrospinal fluid (CSF) or blood, and sometimes in respiratory secretions. *Cryptococcus neoformans* and *C. gattii* grow well at 37^{o}C. On Sabouraud dextrose agar colonies appear soft, creamy, opaque in 3-5 days, then colonies become mucoid and creamy to tan.



Cryptococcus colonies on Sabouraud's dextrose agar

Cryptococcus colonies are brown on bird seed agar, modified tobacco and Eucalyptus leave extract agar as well as on Pal′s medium. Other yeasts develop white to creamy colonies. On canavanine glycine bromthymol blue (CGB) medium, *Cryptococcus neoformans* develop non-coloured colonies and , while *C. gattii* develops blue colonies.

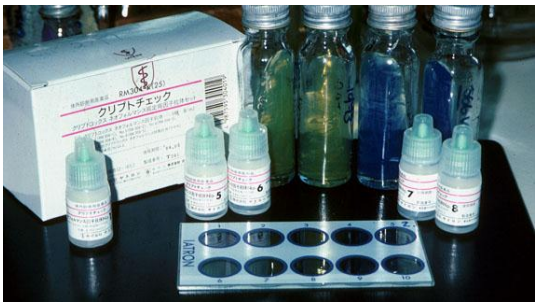
Colonies on bird seed agar *C. gattii* (left) colonies of *C. neoformans* (right)

Biochemical identification

Cryptococcus neoformans and *C. gattii* do not ferment sugars, but assimilate several sugars such as glucose, galactose, sucrose, maltose and inositol, but not lactose or nitrate and hydrolyses urea.

Serotyping of *Cryptococcus neoformans* and *C. gattii*

To determine the antigenic formulas of *Cryptococcus* species, equal volumes of factor serum and heat-killed cell suspension are mixed on a glass slide and rotated for 5 min, and then the results of agglutination are observed. The formation of aggregates within 5 min is considered positive. Smaller clumps are recorded as weakly positive. PSS is used for a negative control.



Iatron serotyping kit can be used to serotype isolates of *Cryptococcus neoformans*.

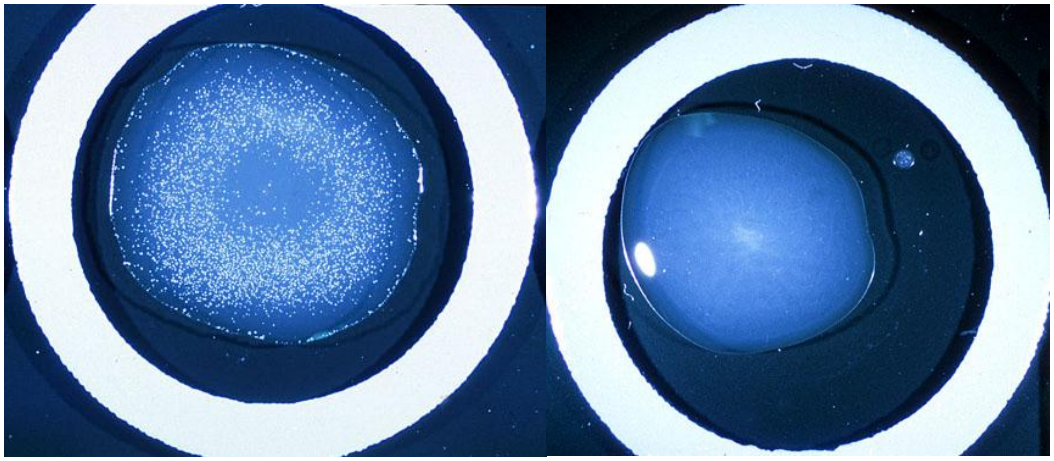
Molecular typing

Numerous molecular techniques have been applied to subtype *C. neoformans* and *C. gattii* strains, only three methods were proved to produce comparable results: PCR Fingerprinting, AFLP, and MLST. M13 PCR Fingerprinting and *URA5* RFLP:

Serological diagnosis of cryptococcosis

Cryptococcal antigen from cerebrospinal fluid is the best test for diagnosis of cryptococcal meningitis in terms of sensitivity. Rapid diagnostic methods to detect cryptococcal antigen by latex agglutination test, lateral flow immunochromatographic assay (LFA), or enzyme immunoassay (EIA).

This qualitative and semi quantitative test detects capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid. It utilizes latex particles coated with anticryptococcal globulin. This latex reacts with the cryptococcal polysaccharide antigen, causing a visible agglutination.



Positive latex test Negative latex test

2.6. Treatment

- Itraconazole is the drug of choice for treating cryptococcosis. This should be given with a fatty meal to enhance absorption of the drug.
- Fluconazole if there is CNS involvement.
- Surgical removal of the lesions in the nasal cavity.
- Supportive care such as a feeding tube if necessary.

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3. Malassezia dermatitis and otitis cats and dogs

3.1. Introduction

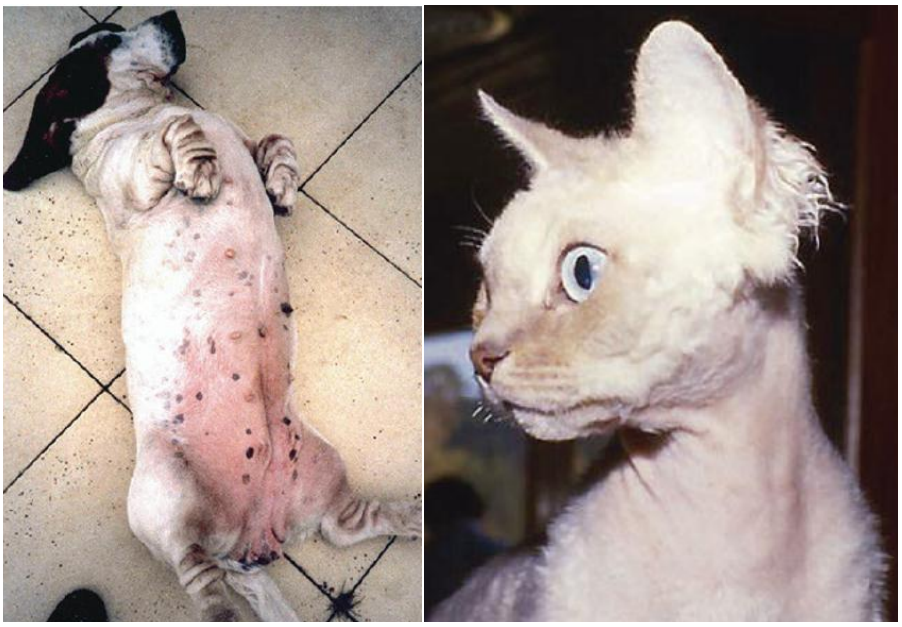
- *Malassezia* yeasts belong to normal cutaneous or mucosal microbiota of many warm-blooded vertebrates. They are recognized as opportunistic pathogens that play a significant role in the development of different human and animal diseases such as otitis externa or seborrheic dermatitis.
- Malassezia dermatitis and otitis occurs most commonly in animals with allergies, endocrinopathies (hypothyroidism, Cushing's disease), immunosuppressive diseases and other skin diseases.
- The genus *Malassezia* comprises 14 species, of which 13 *Malassezia* species show an absolute requirement for long fatty acid chains. These "lipid-dependent" yeasts are therefore seldom isolated in the laboratory unless specific nutrients are provided in the medium. The species *M. pachydermatis* is the only lipophilic yeast that may be isolated in regular media like Sabouraud dextrose agar.
- The most common causative organism is *Malassezia pachydermatis*. It is normal to find a small number of these organisms on cats, dogs and even people. However, overpopulation is common when the normal skin barrier is compromised.

- Dogs of any age, breed, or gender can be affected by yeast dermatitis. Predisposing skin factors for *Malassezia* include warmth, moisture, increased humidity, exaggerated skin folds, obesity and inflamed skin or ears.
- Commonly affected breeds include West Highland White Terriers, Basset Hounds, Cocker Spaniels, Springer Spaniels, and Chinese Shar Peis.
- Although less common than in dogs, yeast dermatitis can occur in cats, especially in Persian cats or cats with underlying internal disease.
- *Malassezia* is not considered to be contagious to other animals or people; however there are very rare reports of immunocompromised humans being at greater risk of infection.

3.2. Commonly affected breeds

- West Highland white terrier,
- dachshund, English setter,
- basset hound,
- American cocker spaniel,
- Chinese Shar Peis.
- Persian cats
- springer spaniel, and
- German shepherd.

West Highland white terriers are predisposed to Malassezia dermatitis (**Service de Parasitologei, ENVA)**

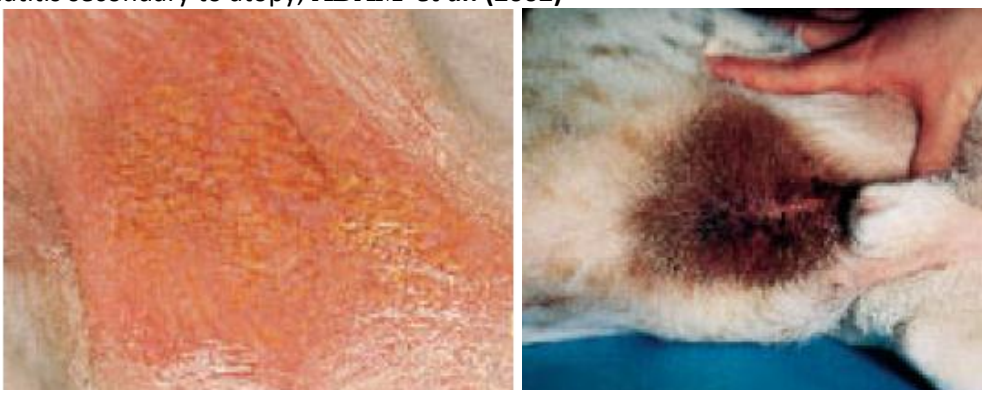


Left:The skin of a basset hounds represents a very favourable biotope for the development of Malassezia yeasts (Service de Parasitologei, ENVA), Right:The skin of Devon Rex cat represents a favourable biotope for the development of Malassezia yeasts. In this breed, high members of malassezia yeasts were detected in different anatomic sites, including axilla, groin, ventral neck and nail folds (**Service de Parasitologei, ENVA**)

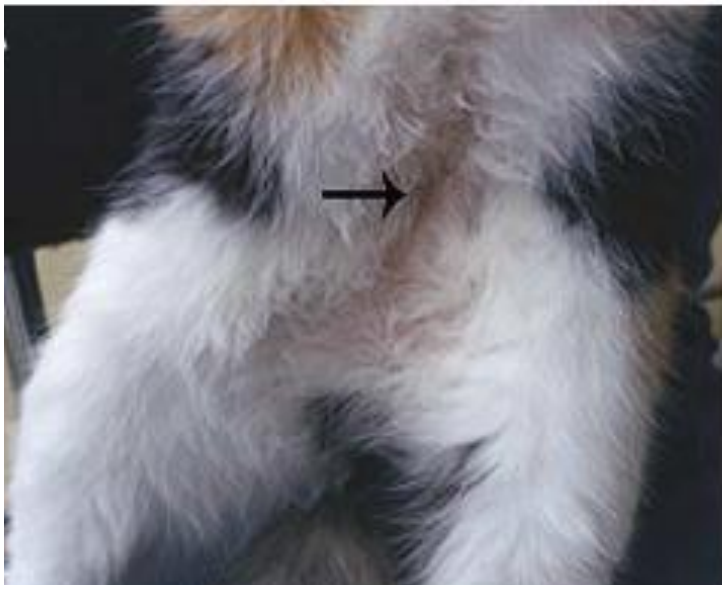
3.3. Clinical Signs

- Moderate to intense pruritus, which may be only partially responsive to corticosteroids and antibiotics.
- Affected animals typically have an offensive odour(yeasty or rancid).
- Dermatitis is manifested either as a generalized or localized dermatitis (lesions involving the ear, muzzle, interdigital areas, nail fold, ventral neck, medial thigh, axilla, perianal region, and intertriginous areas).
- Areas of the body commonly affected in dogs include the feet, nails, underside of the neck, axillae, abdomen, legs and under the tail.
- In cats, yeast infections can involve the chin or face, nails, or occasionally elsewhere on the body.
- Skin lesions are not specific for *Malassezia* dermatitis and reflect the existing seborrhea and pruritus. Lesions may be:
    - erythematous,
    - scaly (yellow to slate gray with or without plaques),
    - greasy or dry,
    - crusty,
    - hyperpigmented,
    - lichenified,
    - saliva-stained and alopecic

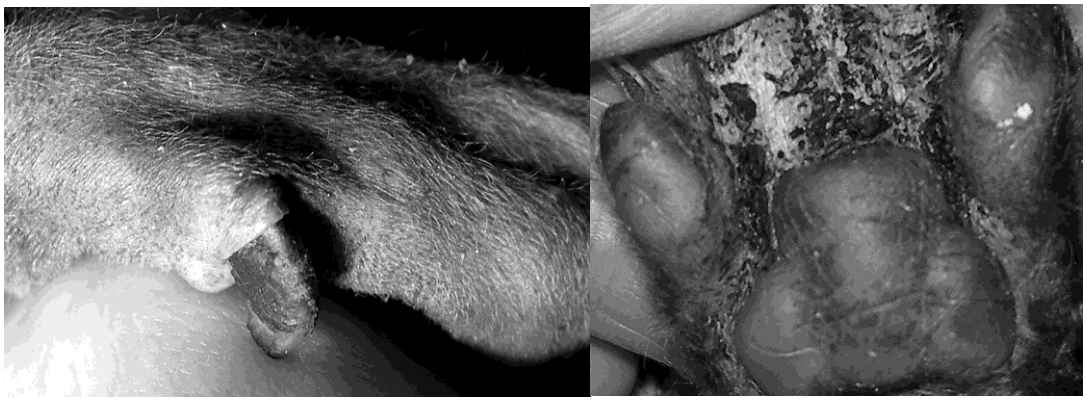
Left:The hind limb of an adult Shih Tzu *Malassezia* dermatitis secondary to demodicosis and hypothyroidism Right: The ventral neck of a West Highland white terrier with *Malassezia* dermatitis secondary to atopy, **ADAM *et al*. (2002)**



Left: axilla of a 6-month-old basset hound with *Malassezia* dermatitis and pyoderma.Right: ventral abdomen of an adult male hound dog with *Malassezia* dermatitis, **ADAM *et al*. (2002)**



Dermatitis of a cat associated with M. pachydermatis (arrow). .**R. Batra et al. (2005)**



Left fore foot of a seborrhoeic Devon Rex cat. Black, greasy material is tightly adhered to the medial aspect of the claw of digit I, Right fore foot of a seborrhoeic Devon Rex cat. Black, greasy material is adherent to the skin of the palmar aspect of the interdigital skin. **Ahman *et al.*(2007)**

Left: Alopecia and seborrheic dermatitis on the face of a cat, Right**:** Erythematous and seborroeic lesions on the ears of a cat, **Tresamol *et al.*, 2012**

Left: Erythema, crusts, and alopecia on the abdomen of a 6-year-old neutered female short-haired cat with *Malassezia* overgrowth Middle: the same cat after the application of a 2% miconazole/2% chlorhexidine shampoo (Malaseb®, Dechra) at 3 days interval for 4 weeks. Right:Brown and greasy material observed in the nail folds of a cat (with *Malassezia* overgrowth. **Crosaz *et al.* (2013)**

Left: Ear showing obvious signs of Malassezia Pachydermtitis, clearly inflammed and showing a

3.4. Predisposing factors:

- Skin microenvironmental factors favourable for yeast overgrowth include excessive sebum production, diminished sebum quality, moisture accumulation, a disrupted epidermal surface, and concurrent dermatoses.
- Diseases that cause cutaneous inflammation and altered sebum production and quality
    - allergies (atopy, food allergy, flea allergy, and contact allergy),
    - keratinization disorders (seborrhea),
    - bacterial skin diseases,
    - endocrinopathies (hyperadrenocorticism,
    - hypothyroidism, diabetes mellitus4),
    - metabolic diseases (zinc-responsive dermatosis and superficial necrolytic dermatitis),
    - cutaneous or internal neoplasia.

3.5. Aetiology:

3.5.1. Historical:

- **Eichstedt in 1846**: was the first to note the fungal nature of the aetiology of Pityriasis versicolor.
- **Robin 1853**: identified it as ***Microsporon furfur***
- **Baillon1889**: created a new genus ***Malassezia*** and replaced the name ***Microsporon furfur*** by ***Malassezia furfur***
- **Rivolta 1873**: placed the organism within the genus Cryptococcus
- **Malassez 1874**: described spherical and oval "spores" in scaly lesions of the scalp and named them ***Saccharomyces ovalis*** and ***Saccharomyces sphaericus***
- **Sabouraud 1904:** established a new genus ***Pityrosporum*** for the above mentioned species.
- **Aldo Castellani and Albert J. Chalmers 1913**: introduced the name ***Pityrosporum ovale***
- **Albert J. Chalmers 1925:** managed to isolate the organism in culture
- **Weidman 1925**: was first to use the name ***Pityrosporum*** *pachydermatis* (Greek for "thick-skin") for a yeast isolated from an Indian rhinoceros (*Rhinocerosus unicornis*) with severe exfoliative dermatis.
- **Panja 1927**: recognized similarities between ***Malassezia*** and ***Pityrosporum*** and he suggested a single genus for these yeasts
- **Dodge 1935**: proposed the name ***Malassezia*** *pachydermatis* for the yeast isoalated by Weidman
- Morris A. Gordon 1951: isolated from PV lesions and healthy skin the double-contour, globose cells that he named ***Pityrosporum orbiculare***.
- **Gustafsson 1955:** was the first, who associated the yeast with canine otitis externa in and gave it the names ***Pityrosporum canis***

- Gordon 1951: described a new species and named it ***Pityrosporum orbiculare***
- Lodder and Kerger-van-Rij 1952: accepted the Genus Pityrosporum of Sabouraud **and** listed 3 species of Pityrosporum, ***P. ovale, P. pachydermatis*** and **P. orbiculare**
- Lodder and Kerger-van-Rij 1984: in the third edition of "The Yeasts", a single generic name was proposed for these yeasts and ***Malassezia*** was accepted.
- The International Commission on the Taxonomy of Fungi 1986 approved the genus ***Malassezia***

3.5.2. Currently recognized species of the genus *Malassezia*

1. *Malassezia furfur* (Robin) Baillon (1889)
2. *Malassezia pachydermatis* (Weidman) Dodge (1925)
3. *Malassezia sympodialis* Simmons & Guého (1990)
4. *Malassezia globosa* Midgley, Guého & Guillot (1996)
5. *Malassezia obtusa* Midgley, Guillot & Guého (1996)
6. *Malassezia slooffiae* Guillot, Midgley & Guého (1996)
7. *Malassezia restricta* Guého, Guillot & Midgley (1996)
8. *Malassezia dermatis* Sugita, Takashima, Nishikawa & Shinoda (2002)
9. *Malassezia japonica* Sugita, Takashima, Kodama, Tsuboi & Nishikawa (2003)
10. *Malassezia nana* Hirai, Kano, Makimura, Yamaguchi & Hasegawa (2004)
11. *Malassezia yamatoensis* Sugita, Tajima, Takashima, Amaya, Saito, Tsuboi & Nishikawa (2004)
12. *Malassezia caprae* Cabañes & Boekhout (2007)
13. *Malassezia equina* Cabañes & Boekhout (2007)
14. *Malassezia cuniculi* Cabañes , Vega &Castellá (2011).

3.5.3. Malssezia species isolated from cats and dogs

3.5.3.1. ***M. pachydermatis***,

- M. pachydermatis is a lipophilic species,
- M. pachydermatis able to grow without supplementation of long-chain fatty acids or their esters.
- All isolates grow well at 37 C,
- M. pachydermatis occurs rarely on humans, although it has been found to cause septic epidemics, usually in neonates receiving intravenous lipid supplementation.
- M. pachydermatis is well-known as a normal cutaneous inhabitant of numerous warm-blooded animals.
- Seborrhoeic dermatitis and otitis associated with this lipophilic yeast are now commonly recognized, especially in dogs.

3.5.3.2. M. sympodialis

- M. sympodialis is characterized by a strong b-glucosidase activity and growth at 37^{o}C,
- Poor growth with cremophor EL as a lipid supplement.
- M. sympodialis cells are small and ovoid.

- M. sympodialis is commonly isolated from healthy as well as diseased skin.
- M. sympodialis is often present in skin lesions
- M. sympodialis has also been isolated from healthy feline skin
- Its role as a pathogen has not yet been elucidated.

3.5.3.3. *M. slooffiae*

- M. slooffiae does not grow with cremophor.
- M. slooffiae is regularly isolated from human skin and is mostly found in association with M. sympodialis or M. globosa.
- M. slooffiae may be a weak human pathogen, and seems better adapted to animals, especially pigs.

3.5.3.4. M. obtusa

- M. obtusa resembles M. furfur morphologically
- M. obtusa does not grow at 37 C,
- M. obtusa cannot utilize any of the five lipids used in the tests.
- M. obtusa darkens esculin medium.
- M. obtusa is a rare species that was known only from healthy human skin.
- M. obtusa was recently isolated from goats, horses and dogs.

3.5.3.5. M. furfur

- *M. furfur* is the cause of pityriasis versicolor in man
- *M. furfur* is morphologically heterogeneous with globose, oval or cylindrical yeast cells.
- *M. furfur* can be identified by its ability to grow at 37 °C, strong catalase activity, absence or a very weak β-glucosidase activity, and equal growth in the presence of cremophor EL (=castor oil) and Tweens 20, 40, 60, 80 as sole lipid sources

3.5.4. Characteristics of Malassezia species

Species: 1, *M. nana* sp. nov.; 2, *M. furfur*; 3, *M. pachydermatis*; 4, *M. sympodialis*; 5, *M. globosa*; 6, *M. obtusa*; 7, *M. restricta*; 8, *M. slooffiae*; 9, *M. dermatis*. Data are from Guého *et al.* (1996), Mayser *et al.* (1997), Hammer & Riley (2000) and Midgley (2000). +, Positive; +/−, weakly positive; −, negative; V, variable; NT, not tested.

Characteristic	1	2	3	4	5	6	7	8	9
Growth on Sab.* at 32 °C	−	−	+	−	−	−	−	−	−
Growth on mDixon at 40 °C	+ or −	+	+	+	−	−	−	+	+
Catalase reaction	+	+	+/− or +	+	+	+	−	+	+
Use of lipid sources:									
Tween 20 (10 %)	+ or −	+	−	−	−	−	−	+/− or +	+
Tween 40 or 60 (0·5 %)	+	+	+	+	−	−	−	+	+
Tween 80 (0·1 %)	+/−	+	+	+	−	−	−	−	+
Cremophor EL	−	+	V	−	−	−	−	−	+
Hydrolysis of aesculin	−	−	V	+	−	+	−	−	−
Precipitate production on mDixon agar	+	−	NT	+	+	−	NT	−	+

*Sabouraud's agar without any lipid supplementation.

Salient characteristics of Malassezia species (R. Batra et al. / FEMS Yeast Research 5 (2005) 1101–1113)

Species	Lipid dependency	Tween 20	Tween 40	Tween 60	Tween 80	Cremophor EL	Catalase	β-Glucosidase	Growth at 37 °C
M. dermatis	+	+	+	+	+	W, (+)	+	?	+
M. furfur	+	+, (−)	+, (−)	+, (−)	+, (−)	+, (−)	+, (−)	−, (w)	+
M. globosa	+	−	−[2]	−[2]	−	−	+	−	−, (w)
M. japonica	+	−	w	+	−	?	+	?	+
M. nana	+	v	+	+	w	?	+	?	+
M. obtusa	+	−	−	−	−	−	+	+	−, (w)
M. pachydermatis	−, (w)	+[1]	+	+	+	+[1]	+, w	+, (−)	+
M. restricta	+	−	−[3]	−[3]	−	?	−	−	v
M. slooffiae	+	+, w, (−)	+	+	−, (w)	−	+	−	+
M. sympodialis	+	−, w[2]	+	+	+	−, (w)	+	+	+
M. vamatoensis	+	+	+	+	+	?	+	?	+

3.5.5. ***Malassezia pachydermatis*** (Weidman) C.W. Dodge, Medical mycology. Fungous diseases of men and other mammals: 370 (1935)

Synonyms:

≡Torulopsis pachydermatis (Weidman) Krassiln.

≡Pityrosporum pachydermatis Weidman, Rep. Lab. Mus. Comp. Path. Zool. Soc. Philad.: 36 (1925)

≡Cryptococcus pachydermatis (Weidman) Nann., Repert sist dei miceti 4: 345 (1934)

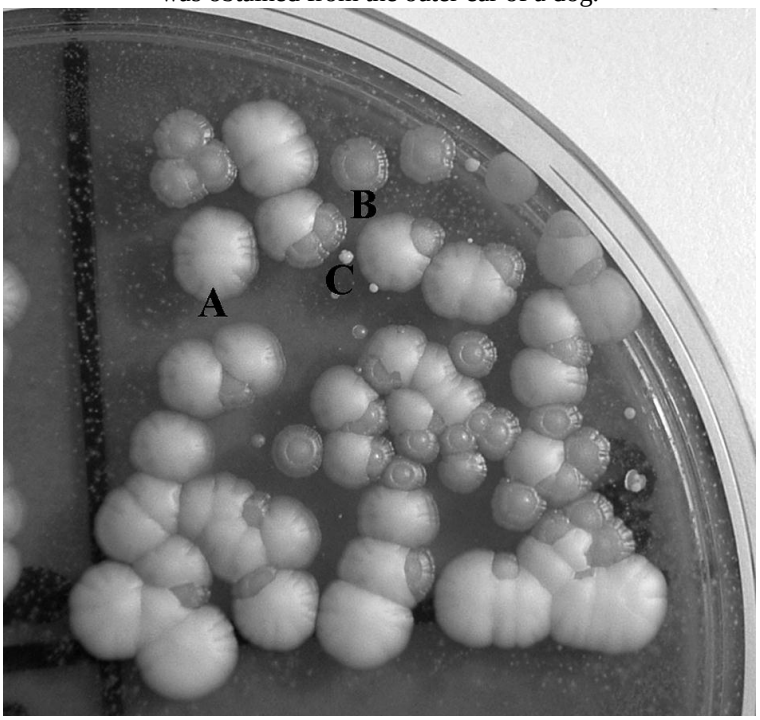
=Pityrosporum rhinocerosum Sabour.

=Pityrosporum canis Gustafson, Otitis externa in the Dog, Stockholm: 46 (1955)

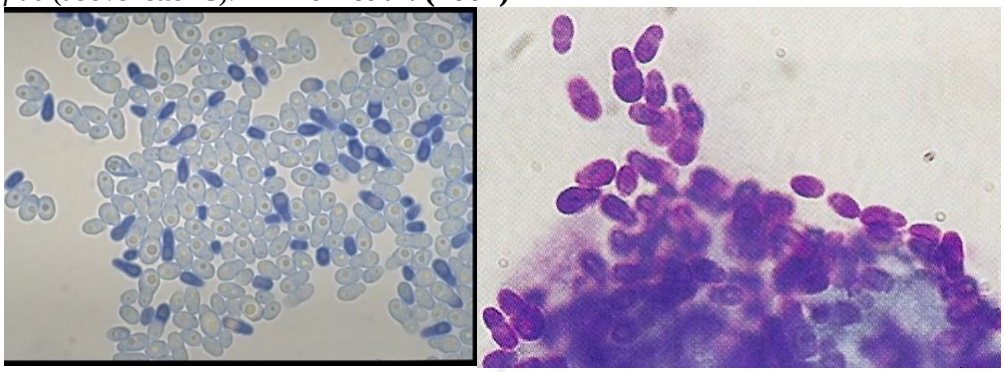
Cultural characteristics

The growth patterns of ***Malassezia pachydermatis*** shows poor growth on Blood Agar and DTM after 72 h at 37 "C, but grows well on SDA, YM and modified malt extract agar after 48 h at 37 "C. On the surface of plates two colony forms are observed: (a) white-creamy, matt, smooth, moist colonies, 0.5-1 mm in diameter (35 strains); and (b) dark creamy, dry, fragile (hard to suspend) colonies,0.2-0.8 mm in diameter. Both types of colonies firmly adhere to the agar surface and assume a dark-brownish colour with age. On BA, a poor growth of pin-point sized colonies is observed after 3-4 days. These colonies are non-haemolytic, but the agar around them shows a dark discolouration.

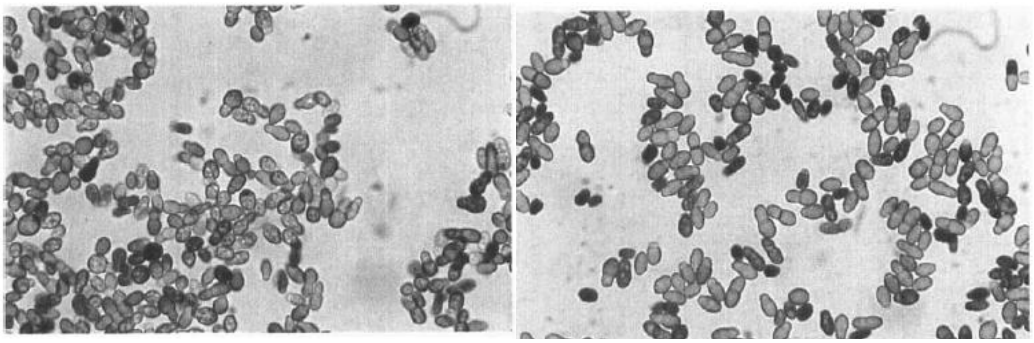
Malassezia pachydermatis, colonies on SDA (left, **ww.scienceopen.com**) and on Potato dextrose agar (right, **www.pf.chiba-u.ac.jp**), supplemented with chloramphenical at 25℃ for 14 days, Culture was obtained from the outer ear of a dog.



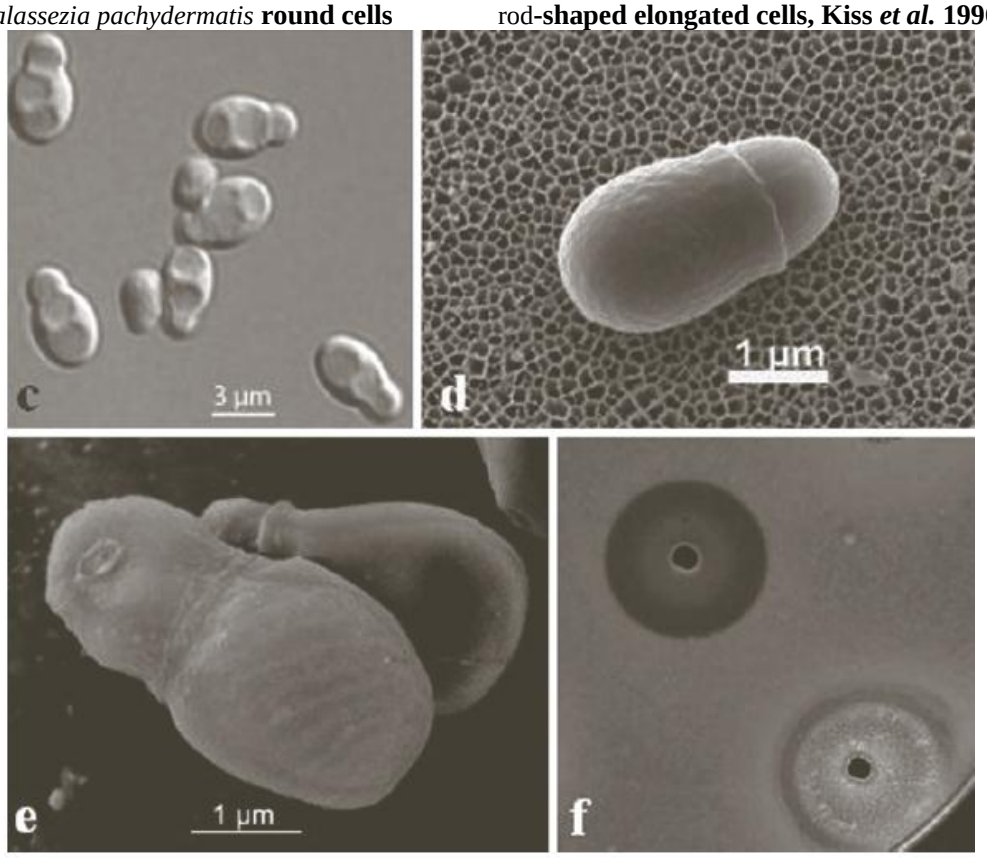
Primary isolation plate composed of modified Dixon's agar inoculated with a swab from the claw fold of the fore foot of a seborrhoeic Devon Rex cat. Two colony types of *M. pachydermatis* are represented; large, domed, entire yellow colonies (above letter A) and smaller, buff-coloured, domed or umbonate colonies (above letter B). The smaller yellow colonies are examples of *M. slooffiae* (above letter C). **Ahman *et al*. (2007)**



*Malassezia pachydermatis*www.pfdb.net (**Y. Nishiyam**) www.studyblue.com



Malassezia pachydermatis **round cells** rod-**shaped elongated cells, Kiss *et al.* 1996**



M. pachydermatis. C,d,e, Nomarski's and SEM micrographs showing ovoid to short cylindrical yeast cells with a broad budding site; (**e**), showing the helicoidal structure of the cell wall; (**f**), details of Cr EL and Tween 40 utilization showing secondary growth within the inhibitory areas, **E. Guého-Kellermann et al., 2010**

Biochemical characteristics:

None of the carbohydrates is fermented. After 48 h glucose is assimilated by all strains, mannitol and sorbitol assimilation is variable. All strains assimilate peptone but none assimilate (NH4)2S04K, N0 3 or ethanol. Urease test on Christensen's agar rapidly gives a positive result, within 24 h at 37 "C. Indole is negative, catalase variable, lecithinase activity on eggyolk positive strong peroxidase activity and negative coagulase (either on a slide or in a tube)

3.5.6. Reports on *Malassezia pachydermatis* in cats and dogs

Baxter (1976) investigated the frequency of ***Malassezia pachydermatis*** within the non-otitic external ear canals of dogs and cats and an assessment of the abundance of the yeast made by microscopic and cultural techniques. It was present in varying

amounts in 49% of clean dog ears and 23% of clean cat ears. Microscopically, 14% of the ears were found to contain the yeast in large numbers. A further 3 % of ears examined showed excessive cerumen production and all these contained the yeast. The prevalence of *M. pachydermatis* in non-otitic ears of both dogs and cats was similar to that previously reported in otitic ears.

Gedek *et al.* (1979) assessed the bacterial and mycotic flora in 158 ears of dogs with otitis externa and in 101 ears of healthy control dogs. ***Malassezia pachydermatis*** occurred in 57 per cent of ears with otitis externa and in 17 per cent of clinically healthy ears. Staphylococci and Pseudomonas aeruginosa were the predominant bacteria in otitic ears, micrococci and Bacillus spp were the most frequent isolates from clinically healthy ears. ***Malassezia pachydermatis*** mainly chronic cases of otitis externa. A combination preparation, containing miconazole, polymyzin B and prednisolone, was highly effective in controlling the clinical signs of otitis externa and eliminating flora from the affected ears. The data presented suggest that yeasts, and especially ***Malassezia pachydermatis***, may be significant pathogens in otitis externa and that antimycotic treatment is an essential part of the treatment of otitis externa in dogs.

Dufait (1983) was the first one to report *Malassezia* yeasts as a cause of generalized dermatitis in dogs. He described a series of 50 dogs with pruritic dermatitis from which the yeasts could be readily recovered by cytology or culture and which responded to antifungal therapy. Skin lesions consisted of erythema and hyperpigmentation that most often affected the ventral abdomen, although the face, feet and perineal regions were also commonly affected.

Gabal (1988) conducted a study on the mechanism of infection and the characterization of ***Malassezia pachydermatis*** in connection with canine otitis externa. The ability of this yeast to grow uninhibited in intimate contact with the diversity of other microbial isolants of the canine aural canal was demonstrated. Canine cerumen seemed to promote the growth of this yeast. Although the source of infection is still obscure, M. pachydermatis managed to survive in soil and dust at different temperatures for four weeks. Among the commonly used diagnostic media, Sabouraud's dextrose agar was shown to be the most supportive for the growth of this yeast.

Plant *et al.* (1992) evaluated the prevalence of cutaneous Malassezia spp in a semi quantitative fashion at 3 sites on 98 dogs examined because of various dermatoses. Thirty (10.2%) of the sites and 19 (19.4%) of the dogs had Malassezia spp amounts higher than that found on grossly normal skin. The prevalence of higher than normal amounts did not correlate significantly with sample site, sex, or age. The factors associated with an increased prevalence of increased Malassezia spp counts were seborrheic dermatitis, recent antibiotic treatment, and breed.

Bond *et al.* (1994) compared the ***Malassezia pachydermatis*** populations of the axilla and groin of 12 normal and 12 atopic dogs using tape-strips and contact plates. When assessed by either method, the mean density of yeasts in the groin of the atopic dogs was significantly greater ($P<0.05$) than that of the normal dogs, suggesting that the cutaneous microenvironment of the groin region of the atopic dogs favoured colonisation by this yeast. Differences between the counts from the axilla were not significant. The frequency of isolation of yeasts from both dogs and sites was

significantly higher ($P<0.05$ and $P<0.001$, respectively) in the atopic group. There was a very highly significant correlation ($P<0.001$) between the tape-strip counts and contact plate counts in the atopic group only. This study suggests that isolation of numerous *M pachydermatis* colonies from the axilla and groin of dogs using contact plates is indicative of elevated skin surface populations. The simplicity of the contact plate method makes it suitable for the routine quantitative culture of cutaneous *M pachydermatis* populations in dogs with dermatological disease.

Bond *et al.* (1995) sub-cultured 244 Malassezia colonies which had been isolated from a colony of Beagle dogs using modified Dixon's agar on Sabouraud's dextrose agar to determine their lipid dependence, 30 showed poor growth resembling M. furfur, whereas the remainder were typical of ***M. pachydermatis***. Eight of the 10 poor growing isolates selected for further study formed colonies typical of M. pachydermatis after five passages on Sabouraud's dextrose agar at 4 d intervals and two continued to show poor growth. Nine isolates had enzyme profiles identical to those of typical M. pachydermatis isolates, and one resembled M. furfur. However, seven of the poor growing isolates which were karyotyped had patterns typical of M. pachydermatis. Poor growing isolates and their non-lipid-dependent 'revertants' had identical restriction fragment length polymorphism patterns and poly(GT) hybridization profiles. These observations show that some M. pachydermatis isolates grow poorly when sub-cultured onto Sabouraud's dextrose agar and may be incorrectly identified as M. furfur if further studies are not performed.

Bond *et al.* (1995) studied the skin and mucosal carriage of ***Malassezia pachydermatis*** in 20 healthy pet dogs of various breeds and in 20 kennelled beagles. Using swabs, anal carriage was detected in 10 pet dogs and 11 beagles and the nose, mouth, prepuce and vulva were shown to be infrequently colonised. *M pachydermatis* was isolated from the external ear canal of 11 beagles and two pet dogs; both the population sizes and frequency of isolation were significantly ($P<0{\cdot}05$) greater in the beagles. The yeast was infrequently isolated from the axilla and groin in low numbers using contact plates and detergent scrub samples but was often cultured from the lower lip and the dorsal interdigital spaces; isolation frequencies and population sizes in the two groups of dogs were not significantly different. These results demonstrate that the anus, external ear canal and lip and interdigital skin of healthy dogs are frequently colonised by *M pachydermatis.*

Åkerstedt**, J. and** I. Vollset **(1996)** reviewed the clinical manifestation, aetiology, diagnosis and treatment of disease conditions in dogs caused by ***M. pachydermatis***. They mentioned that a review of the diseases caused by *Malassezia pachydermatis* has led to the conclusion that the yeast is an opportunistic pathogen that depends on predisposing host factors and different immune suppressive mechanisms for clinical manifestation. Until recently, the role of *M. pachydermatis* in seborrhoeic dermatitis and otitis externa in dogs has been largely unrecognized.

Bond *et al.* (1996) investigated the carriage of Malassezia yeasts was investigated in 17 cats in two colonies using a lipid-supplemented culture medium. ***Malassezia pachydermatis*** was isolated from one cat. Lipid-dependent Malassezia yeasts with electrophoretic karyotypes consistent with M. sympodialis were isolated from all six cats in one group and from one of 11 in the second group. To our knowledge, this is the first report of the isolation of lipid-dependent yeasts from cats.

Kennis *et al.* (1996) performed a study to define the extent to which Malassezia organisms can be recovered from the skin of clinically normal dogs and to assess differences in organism recovery related to anatomic sampling site and to method of collection. The number of **Malassezia pachydermatis** organisms were determined in fungal cultures of samples obtained from the skin of 19 clinically normal dogs, using an adhesive tape method to obtain samples from 10 sites/dog. Additionally, 3 methods (direct impression, swabbing technique, and superficial skin scraping) that are commonly used for obtaining samples for cytologic examination were evaluated. Malassezia organisms were found in low numbers as part of the microflora of the skin of clinically normal dogs. Number of organisms differed significantly for various anatomic locations (chin, highest number; inguinal and axillary regions, lowest number). Malassezia organisms were identified more frequently by use of adhesive tape and fungal culturing than by the methods used for cytologic examination. However, comparing methods used for obtaining samples for cytologic examination with each other, marked differences were not detected in our ability to recover yeast organisms among the 3 techniques.

Kiss *et al.* (1996) studied the morphological, cultural and biochemical characteristics of 80 *M. pachydermatis* strains isolated from cases of canine otitis externa. Microscopically, the strains could be subdivided into two phenotypes. All *M. pachydermatis* strains grew well on Sabouraud glucose, yeast morphology and modified malt extract agar, but formed two distinct colony types. All strains were characterized by no fermentation. Assimilation of glucose, mannitol (42 strains), sorbitol (40 strains) and peptone was observed, but no ethanol assimilation. Urease and catalase tests were positive, while indole and acetoin production was not detected. All strains showed proteinase, caseinase, lecithinase and peroxidase positivity but to varying extents. Esterase activity was observed for all *Malassezia* strains when using Tween 20, 40 and 60, whereas Tween 80 was hydrolysed by only 42 strains. No coagulase or haemagglutinating activities were detected. When compared for satellite phenomenon and vitamin requirements, some *Malassezia* strains could not grow in the absence of nicotinic acid but grew well in the presence of staphylococci. In susceptibility tests, all strains showed the highest susceptibility to ketoconazole. On the basis of the biochemical differences, *M. pachydermatis* seems to be a heterogeneous species and can be divided into two groups.

Bernardo *et al.* (1998) examined 130 dogs of different breeds and with different clinical forms of **external otitis** were mycologically and bacteriologically. Forty six of those dogs showed abnormal cerumen with a high yeasts contamination. These yeasts belong to four species: ***Malassezia pachydermatis*** (80.4). The most affected dogs were a pendulous ears breeding (65,7%) and males (86,8%). Some dogs had other cutaneous disorders (seborrhoeic dermatitis, pemphigus). In vitro tests, using seven different antifungal drugs were systematically performed. All strains revealed to be 5-fluorocytosine-resistant and 32% of them were also resistant to nystatin. One M. pachydermatis isolated was resistant to all of the tested antifungal drugs

Morris (1999) stated that dermatitis and otitis caused by the yeast *Malassezia pachydermatis* is common in dogs, but unusual in cats. These conditions are extremely pruritic and may occur in conjunction with concurrent or predisposing diseases such as allergic dermatitis or endocrinopathy.

Oliveira *et al.* (2001) evaluated the frequency of ***Malassezia pachydermatis*** infection and other infectious agents in dogs with external otitis and with healthy auditory tubes. Samples from the auditory tube of 102 dogs with otitis and from 32 healthy dogs were submitted to direct microscopic examination and cultured in blood agar and Sabouraud dextrose agar with chloramphenicol and cycloheximide. Direct examination showed more than ten cells of *M. pachydermatis* in 52.0% of the samples from dogs with otitis, but in only 21.8% of the healthy auditory tube samples. *M. pachydermatis* was isolated in 37.5% of the samples from dogs with healthy auditory tube and 76.5% ($p<0.01$) of the samples from dogs with otitis. There was an association between *M. pachydermatis* and *Staphylococcus aureus* ($p<0.01$), but not with *Pseudomonas aeruginosa* ($p>0.05$). Infection by *M. pachydermatis*was prevalent in the following breeds: Cocker Spainel, German Shepherd and Brazilian Fila. No differences were found in frequency of the infection in relation to age, sex and ear anatomy of the dogs. Otomycosis were predominantly ceruminous and erythematous. *M. pachydermatis* was the most frequent agent in external otitis.

Crespo *et al.* (2002) studied the lipophilic microbiota of the external ear canals of 332 animals (264 dogs and 68 cats), with and without otitis externa, over an 11-year period from 1988 to 1999. ***Malassezia pachydermatis*** was isolated from 62.2% and 50% of dogs with and without otitis externa, respectively, and from 41.2% and 17.6% of cats with and without otitis externa, respectively. In the group of animals studied for lipid-dependent species, these yeasts were isolated from 4.5% of dogs with otitis externa and from 23.1% and 8.9% of cats with and without otitis externa, respectively. *M. sympodialis* and *M. furfur* were isolated from cats and M. furfur and *M. obtusa* from dogs. Our findings show that lipid-dependent Malassezia species may contribute to the etiology of otitis externa in dogs and cats.

Prado *et al. (2004)* *examined* 19 dogs with unilateral or bilateral corneal ulcers and 60 healthy dogs for the presence of ***Malassezia pachydermatis*** . A total of 158 clinical specimens from both the groups were obtained from the conjunctival sac of each eye by a calibrated platinum loop. The samples were placed on Dixon and blood agar, incubated at 35 °C, and examined daily for 15 days. Then, the strains were subcultured on Sabouraud agar. Of 22 clinical specimens collected from the eyes with corneal ulcers, five cultures (23%) were positive for *M. pachydermatis*. Of 16 samples collected from the contralateral healthy eye, cultures were positive in three samples (19%). Three animals had unilateral corneal ulcer and positive cultures for *M. pachydermatis* in both the eyes. Two dogs had unilateral corneal ulcer and positive cultures for *M. pachydermatis* in the same eye. However, from the 120 samples of 60 healthy dogs, only four clinical specimens (3%) had positive cultures for *M. pachydermatis*. The findings of *M. pachydermatis*, in a considerable percentage of clinical specimens from dogs with corneal ulcer, suggest its possible role at least as an aggravating factor in the pathophysiology of this disease.

Cafarchia *et al.* (2005) mentioned that out of the 413 isolates obtained from animals with and without otitis, 403 (97.6%) were identified as ***M. pachydermatis*** and 10 (2.4%) as *M. globosa*. A statistical evaluation of the occurrence of Malassezia yeasts in dogs and cats revealed that predisposing factors for Malassezia infections are sampling period for cats, and type of ear for dogs. The largest population of Malassezia yeasts was detected in animals with otitis, suggesting a role in the occurrence of lesions**.**

Ahman *et al.* (2007) investigated skin and anal mucosal carriage of *Malassezia* spp. yeasts in 21 healthy Devon Rex cats (DRC) and in 9 seborrhoeic DRC using swabs and contact plates. ***M. pachydermatis*** was isolated from 26 cats and lipid-dependent *Malassezia* spp. isolates were recovered from the claw fold of 5 healthy and 3 seborrhoeic DRC. The frequencies of isolation and population sizes of *M. pachydermatis* in the axillae, left groin and claw fold in seborrhoeic DRC significantly exceeded (P<0.05) those of healthy animals. The frequencies of isolation and population sizes of *M. pachydermatis* in the axillae and groin in both groups of DRC, and the frequencies of isolation and population sizes of *M. pachydermatis* in the claw fold of the seborrhoeic DRC, exceeded those of healthy Domestic short-haired cats. Using polymerase chain reaction – restriction enzyme analyses (PCR-REA) based on amplification of the large subunit rRNA gene, all eight lipid-dependent isolates had profiles that were indistinguishable from that of *M. slooffiae* CBS 7956. These data indicate that DRC are frequently colonized by *M. pachydermatis* and that the claw folds may also be colonized by *M. slooffiae*.

Ahman *et al*. (2007) described Two distinct *M. pachydermatis* colony morphologies, i.e., 'type A' colonies were large, domed, entire yellow colonies, often surrounded by precipitates in the medium, that grew well when sub-cultured on Sabouraud's dextrose agar as opposed to 'type B' colonies which were smaller, buff-coloured, domed or umbonate with precipitates, which grew quite poorly but could be maintained on Sabouraud's dextrose agar

Dizotti and Coutinho (2007) studied 45 cats, 20 with and 25 without **otitis externa (OE).** Cerumen or secretion from external ear canal samples was cultured on modified Mycosel agar and sterile olive oil was added to the surface of the medium before specimen seeding. The isolates were analysed for macro- and micromorphology and identified by catalase tests and on the basis of growth on Tween 20, 40, 60 and 80. *Malassezia* spp. were isolated from 15 out of 20 (75%) animals with otitis and from 7 out of 25 (28%) cats without OE; the difference between the two groups was statistically significant ($P \leq 0.05$). ***Malassezia pachydermatis*** and *M. sympodialis* were isolated from 60% (12/20) and 40% (8/20) of cats with otitis, respectively, with no significant difference in the frequency of isolation between the two species. In the microflora of the healthy ear canal *M. pachydermatis* was significantly more common (6/25, 24%) than *M. sympodialis* (1/25, 4%).

Ahman and **Bergström (2009)** investigated cutaneous carriage of ***Malassezia species*** yeast in 32 Sphynx cats, and in 10 domestic shorthair (DSH) cats. Samples for mycological culture were taken using contact plates and swabs at seven sites in each cat (left and right axillae and groin, left ear, claw fold on left front paw and the interdigital palmar web of the left front paw). Malassezia species were isolated from 26/32 Sphynx cats (81%) and from 0/10 DSH control cats. In five cases Malassezia species yeasts were isolated at a single site, in the remaining 21 Sphynx cats at multiple sites. A total of 73 Malassezia species isolates were identified, of which 68 were ***Malassezia pachydermatis*** and five were lipid-dependent Malassezia. Five out of the 32 Sphynx had greasy seborrhoea, and all seborrhoeic cats had M pachydermatis isolated from their skin, at multiple sites. None of the 32 Sphynx had Malassezia species isolated from the ears. The difference in population sizes between Sphynx and DSH cats was significant (P<or=0.05) for the axillae, groins and claw

fold. The difference in frequency of isolation was significant (P<or=0.05) for the axillae and right groin. The level of cutaneous carriage of Malassezia species in Sphynx was similar to that previously reported for Devon Rex cats (DRC) The poor recovery of Malassezia species from ears in both Sphynx and DRC, has clinical implications for dermatological sampling in these breeds.

Čonková *et al.* (2011) performed a study to evaluate the prevalence of yeast ***Malassezia pachydermatis*** in dogs from Slovakia in relation to different predisposition factors (sex, age, body localisation, hair type, and season). Samples of ear swabs (58) and dermal swabs (131) from 147 dogs with clinical symptoms of suspected yeast dermatitis and/or otitis, were examined between June 2005 to June 2007. Relatively higher prevalence of *M. pachydermatis* was found in samples taken from males (45.2%) than in females (35.2%), and in geriatric dogs (63.6%) than in young (42.5%) or adult (38.5%) dogs. *Malassezia pachydermatis* was isolated more often from ear swabs (44.8%) than from skin swabs (38.9%). Prevalence of *M. pachydermatis* was significantly higher ($p < 0.05$) in samples from the trunk area (60.3%) than in samples from other skin areas. Significantly higher prevalence was found in samples from long-haired (51.5%) and short-haired (45.9%) dogs compared to smooth-haired (21.4%) dogs. The prevalence was relatively higher in the samples taken in autumn (52.6%), than the other seasons: spring (36.1%), summer (27.3%), winter (45.7%); those differences were not significant. *Malassezia pachydermatis* is one of the most frequent yeasts isolated in dogs. Knowledge of factors predisposing to development of infection is valuable attribute of the correct diagnostic approach and case management.

Petrov and Mihaylov (2007) investigated 48 swab samples from dogs with otitis externa by means of parallel cultivation on blood agar and Sabouraud dextrose agar, supplemented with chloramphenicol and cycloheximide. Thirty four plasmocoagulase-positive Staphylococcus spp., 12 M. pachydermatis, 7 Streptococcus spp., 4 Escherichia coli, 4 Proteus mirabilis, and 3 Pseudomonas aeruginosa strains were isolated. Association between: M. pachydermatis and Staphylococcus spp. (in 9 samples), M. pachydermatis and Streptococcus spp. (2 samples) and M. pachydermatis and Escherichia coli, (in one sample) were determined. There were not coinfections of M. pachydermatis with either Pseudomonas aeruginosa or Proteus mirabilis.

Eidi *et al.* (2011) examined 62 healthy and 90 diseased privately owned dogs with otitis externa (n = 66) or skin lesions (n = 24) localized on only 1 anatomical site were Samples were collected from 8 anatomical sites (i.e. scalp, periorbital, perioral, back, trunk, groin, interdigital, and external ear canal) using sterile cotton swabs moistened with sterile saline solution (0.9% NaCl) for the external ear canal, and scraping with a scalpel for other body sites. The most commonly isolated species in the diseased group with culture-positive results was ***M. pachydermatis*** (55.2%).

Hernández-Escareño (2012) collected samples from 125 dogs from the external canal of the left and right ear (n=250 ears), of which 180 were positive to ***Malassezia pachydermatis***, representing 72% (180/250) and 70 negatives or 28% (70/250). Parameters considered for this study were: kind of ear, length of hair and size of the animal. Samples were taken using sterile cotton swaps. Samples were cultured in potato dextrose agar media with cycloheximide and chloramphenicol.

Crosaz *et al.* (2013) observed six cases of generalized dermatitis associated with *Malassezia* overgrowth in cats presented to the Veterinary College of Alfort,

France. Elevated numbers of yeasts were observed in lesional skin by cytology and culture. Skin lesions occurred on the face, ventral neck, abdomen and ear canals and were characterized by some degree of alopecia, erythema and crusting. In most cases, pruritus was intense. Contact plates filled with modified Dixon's medium were applied on lesional skin. Plates were incubated at 32 °C for 5 days. *Malassezia* yeasts were identified by microscopic examination of the cells and by physiological tests. For all the cases, positive subculture on Sabouraud dextrose agar confirmed that the colonies belonged to the non lipid-dependent species ***M. pachydermatis*.**

3.5.7. Reports on lipid-dependant *Malassezia* species in cats and dogs

culture medium. *Malassezia pachydermatis* was isolated from one cat. Fifteen of the 20 lipid-dependent isolates (one to three isolates from each of the seven affected cats) were karyotyped. All had profiles that were consistent with that of the type culture of ***M. sympodialis*** CBS 7222 and with those of 30 M. sympodialis isolates from humans. Seven bands of approximate sizes 1.62, 1"47, 1-29, 0"96, 0"76, 0-65 and 0"59 Mbp were resolved. The second largest band stained with twice the predicted intensity when measured using scanning densitometry, suggesting that this band represented two unresolved chromosomes of similar size. The single M. pachydermatis isolate was karyotyped and found to have the typical six-band profile of this species which was consistent with that of the type culture of M. pachydermatis CBS 1879 Fifteen of the 20 lipid-dependent isolates (one to three)

Bond *et al.* (1996) investigated the carriage of Malassezia yeasts was investigated in 17 cats in two colonies using a lipid-supplemented culture medium. Malassezia pachydermatis was isolated from one cat. Lipid-dependent Malassezia yeasts with electrophoretic karyotypes consistent with ***M. sympodialis*** were isolated from all six cats in one group and from one of 11 in the second group. To our knowledge, this is the first report of the isolation of lipid-dependent yeasts from cats.

Raabe *et al.* (1998) isolated 47 wild-type isolates of the genus Malassezia from dog and cat specimens by means of a simple differentiating system recently published. The purpose was to determine whether any of the other seven Malassezia spp. apart from M. pachydermatis occur in carnivores. Of the 47 isolates, three had been obtained from cats (ear 2, skin 1) and 44 from dogs (ear 37, skin 3, faeces 2, claw and paw 2). After primary isolation, they were subcultured on Dixon agar and then purified and differentiated by means of assimilation of Cremophor EL, splitting of esculin, growth on lipid-free medium and formation of tryptophandependent pigments and fluorochromes. Thus, a total of 100 strains could be obtained from the 47 primary isolates. Referring to the source material, M. pachydermatis was found in 83%, ***M. furfur*** in 45% and ***M. sympodialis*** in 75%. More than 80% of cultures were mixed, comprising two or all three species; a single species was isolated in only nine cases. This shows that animals are not colonized by M. pachydermatis alone, as has been thought until now, but in nearly all cases by mixed cultures. Thus, (domestic) animals could well be a reservoir for other Malassezia species such as M. furfur and M.

sympodialis. Surprisingly, Malassezia yeasts were also isolated from dog faeces, indicating that they apparently pass through the gastrointestinal tract.

Crespo *et al.* (1999) isolated ***Malassezia furfur*** during a survey of the occurrence of *Malassezia* species in the external ear canals of cats without otitis externa.

CRESPO *et al.* (2000) reported otitis externa associated with the lipid-dependent species ***M. sympodialis*** in two cats. **Case 1.** A 10-year-old female Persian cat presented with acute otitis externa in the right ear. The animal had pruritus and an excessive aural discharge. On otoscopic examination, a generalized erythema was seen, and the external meatus was full of flaky black wax. **Case 2.** A 14-year-old female Angora cat presented with a chronic otitis externa in the left ear with pruritus. The external ear canal was full of brownish wax, and erythema was seen on otoscopic examination. Smears from the external ear canals of the two cats stained with Gram and Diff-Quick stains revealed the presence of numerous *Malassezia* cells, more than 10 organisms per high-power field. Hyphae were not seen. Buds were formed on a narrow base, which differed from the monopolar budding on a broad base typical of *M. pachydermatis*. No bacteria were detected in any case. Cultures on Sabouraud glucose agar (SGA), SGA supplemented with olive oil (10 ml/liter), Leeming's medium blood agar and MacConkey agar. Cultures on SGA supplemented with olive oil and Leeming's medium yielded numerous yeast colonies at 5 days of incubation. Two different types of colonies were isolated on SGA supplemented with olive oil: one type was yellow with a creamy texture, and the other was white with an oily texture. On Leeming's medium, all colonies were white with a soft texture. Cultures on SGA were negative at 14 days of incubation. Bacteriological culture was negative at 3 days of incubation (case 2). These lipid-dependent yeasts were identified as *M. sympodialis*.

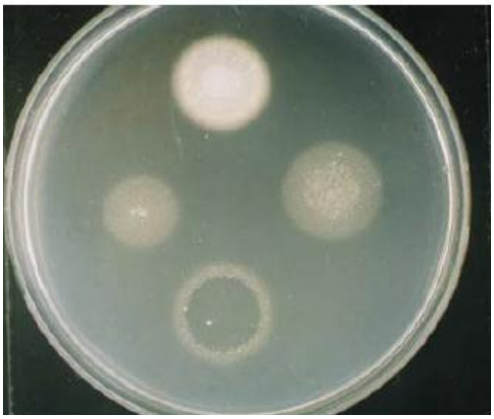
CRESPO *et al.* (2002) studied the lipophilic microbiota of the external ear canals of 332 animals (264 dogs and 68 cats), with and without otitis externa, over an 11-year period from 1988 to 1999. Malassezia pachydermatis was isolated from 62 2% and 50% of dogs with and without otitis externa, respectively, and from 41 2% and 17 6% of cats with and without otitis externa, respectively. In the group of animals studied for lipiddependent species, these yeasts were isolated from 4 5% of dogs with otitis externa and from 23 1% and 8 9% of cats with and without otitis externa, respectively. ***M. sympodialis*** and ***M. furfur*** were isolated from cats and M. furfur and ***M. obtusa*** from dogs. Our □ndings show that lipid-dependent Malassezia species may contribute to the etiology of otitis externa in dogs and cats

Hirai *et al*. (2004) isolated 5 isolates of a novel species of the yeast genus Malassezia from animals in Japan and Brazil. Phylogenetic trees based on the D1/D2 domains of the large-subunit (26S) rDNA sequences and nucleotide sequences of the internal transcribed spacer 1 region showed that the isolates were conspecific and belonged to the genus Malassezia. They were related closely to Malassezia dermatis and ***Malassezia sympodialis***, but were clearly distinct from these two species and the other six species of Malassezia that have been reported, indicating that they should be classified as a novel species, ***Malassezia nana*** sp. nov. Morphologically and physiologically, M. nana resembles M. dermatis and M. sympodialis, but can be distinguished from these species by its inability to use Cremophor EL (Sigma) as the

sole lipid source and to hydrolyse aesculin. The type strain of M. nana is NUSV 1003T (=CBS 9557T =JCM 12085T).

Cafarchia *et al.* (2005) mentioned that out of the 413 isolates obtained from animals with and without otitis, 403 (97.6%) were identified as ***M. pachydermatis*** and 10 (2.4%) as ***M. globosa***. A statistical evaluation of the occurrence of Malassezia yeasts in dogs and cats revealed that predisposing factors for Malassezia infections are sampling period for cats, and type of ear for dogs. The largest population of Malassezia yeasts was detected in animals with otitis, suggesting a role in the occurrence of lesions**.**

Dizotti and Coutinho (2007) studied 45 cats, 20 with and 25 without **otitis externa (OE).** Cerumen or secretion from external ear canal samples was cultured on modified Mycosel agar and sterile olive oil was added to the surface of the medium before specimen seeding. The isolates were analysed for macro- and micromorphology and identified by catalase tests and on the basis of growth on Tween 20, 40, 60 and 80. *Malassezia* spp. were isolated from 15 out of 20 (75%) animals with otitis and from 7 out of 25 (28%) cats without OE; the difference between the two groups was statistically significant ($P \leq 0.05$). ***Malassezia pachydermatis*** and ***M. sympodialis*** were isolated from 60% (12/20) and 40% (8/20) of cats with otitis, respectively, with no significant difference in the frequency of isolation between the two species. In the microflora of the healthy ear canal *M. pachydermatis* was significantly more common (6/25, 24%) than *M. sympodialis* (1/25, 4%). **The investigation confirmed that *M. sympodialis* can also act as an aetiological agent of feline OE**,

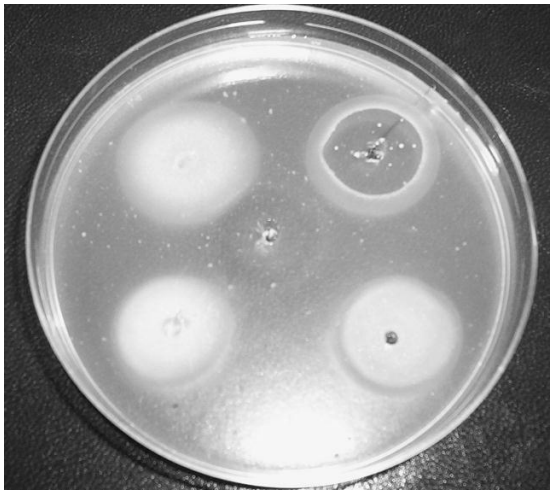


Behaviour of *M. sympodialis* in response to Tween 20 (below), 40,60 and 80 clockwise direction), **DIZOTTI and** COUTINHO **(2007)**

Perrins *et al.* (2007) used a polymerase chain reaction-restriction enzyme analysis (**PCR**-REA) method that differentiated the 11 species of Malassezia spp was used to identify the lipid-dependent isolates that were obtained from two cats with diabetes mellitus, two cats with hyperthyroidism and one cat with multicentric lymphoma. Six isolates had PCR-REA patterns that were indistinguishable from ***M. slooffiae*** CBS 7956 and three matched M. nana CBS 9557.

Perrins *et al.* (2007) described field isolates of ***M. nana*** recovered from cats. On primary isolation they formed small yellow colonies composed of small ovoid cells. These isolates failed to grow on Sabouraud's dextrose agar at 32°C. Two isolates showed profuse growth on modified Dixon's agar at 37°C whereas the other grew

poorly at this temperature. They were catalase-positive, failed to hydrolyse aesculin, did not assimilate Cremophor EL but grew well in the presence of Tweens 40, 60 and 80. None of the isolates assimilated Tween 20, although each showed a ring of slight growth some distance away from the well, resembling the inhibitory effects of high Tween 20 concentrations described for *M. sympodialis*. The type culture CBS 9557 showed the same phenotype as the field isolates, as described previously , although growth at 37°C was quite poor and a ring of distant growth was noted around the well inoculated with Tween 20.



Growth around wells inoculated with Tweens (clockwise from top right: Tween 20, 40, 60, 80) and Cremophor EL (centre) of a field isolate of *Malassezia nana* obtained from the left ear of a hyperthyroid cat incorporated into Sabouraud's dextrose agar, **Perrins *et al.* (2007)**

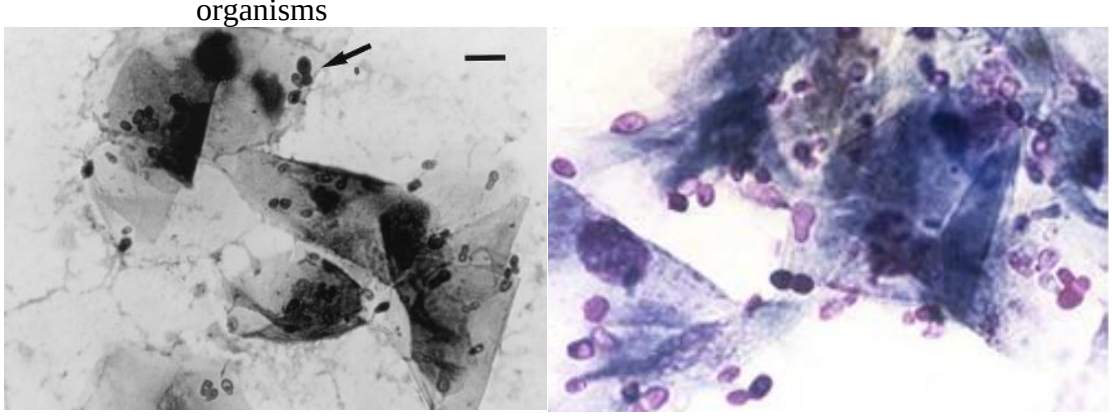
Eidi *et al.* (2011) examined 62 healthy and 90 diseased privately owned dogs with otitis externa (n = 66) or skin lesions (n = 24) localized on only 1 anatomical site were Samples were collected from 8 anatomical sites (i.e. scalp, periorbital, perioral, back, trunk, groin, interdigital, and external ear canal) using sterile cotton swabs moistened with sterile saline solution (0.9% NaCl) for the external ear canal, and scraping with a scalpel for other body sites. The most commonly isolated species in the diseased group with culture-positive results was *M. pachydermatis* (55.2%), followed by ***M. sympodialis*** (25.9%), ***M. furfur*** (10.3%), ***M. obtusa*** (5.2%), ***M. globosa*** (1.7%), and ***M. restricta*** (1.7%), and in the healthy group with culture-positive results, the most commonly isolated species was M. pachydermatis (58.8%), followed by M. sympodialis (35.3%) and M. obtusa (5.9%). A total of 75 strains from 6 Malassezia species isolated from both groups and their species were detected with a frequency rate as follows: M. pachydermatis (56%), M. sympodialis (28%), M. furfur (8%), M. obtusa (5.4%), M. globosa (1.3%), and M. restricta (1.3%).

3.6. Diagnosis

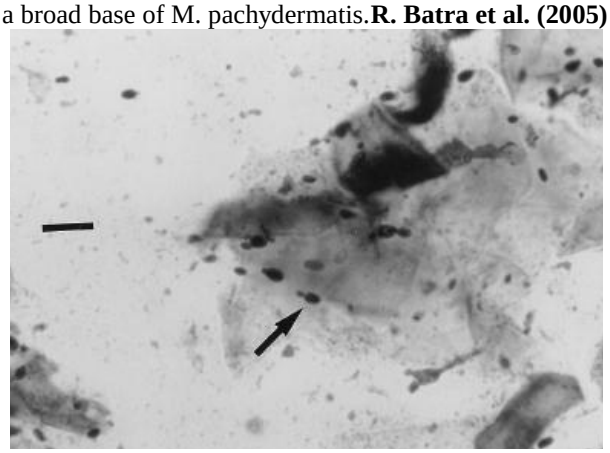
3.6.1. Direct microscopic examination

- Cotton swab smears, skin scrapings, direct impression smears, and acetate tape impressions are all routinely used to identify M. pachydermatis cytologically.

- Smears are fixed then stained with a modified Wright's stain or ink
- The tape is not fixed, stained with a modified Wright's stain or ink
- The stained tape Is then applied to a glass slide with the adhesive side down.
- The slide is examined under oil immersion, looking for unipolar budding yeast that are described as peanut-, footprint-, or bottle-shaped organisms



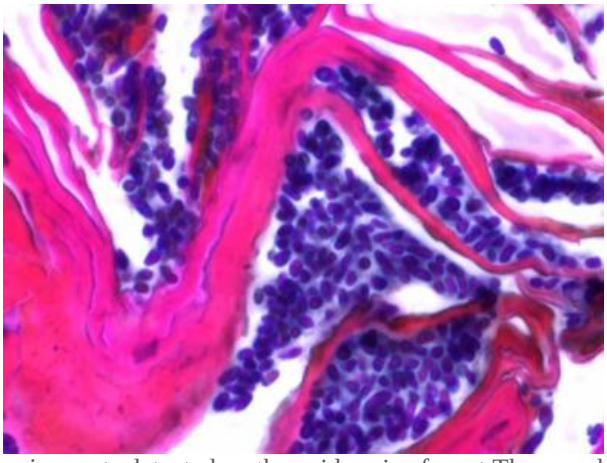
Diff-Quick stain of a smear from an ear swab of a dog with otitis externa showing the typical monopolar budding on a broad base of M. pachydermatis.**R. Batra et al. (2005)**



Gram-stained smear from otic swab showing the presence of numerous M.sympodialis cells

3.6.2. Histopathologic examination

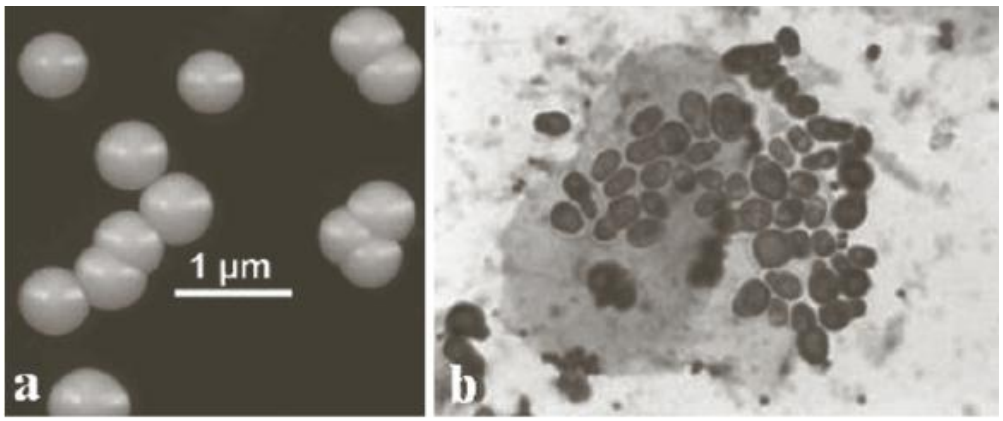
- histopathologic examination has a low sensitivity in detecting Malassezia species, because the yeast are removed from the skin surface during processing.
- histopathology may sometimes show the yeasts on the surface of the epidermis and in the infundibula, particularly in PAS stained sections (although they are occasionally visible on HE stained sections).



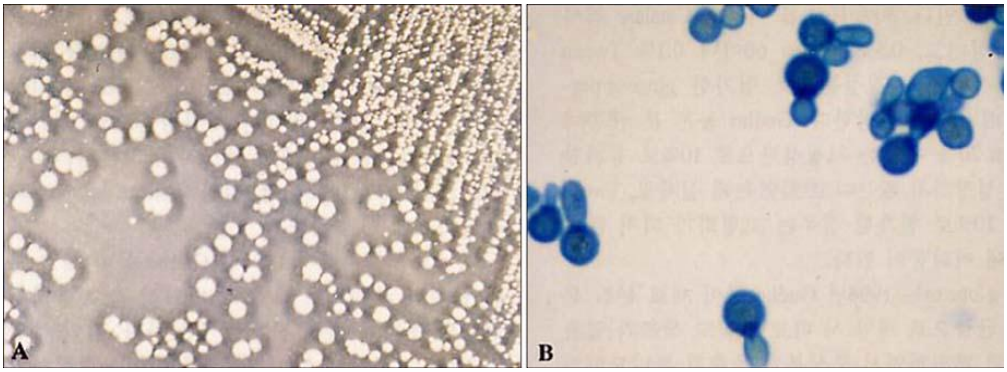
Large number of *Malassezia* yeasts detected on the epidermis of a cat The sample was stained with Periodic Acid–Schiff. Bar=10 µm (Pathology Department, Ecole Nationale Vétérinaire d'Alfort), .
Crosaz *et al.* (2013)

3.6.3. . Isolation on Sabouroud Dextrose Agar, with and without lipid supplementation

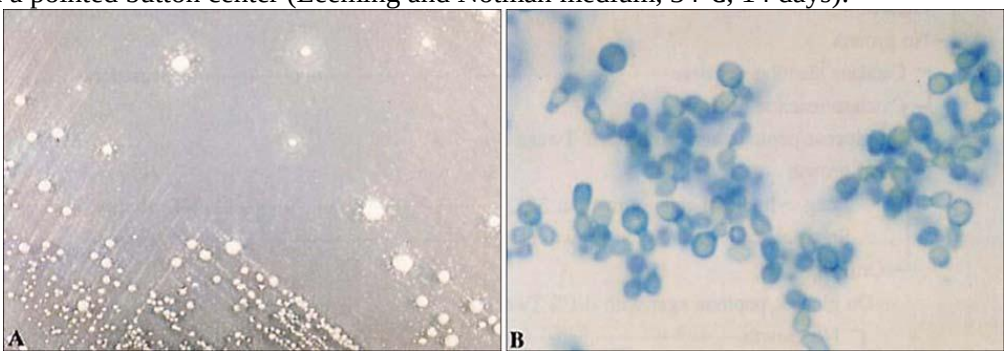
- Colonies are sub-cultured in modified Dixon's agar for identification at the species level.
- The morphological identification criteria used to identify Malassezia spp. are the cell shape, size, and the budding pattern.
- Lipid-dependent species are identified by the Tween assimilation method (i.e. 20, 40, 60, 80).
- Catalase reaction, tryptophan, cremophor assimilation tests and esculin splitting were used as additional tests to differentiate the species.



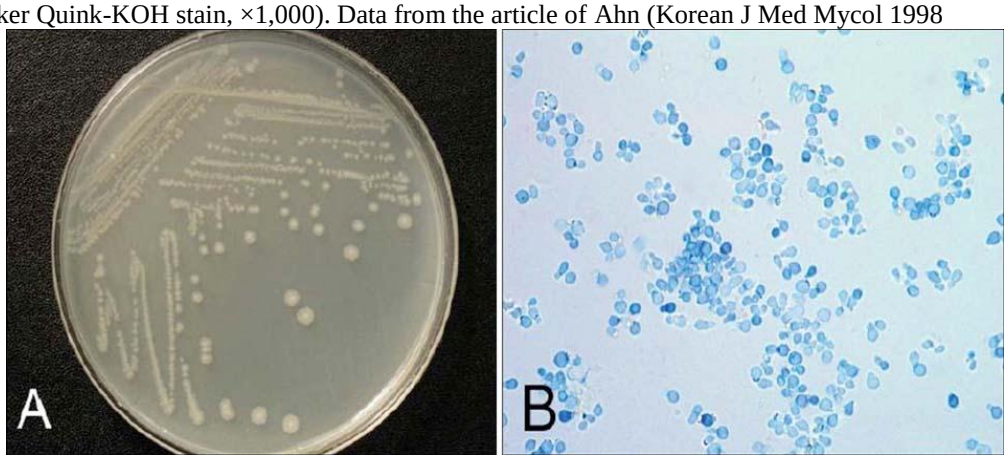
M. pachydermatis. (**a**) Convex colonies with an entire margin; (**b**) ovoid Gram stained yeasts in dog ear cerumen (picture by the courtesy of Guillot);



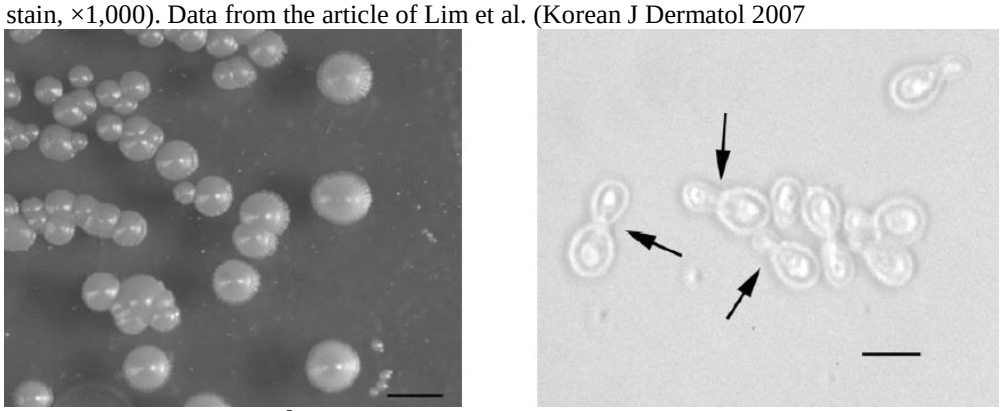
Malassezia globosa. (A) Medium-sized, lighter in color, friable and crenated flat colonies with a pointed button center (Leeming and Notman medium, 34℃, 14 days).



Malassezia restrica. (A) Small-sized, circular, umbonate, entire, dull colonies (Leeming and Notman medium, 34℃, 14 days). (B) Small, spherical or oval cells with buds on a relatively narrow base (Parker Quink-KOH stain, ×1,000). Data from the article of Ahn (Korean J Med Mycol 1998



Malassezia dermatis. (A) Large-sized, circular, smooth colonies (Leeming and Notman medium, 34℃, 14 days). (B) Spherical, oval, or ellipsoidal vegetative cells with monopolar budding (Parker Quink-KOH stain, ×1,000). Data from the article of Lim et al. (Korean J Dermatol 2007



M. nana cultured on agar at 32°C for 1 week, Cells of M. nana, small ovoid to globose with a narrow end and monomorphic budding. Hirai et al.,

3.6.4. Molecular diagnosis

Molecular techniques

Molecular techniques may be preferable to these generally time-consuming phenotypic methods since they may lack the ability to reliably discriminate between the newly-described species and lipid-dependent isolates from animals.

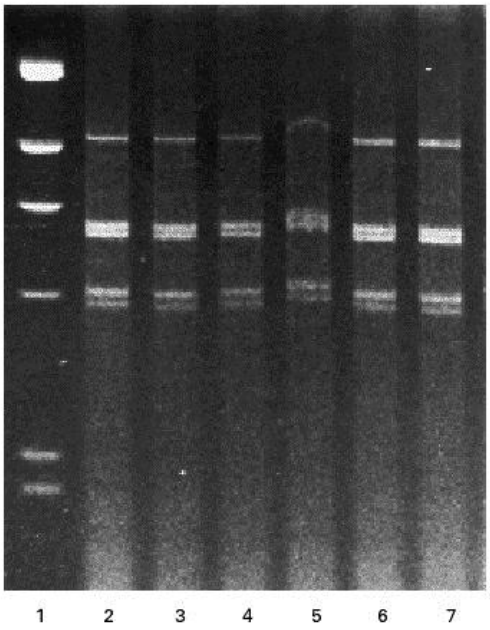
- **Polymerase chain reaction (PCR) and restriction endonuclease analyses (REA)** which usually involve amplification and subsequent restriction of portions of the highly variable ribosomal RNA gene are potentially applicable for routine laboratory use
- Specific molecular methods have also been developed for the identification of Malassezia isolates, such as
    - **pulsed-field gel electrophoresis (PFGE),**
    - **random amplification of polymorphic DNA (RAPD),**
    - **DNA sequence analysis,**
    - **restriction analysis of PCR amplicons of ribosomal sequences,**
    - **amplified fragment length polymorphism (AFLP),**
    - **denaturing gradient gel electrophoresis (DGGE), and**
    - **terminal fragment length polymorphism** (tFLP)

- Of the molecular methods, PFGE and DGGE have met with limited success due to the need for specialized equipment and training.
- AFLP has been successfully applied to the identification of Malassezia isolates and yields highly-specific genotypic information about each strain.
- The detailed ‘‘fingerprint’’ achieved from careful AFLP analysis has revealed multiple genetic subgroups in each Malassezia species, and may lead to the differentiation of clinically-important subgroups or even new species.
- The new method, named terminal fragment length polymorphism (tFLP), can be used on non-invasively acquired swab samples. This method uses only three primer sets, and therefore minimizes the potential bias related to amplification efficiency. It is sensitive enough to detect Malassezia with as few as 100 cells per sample, either as spiked directly onto the swab to control the extraction procedure, or from samples collected from 1 cm2 of skin surface sample using a swab.

Reports

Huan *et al*. (1998) reported an outbreak of ***Malassezia pachydermatis*** in an Intensive Care Nursery, eight patients with bloodstream infections, two with urinary tract infections, one with meningitis, and four with asymptomatic colonization. In a point-prevalence survey, 9 additional infants, 1 health care worker, and 12 of the health care workers' pet dogs had positive cultures for *M. pachydermatis.* The isolates from all case patients, the 9 additional colonized infants, 1 health care worker, and 3 of the 12 dogs had identical patterns of restriction-fragment–length polymorphisms. In this outbreak, it was likely that *M. pachydermatis* was introduced into the intensive care

nursery on health care workers' hands after being colonized from pet dogs at home. The organism persisted in the nursery through patient-to-patient transmission.

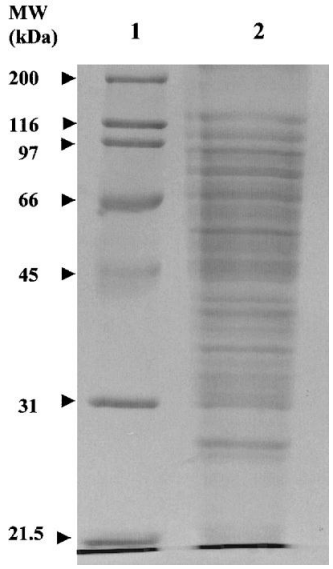


Pulsed-Field Gel Electrophoretic Patterns with Restriction Enzyme*Hae* III in *M. pachydermatis* Isolates from Infants in the Intensive Care Nursery and Health Care Workers' Dogs. **Huan *et al.* (1998)**

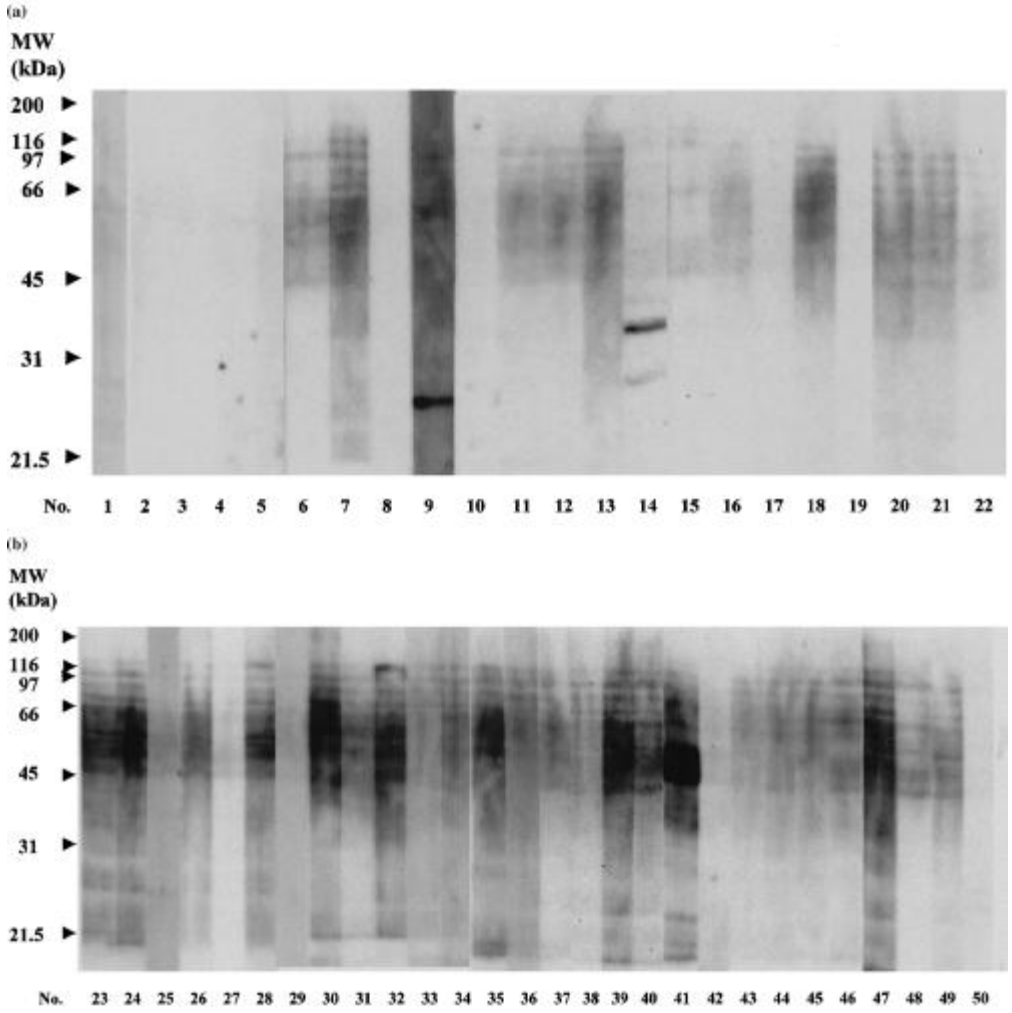
Aizawa *et al.* (1999) carried out molecular investigations of 16 strains, conventionally identified to be *Malassezia pachydermatis*, isolated from dogs in Japan was by random amplification of polymorphic DNA (RAPD) and chitin synthase 2 (*CHS2*) gene sequence analyses. The RAPD band patterns of 13 clinical isolates were identical to that of standard strain of ***M. pachydermatis*** (CBS-1879). The other three clinical isolates were different from the standard strain of *M. pachydermatis* in RAPD patterns, and two of the three isolates were identical. About 620 bp genomic DNA fragments of the *CHS2* gene were amplified from the same 16 clinical isolates of *M. pachydermatis* by polymerase chain reaction (PCR) and sequenced. The phylogenetic analysis of the nucleotide sequences of *CHS2* gene fragments of the 16 clinical isolates revealed that the 13 strains were genetically very close to the standard strain of *M. pachydermatis* and the other two isolates were genetically close to the standard strain of *M. furfur* rather than *M. pachydermatis*. The remaining one isolate was phylogenetically distinct from all the seven *Malassezia* species reported so far.

Aizawa *et a*l. (2001) carried out **molecular investigation** of 110 clinical isolates of non-lipid-dependent ***Malassezia pachydermatis*** from dogs and cats by random amplification of polymorphic DNA (RAPD) and chitin synthase 2 (CHS2) gene sequence analyses. The RAPD analysis indicated that the clinical isolates of M. pachydermatis constituted four distinct genetic types (A, B, C and D). Moreover, the results from CHS2 gene analysis completely agreed with those from the RAPD analyses. The clinical isolates of M. pachydermatis were obtained from normal external ears, lesions of atopic dermatitis, flea allergic dermatitis, otitis externa, pyoderma and seborrheic dermatitidis in dogs and cats. Type A consisted of 93 clinical isolates as well as the ex-neotype strain of M. pachydermatis. The isolates of type A M. pachydermatis originated from lesions of all kinds of diseases. They were predominant on dog and cat skin. The other types, B, C, and D were isolated mainly from otitis externa.

Chen *et al.* (2002) performed a study with the aim of this study was to compare IgE responses to separated proteins of ***M. pachydermatis*** in 28 atopic dogs with *Malassezia* dermatitis and 22 clinically normal dogs using Western immunoblotting. Six different detection systems were evaluated in order to assess sensitivity and eliminate nonspecific binding and cross-reactivity. The protocol yielding the best results utilized a monoclonal mouse antidog IgE, an alkaline phosphatase conjugated goat antimouse IgG which had been passed through a canine IgG column 3 times, a chemiluminescent substrate and a digital imaging system. Proteins of 45, 52, 56 and 63 kDa were recognized by more than 50% of the atopic dog sera and thus represented major allergens. Only a minority of normal dogs showed faint IgE binding to these proteins. The results indicate that the majority of atopic dogs with *Malassezia* dermatitis have a greater IgE response than normal dogs, suggesting an IgE-mediated immune response may be clinically important in the pathogenesis of the disease.



Coomassie Brilliant Blue stained *M. pachydermatis* extracts on a 10% separating polyacrylamide gel. Lane 1: molecular weight markers; lane 2: *Malassezia* extract., **Chen *et al.* (2002)**

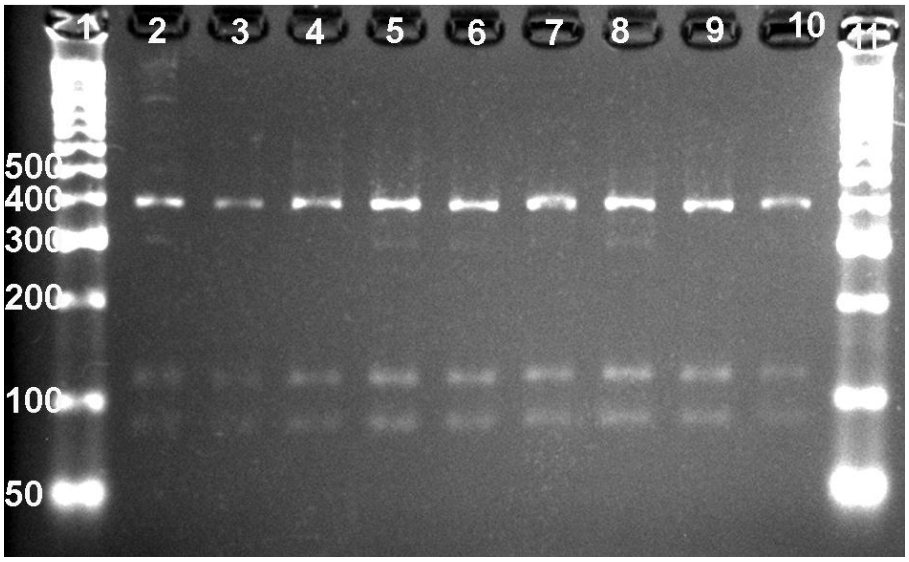


IgE-binding proteins in extracts of *M. pachydermatis* detected by immunoblotting with dog sera from 2 groups: (a) normal dogs; and (b) atopic dogs with *Malassezia* dermatitis. The images of blots were captured by a digital imaging station and printed out directly from the computer. The numbers along the bottom signify the strips probed with individual dog sera. The molecular weight markers on the left are 200, 116, 97, 66, 45, 31 and 21.5 kDa., **Chen *et al.* (2002)**

Hirai *et al*. (2004) isolated 5 isolates of a novel species of the yeast genus Malassezia from animals in Japan and Brazil. Phylogenetic trees based on the D1/D2 domains of the large-subunit (26S) rDNA sequences and nucleotide sequences of the internal transcribed spacer 1 region showed that the isolates were conspecific and belonged to the genus Malassezia. They were related closely to Malassezia dermatis and ***Malassezia sympodialis***, but were clearly distinct from these two species and the other six species of Malassezia that have been reported, indicating that they should be classified as a novel species, ***Malassezia nana*** sp. nov. Morphologically and physiologically, M. nana resembles M. dermatis and M. sympodialis, but can be distinguished from these species by its inability to use Cremophor EL (Sigma) as the sole lipid source and to hydrolyse aesculin. The type strain of M. nana is NUSV 1003T (=CBS 9557T =JCM 12085T).

Ahman *et al*. (2007) mentioned that lipid-dependent *Malassezia* spp. isolates were recovered from the claw fold of 5 healthy and 3 seborrhoeic DRC cats. Using polymerase chain reaction – restriction enzyme analyses (PCR-REA) based on

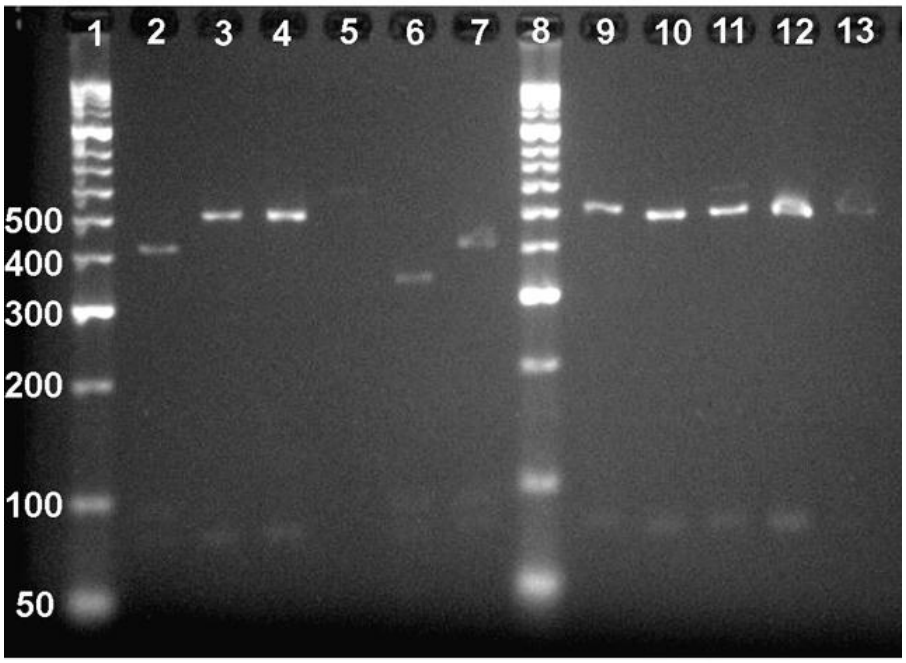
amplification of the large subunit rRNA gene, all eight lipid-dependent isolates had profiles that were indistinguishable from that of *M. slooffiae* CBS 7956.



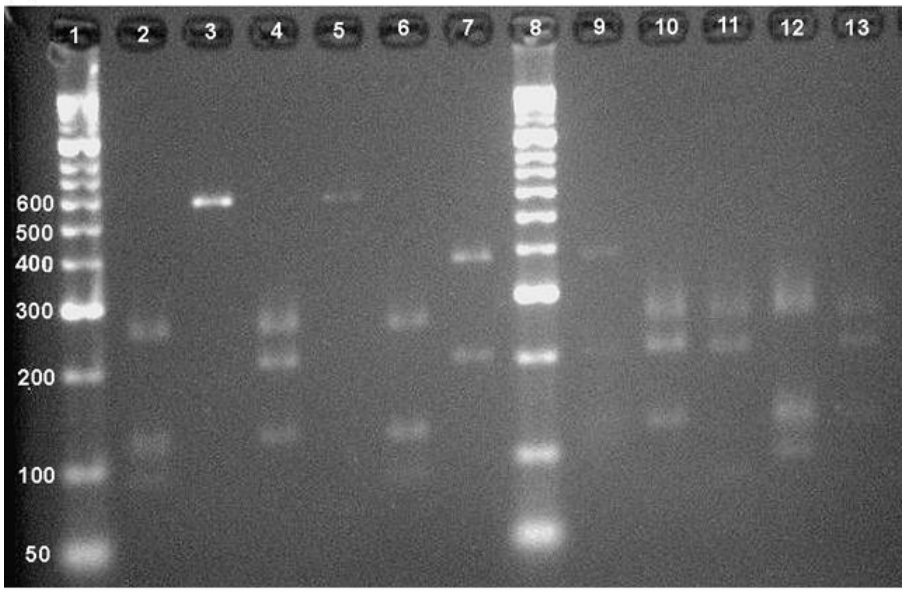
Restriction enzyme patterns obtained by *Msp*I digestion of an amplicon of the large subunit rRNA gene of ***M. slooffiae*** CBS 7956 and field isolates of *Malassezia* spp. obtained from cats. Tracks 1 and 11, molecular weight marker (base pairs); 2, *M. slooffiae* CBS 7956; tracks 3–10, field isolates of *M. slooffiae* from Devon Rex cats. **Ahman *et al*. (2007)**

Perrins *et al.* (2007) used a polymerase chain reaction-restriction enzyme analysis (PCR-REA) method that differentiated the 11 species of *Malassezia* spp was used to identify the lipid-dependent isolates that were obtained from two cats with diabetes mellitus, two cats with hyperthyroidism and one cat with multicentric lymphoma. Six isolates had PCR-REA patterns that were indistinguishable from ***M. slooffiae*** CBS 7956 and three matched ***M. nana*** CBS 9557. An amplicon of approximately 600 base pairs (bp) was obtained from each of field isolates of lipid-dependent *Malassezia* spp. and from each of the 11 CBS-derived cultures. Agarose gel electrophoresis of the restriction digests using the three enzyme system produced distinct restriction patterns for each of the 11 CBS cultures examined. The four new species (***M. dermatis, M. nana, M. japonica,* and *M. yamatoensis***) could be differentiated from each other and from the seven previously reported species using the combination of the three restriction enzymes

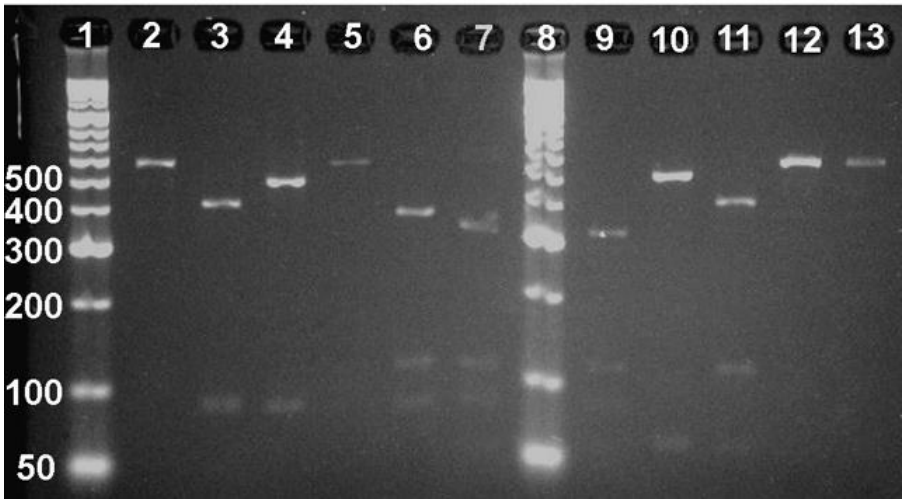
(a)


1 2 3 4 5 6 7 8 9 10 11 12 13
500
400
300
200
100
50

(b)


1 2 3 4 5 6 7 8 9 10 11 12 13
600
500
400
300
200
100
50

(c)


1 2 3 4 5 6 7 8 9 10 11 12 13
500
400
300
200
100
50

Restriction patterns obtained by (a) *Ban*I, (b) *Hae*II, and (c) *Msp*I digestion of an amplicon of the large subunit rRNA gene of 11 species of *Malassezia*. Tracks 1 and 8, molecular weight marker (base pairs); 2, *M. furfur* CBS 1878; 3, *M. globosa* CBS 7966; 4,*M. obtusa* CBS 7876; 5, *M. restricta* CBS 7877; 6, *M. slooffiae* CBS 7956; 7, *M. sympodialis* CBS 7222; 9, *M. dermatis* CBS 9169; 10,*M. japonica* CBS 9432; 11, *M. nana* CBS 9557; 12, *M. yamatoensis*CBS 9725; 13, *M. pachydermatis* CBS 1879. **Perrins *et al.* (2007)**

Kobayashi *et al.* (2011) performed molecular characterization of isolates of *Malassezia pachydermatis* from healthy dog skin and from dogs with atopic dermatitis using internal spacer 1 (IGS1) region analyses, and their phospholipase A2 activity and pH growth profiles were then characterized *in vitro*. The percentage of isolates from healthy dogs that had the following IGS1 subtypes (isotype, %) were as follows: 1A, 6%; 1B, 27%; 1C, 11%; 2A, 6%; 2B, 6%; 3A, 11%; 3C, 3%; and 3D, 24%. In contrast, 9% of isolates from dogs with atopic dermatitis were isotype IB and 91% were isotype 3D, indicating that isolates of subtype 3D were the most prevalent in dogs with atopic dermatitis. Production of phospholipase A2 was statistically higher in isolates of subtype 3D than in the other subtypes. The subtype 3D isolates showed enhanced growth on alkaline medium compared with non-3D subtype isolates. The main clinical sign of canine *Malassezia* dermatitis is waxy exudates on the skin, which predispose the patient to development of a yeast overgrowth of the subtype 3D. Increased phospholipase A2 production may be involved in the inflammatory process associated with *Malassezia* dermatitis.

Hernández-Escareño (2012) collected samples from 125 dogs from the external canal of the left and right ear (n=250 ears), of which 180 were positive to ***Malassezia pachydermatis***, representing 72% (180/250) and 70 negatives or 28% (70/250). Parameters considered for this study were: kind of ear, length of hair and size of the animal. Samples were taken using sterile cotton swaps. Samples were cultured in potato dextrose agar media with cycloheximide and chloramphenicol. **PCR** tests amplifying regions D1 and D2, coding for LSU rRNA were also performed and were compared with the reference strain CBS 1879NT of M. pachydermatis. Amplified product yielded a 600 bp product characteristic for Malassezia. Most predominant dog breeds for external otitis were Cocker Spaniel, French Poodle and Criolla. In Mexico, as well as in the state of Nuevo Leon, no documented evidence exists about the presence of this yeast in dogs with external otitis. The objective was to analyze dogs with this pathology and to inform the presence of M. pachydermatis by means of culture and amplification of D1 and D2 regions of gen 26S rRNA.

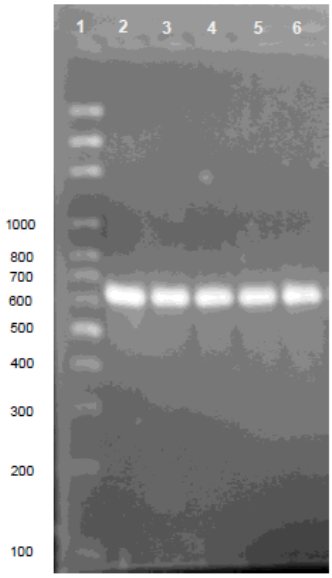


Figure 5. Five band patterns of the several strains of *Malassezia pachydermatis*.
Lanes 1: molecular weight marker (100 bp); lane 2: strain *M pachydermatis* CBS 1879^{NT} (600bp) Lane 3, 4, 5 and 6 strains.
Hernández-Escareño (2012)

3.7. Allergenic role of the *Malassezia pachydermatis*

- The lipophilic but not lipodependent yeast Malassezia pachydermatis is a component of the normal cutaneous flora of the dog. Around 50 % of healthy dogs are carriers (external ear canal, skin - anal area, lips and extremities, haircoat).
- The response of the host to the yeast includes non-specific defense mechanisms (phagocytosis by neutrophils) as well as cell-mediated specific defense mechanisms. Local delayed hypersensitivity responses and/or innate immune mechanisms (transferrin limiting microbial access to iron) play an important role.
- Malassezia produce many enzymes (including lipases and proteases) which can contribute to cutaneous inflammation through proteolysis, lipolysis (which alters the lipid cutaneous fi lm), changes of cutaneous pH, eicosanoid release and complement activation.
- Malassezia pachydermatis could play an allergenic role in regard to a type 1 (immediate) hypersensitivity. Skin-testing with a Malassezia extract may show immediate hypersensitivity reactions.
- Recently, the functionality of anti Malassezia IgE has been demonstrated through passive transfer using the Prausnitz-Küstner technique.
- Some major allergens of Malassezia pachydermatis have been identified: proteins with 45, 52, 56 and 63 kDa molecular weight.
- The delayed hypersensitivity response is less well known. Patch-testing (epicutaneous tests) has been evaluated recently and may be a good tool to explore delayed hypersensitivity caused by the yeast.

Reports:

Bond *et al.* (1998) reported that the in vitro proliferative responses of peripheral blood mononuclear cells from seven healthy basset hounds exposed to Malassezia pachydermatis antigen (500 micrograms/ml) exceeded ($P < 0.05$) those of seborrhoeic basset hounds with high populations of M pachydermatis and eight Irish setters with gluten-sensitive enteropathy. The stimulation indices in the latter two groups and in eight healthy beagles were comparable. The stimulation indices of the four groups after exposure to phytohaemaglutinin did not differ significantly. The serum titres of M pachydermatis-specific IgG and IgA measured by enzyme-linked immunosorbent assays (ELISA) in 21 seborrhoeic basset hounds and 11 affected dogs of various breeds exceeded those of 14 healthy basset hounds and eight healthy beagles ($P < 0.01$ for IgG, $P < 0.05$ for IgA). Total serum IgA concentrations measured by ELISA in the affected dogs were not lower than those of healthy dogs.

Morris *et al.* (1998) investigated the potential allergenic role of the Malassezia pachydermatis in 5 clinically normal nonatopic dogs, 10 atopic dogs with cytologic evidence of Malassezia dermatitis, and 12 atopic dogs without cytologic evidence of Malassezia dermatitis.. A crude yeast extract was produced by disrupting the cell wall of M pachydermatis. The crude extract and 8 of its fractions, which were generated by fractionation in a high-performance liquid chromatography column, were injected along with 46 commercial allergens for intradermal allergy testing of normal and atopic sample populations. Significant difference between atopic populations was evaluated, using a threshold concentration of crude yeast extract that failed to induce wheal-and-flare responses in normal nonatopic dogs. Atopic dogs with cytologic evidence of Malassezia dermatitis had significantly greater wheal-and-flare reactions to intradermal injection of crude extract of M pachydermatis than did atopic dogs without cytologic evidence of Malassezia dermatitis.

Nuttall and Halliwell (2001) compared the serum IgG and IgE response to *Malassezia* in atopic dogs with and without clinical evidence of *Malassezia* dermatitis and/or otitis, nonatopic dogs with clinical evidence of *Malassezia* dermatitis and/or otitis and healthy dogs. Cytology was used to diagnose clinically significant *Malassezia* dermatitis and otitis. Contact plate cultures confirmed the validity of this technique. Reproducible enzyme-linked immunosorbant assays for *Malassezia*-specific IgG and IgE in canine serum were established. Atopic dogs had significantly higher serum IgG and IgE levels than either healthy dogs or nonatopic dogs with clinical evidence of *Malassezia* dermatitis and/or otitis. There was no significant difference in IgG and IgE levels between atopic dogs with and without clinical evidence of *Malassezia* dermatitis and/or otitis

Bond *et al.* (2002) tested atopic dogs intradermally tested using extracts prepared from ***M pachydermatis***. The group comprised four entire males, five neutered males, two entire females and seven neutered females aged between one and 11 years (median four years). These dogs were diagnosed as having atopic disease based on a relapsing or persistent history of pruritic dermatitis affecting the face, ears, feet and/or ventrum which did not completely resolve following antimicrobial and antiparastic therapy, and fulfilment of the criteria of Willemse (1986. Skin lesions of varying severity were present, comprising erythema and the consequences of self-trauma. Concurrent or previous Malassezia otitis externa was diagnosed in nine dogs, Malassezia dermatitis in nine dogs, and six dogs had both otitis and dermatitis. These disorders were diagnosed on the basis of suggestive clinical signs (greasy, erythematous, otitis externa

or dermatitis), cytological evidence of high M. pachydermatis populations, and a clinical response to topical antifungal therapy. Ear populations were assessed cytologically by microscopical examination of Diff-Quik-stained specimens made by smearing ear wax on glass slides. Skin populations were assessed by microscopical examination of tape-strip specimens stained with Diff-Quik. Populations were considered high when the yeast was readily detected in multiple high-powered fields. Dogs were sedated for intradermal testing using medetomidine (Domitor; Pfizer) injected intravenously or intramuscularly at 10 rig/kg. Hair was clipped from the lateral thorax, injection sites marked using a felt-tipped pen, and 0-05 ml of test or control solution was injected intradermally. Histamine phosphate (1:100,000) and sterile 0 5 per cent phenol saline served as positive and negative controls, respe

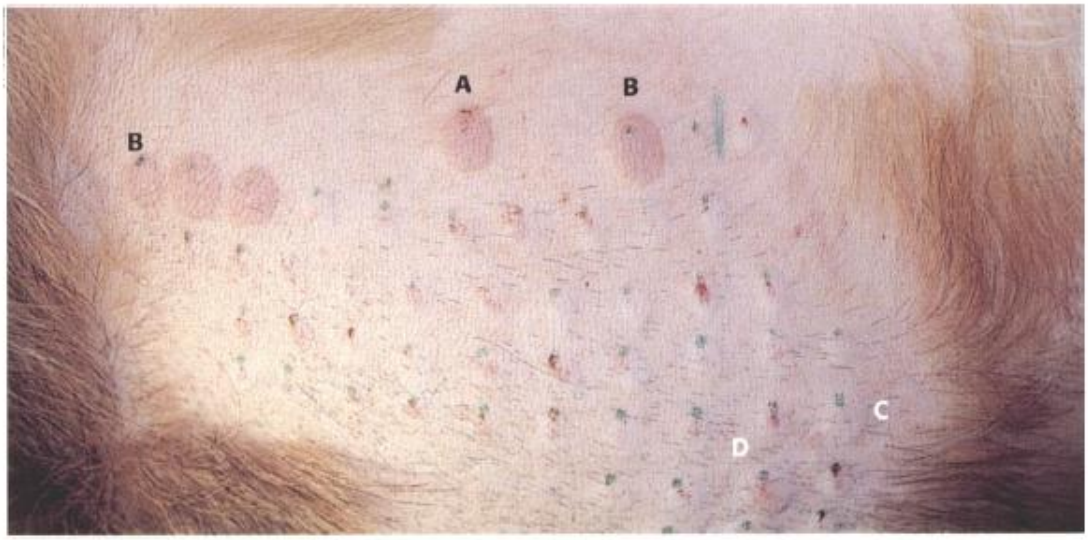


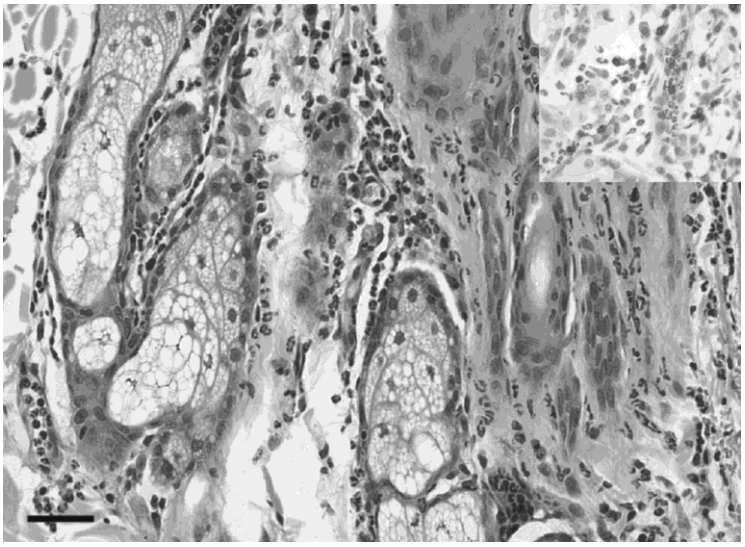
FIG 1: Intradermal test in an atopic dog (dog 1) using environmental and *Malassezia pachydermatis* allergens. An erythematous wheal is apparent at the site of a histamine (positive control) injection (A). Four markedly erythematous wheals are apparent at the 200 and 20 µg/ml dilutions of both *M pachydermatis* extracts (B). Non-erythematous wheals can be seen at the site of injection of grass mix (C) and *Dermatophagoides farinae* (D)

Bond *et al.* (2002)

Chen *et al.* (2002) performed a study aiming to compare IgE responses to separated proteins of ***M. pachydermatis*** in 28 atopic dogs with Malassezia dermatitis and 22 clinically normal dogs using Western immunoblotting. Six different detection systems were evaluated in order to assess sensitivity and eliminate nonspecific binding and cross-reactivity. The protocol yielding the best results utilized a monoclonal mouse anti-dog IgE, an alkaline phosphatase conjugated goat anti-mouse IgG which had been passed through a canine IgG column 3 times, a chemiluminescent substrate and a digital imaging system. Proteins of 45, 52, 56 and 63 kDa were recognized by more than 50% of the atopic dog sera and thus represented major allergens. Only a minority of normal dogs showed faint IgE binding to these proteins. The results indicate that the majority of atopic dogs with Malassezia dermatitis have a greater IgE response than normal dogs, suggesting an IgE-mediated immune response may be clinically important in the pathogenesis of the disease.

Bond *et al.* (2004) evaluated the effects of the daily application for 7 days of suspensions of ***Malassezia pachydermatis*** to normal canine skin in 10 beagle dogs.

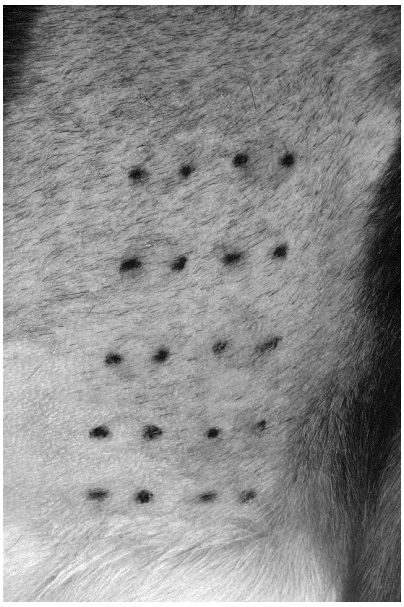
Four out of six dogs challenged without occlusion developed transient lesions generally characterized clinically by mild erythema with papules and histologically by mild epidermal hyperplasia and a superficial perivascular dermatitis. Saline-treated control sites showed no clinical signs. In four dogs challenged with occlusion, skin lesions occurred at both yeast and saline-treated sites; erythema and papules were more severe at the yeast-treated sites in three dogs. Occlusion induced more persistent lesions, which resolved within 24 days. Population densities of the yeast were highest at day 8 and declined rapidly following cessation of application. Peripheral blood mononuclear cell proliferation indices following M. pachydermatis exposure in vitro and serum concentrations of M. pachydermatis-specific IgG antibodies did not vary significantly during the study. Delayed (24 h) intradermal test reactivity to M. pachydermatis antigens developed in all eight dogs with clinical signs following yeast exposure. This study suggests that the resistance of healthy canine skin to infection by M. pachydermatis is mediated by local delayed hypersensitivity responses and, or innate epidermal immune mechanisms.



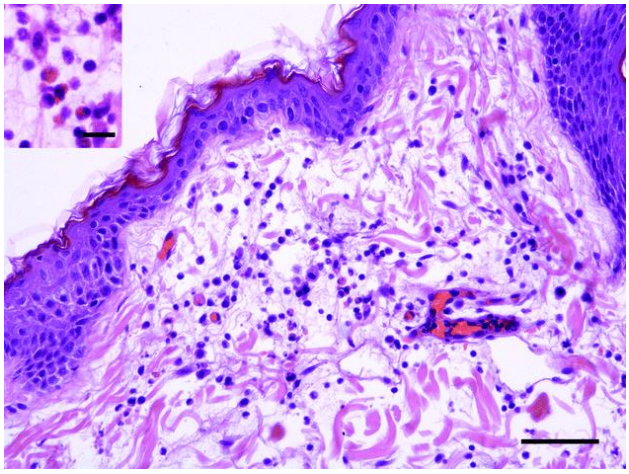
Skin biopsy specimen from the reactive patch test site of dog 1, a basset hound with *Malassezia* dermatitis. There are moderate periadnexal and perivascular inflammatory cell infiltrates with predominance of neutrophils (Haematoxylin and eosin, bar=30 microns). Inset: numbers of CD3+ lymphocytes are also significantly increased (immunoperoxidase) **Bond *et al.* (2004)**

Bond *et al*. (2006) evaluated the effects of the patch test application of Malassezia pachydermatis extracts to normal canine skin in eight healthy beagle dogs. Antigens (4 and 0.4 mg/ml) and saline controls were applied for 48 h using filter paper discs in Finn chambers. At the first test, two dogs showed patch test reactivity 20 min and 24 h after patch removal. Four out of six dogs that did not react to the first patch test showed reactivity when re-tested on day 8. Two remaining dogs were patch tested for a third time on day 15, after 7 days of cutaneous challenge with suspensions of M. pachydermatis cells, but failed to display reactivity. Positive patch test reactions were characterized histologically by mild epidermal hyperplasia, superficial dermal oedema and mild to moderate perivascular, periadnexal and interstitial infiltrates of neutrophils and CD3+ lymphocytes. Four dogs showed delayed intradermal test reactivity to M. pachydermatis antigens but intradermal and patch test reactivity did not correlate. This study indicates that patch test reactivity to M. pachydermatis

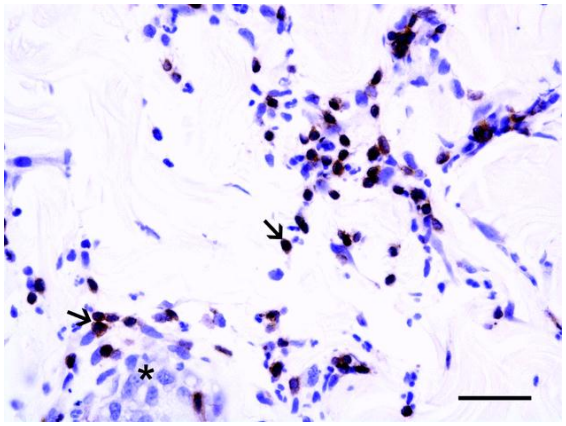
antigen occurs in some healthy dogs exposed to the yeast, or may develop after a short period of antigen exposure.



Patch test reactivity to *Malassezia pachydermatis* antigens in a healthy beagle dog (dog 5, week 2) 20 min after removal of patches applied for 48 h. Reading from left to right and top to bottom, no reactivity is present at sites exposed to the saline control (sites 1, 4 and 7). There is a moderate (grade + + +) reaction to antigen at site 6 (tape-stripped, 4 mg/ml) and weak (grade +) reactions at sites 2 (scarified, 0.4 mg/ml), 3 (scarified, 4 mg/ml) and 8 (no treatment, 0.4 mg/ml), **Bond *et al*. (2006)**



Skin biopsy specimen from a dog (dog 5, wessek 2) with a moderate (grade + + +) reaction to a 48 h patch test with a*Malassezia pachydermatis* antigen. The specimen was obtained 1 h after patch removal. There is mild, irregular epidermal hyperplasia, spongiosis, superficial dermal oedema and an interstitial dermal infiltrate composed of neutrophils with smaller numbers of eosinophils, lymphocytes, plasma cells and macrophages (Haematoxylin and eosin, bar = 50 µm). The inset shows a higher power field of the inflammatory cell infiltrate (Haematoxylin and eosin, bar = 10 µm). **Bond *et al*. (2006)**



Skin biopsy specimen from a dog (dog 5, week 2) with a moderate (grade + + +) reaction to a 48 h patch test with a*Malassezia pachydermatis* antigen. Many of the dermal inflammatory cells are CD3+ T-cells (arrows). The asterisk indicates a sebaceous gland. (EnVision™ polymeric two-step labelling method, Gill's haematoxylin counterstain, bar = 25 um). **Bond *et al*. (2006)**

Bond (2013) reviewed the role of fungi in the pathogenesis of human and canine atopic dermatitis (AD)/ He emphasized similarities and differences, where known, between the two host species. He mentioned that Malassezia spp. yeasts predominate amongst the resident skin mycobiota of both hosts and these yeasts may proliferate and trigger clinically relevant immediate hypersensitivity responses, although dogs are colonized by Malassezia pachydermatis whereas humans are not.

Glatz *et al.* (2015) mentioned that *Malassezia* spp. produces a variety of immunogenic proteins that elicit the production of specific IgE antibodies and may induce the release of pro-inflammatory cytokines. In addition, *Malassezia* spp. induces auto-reactive T cells that cross-react between fungal proteins and their human counterparts. These mechanisms contribute to skin inflammation in atopic dermatitis and therefore influence the course of this disorder. Finally, we discuss the possible benefit of an anti-*Malassezia* spp. treatment in patients with atopic dermatitis.

3.8. Treatment

- Predisposing factors that can lead to yeast overgrowth should be removed Failure to treat concurrent problems may result in partial treatment success, treatment failure, or a relapse of Malassezia dermatitis.
- To treat generalized Malassezia dermatitis, use topical agents alone or in combination with systemic antifungals.
- Prophylactic use of topical agents (shampoos, dips, creams, lotions) at least twice a week to prevent recurrence is beneficial in relapsing cases once the active infection is eliminated.
- Use degreasing antiseborrheic agents (Benzoyl peroxide, benzoyl peroxide with sulfur, 1%selenium sulfide, and tar shampoos) before applying the topical antifungal to enhance the effectiveness of the antifungal agent.
- Use antiseborrheic agents (sulfur, salicylic acid, and 1% selenium sulfide)
- Use antifungal agents (Ketoconazole, miconazole, clotrimazole, and chlorhexidine)

- Antifungal shampoos (ketoconazole, miconazole, 3% or 4% chlorhexidine, miconazole-chlorhexidine combination, or ketoconazole-chlorhexidine combination) are applied to the coat and skin and left on for 10 to 15 minutes before rinsing. Several new shampoos containing boric and acetic acid acidify the cutaneous microenvironment, making the skin less favorable to yeast growth.
- A solution of equal parts of white vinegar and water is an inexpensive acidifying topical agent with residual activity. It is applied to the skin and left to dry.
- Systemic antifungal therapy ((ketoconazole, itraconazole, and fluconazole) in severe cases of Malassezia dermatitis or when topical therapy is unsuccessful, has shown efficacy against Malassezia species.
    - **ketoconazole** is the most common one used to treat Malassezia dermatitis. The dosage is 5 to 10 mg/kg orally once a day (or divided twice daily) for 30 days. Dosages of 5 to 10 mg/kg daily for 10 days followed by alternate-day therapy at the same dose for another 10 days have been reported to be successful.
    - **Ketoconazole**
        - is excreted through sebum and eccrine glands,
        - has immunomodulatory and anti-inflammatory effects,
        - is better absorbed in an acidic environment and when taken with food.
        - can alter the metabolism of some drugs, because it inhibits cytochrome P-450 enzymes.
        - is contraindicated in pregnant bitches and in patients with liver dysfunction,
        - Adverse effects include anorexia, nausea, vomiting, diarrhea, elevated serum liver enzyme activities, icterus, pruritus, and a reversible lightening of the haircoat

Reports:

Uchida *et al.* (1992) experimentally inoculated 8 beagles intraotally with ***Malassezia pachydermatis*** to induce acute otitis externa. Three or 4 days after the inoculation, the animals showed the symptoms of otitis externa. All ear canals were erythematous and the dogs were shaking their heads. A large number of M. pachydermatis was noticed in exudate taken from every ear canal. Clinical signs of otitis externa were reduced after treatment with 0.1 ml (per canal) of 1% pimaricin suspension twice a day for 3 days. The amount of exudate decreased gradually and 12 of the 16 ear swabs examined, thereafter, were found to be negative for M. pachydermatis within 10 days. No side effects were observed in all the treated cases. These results suggested that M. pachydermatis could induce the canine otitis externa, and that pimaricin is effective agent for M. pachydermatis infection in ear canals.

Gupta *et al.*(2000) reported MICs ranging from 0.03 to 0.25 μg/mL. for ketoconazole

Hammer *et al.*(2000) reported the lowest MICs with ketoconazole,

Nakamura *et al.*(2000) tested seven *Malassezia* species and all were susceptible to itraconazole; however, *M. pachydermatis* exhibited the highest MIC range among the species evaluated.

Nègre *et al.* (2009), in a recent evidence-based review of the treatment of Malassezia dermatitis in dogs concluded that there was good evidence for the twice-weekly use of a 2% miconazole/2% chlorhexidine shampoo. The authors concluded that there was fair evidence for the use of oral ketoconazole (10 mg/kg, once daily) and oral itraconazole (5 mg/kg, once daily) for 3 weeks. Itraconazole might be preferred to ketoconazole because it is better tolerated.

Pistelli *et al.* (2012) investigated the antifungal activity and the chemical composition of essential oils (EOs) from some Mediterranean autochthonous plants were investigated against ***Malassezia pachydermatis***. Minimum inhibitory concentrations (MICs) of Anthemis nobilis, Citrus limon, Citrus paradisi, Illicium verum, Lavandula hybrida, Mentha piperita, Ocimum basilicum, Origanum vulgare, Origanum majorana, Rosmarinus officinalis, Salvia sclarea, Thymus serpillum were assessed by microdilution test; minimum fungicidal activity (MFC) was also determined. O. vulgare, T. serpillum and O. basilicum showed the lowest MIC and MFC values (0.8%) followed by C. limon and M. piperita (1%). EOs from the tested plants showed variable degrees of anti-malassezia activity, putatively related to their chemical composition. The effectiveness, manageability and pleasant organoleptic properties of O. vulgare, T. serpillum, O. basilicum, C. limon and M. piperita EOs make them advisable as promising new natural antifungal drugs in the management of M. pachydermatis otitis in dog.

Figueredo *et al.* (2013) performed a study aims evaluate the *in vitro* antifungal susceptibility of *M. pachydermatis* strains, in both their planktonic and sessile forms, to fluconazole, miconazole, ketoconazole, itraconazole, posaconazole, terbinafine and voriconazole using the XTT assay and Clinical and Laboratory Standards Institute (CLSI) microdilution method. The minimum inhibitory concentration (MIC) values recorded for each drug were significantly higher for sessile cells relative to planktonic cells to the extent that ≥ 90% of *M. pachydermatis* strains in their sessile form were classified as resistant to all antifungal agents tested. Data suggest that *M. pachydermatis* biofilm formation is associated with antifungal resistance, paving the way towards investigating drug resistance mechanisms in *Malassezia* spp.

Weiler *et al.* (2013) studied the sensitivity to antifungal drugs of two groups of *M. pachydermatis* isolates taken from dogs and cats at the Cellular and Molecular Biology Laboratory of the Paulista University (UNIP, São Paulo, Brazil). Group 1 (G1) was comprised of 40 isolates recovered from the ear canals of animals with otitis externa; group 2 (G2) was comprised of 40 isolates recovered from the ear canals of healthy animals. Isolate identification was confirmed by randomly amplified polymorphic DNA using the Mpa-F (CTGCCATACGGATGCGCAAG) and 58S-R (TTCGCTGCGTTCTTCATCGA) primers (Sugita *et al.*, 2003). Stock solutions of antifungal agents were obtained from the dilution of each antifungal drug in dimethyl sulfo-xide or sterile distilled water for fluconazole. The drugs were serially diluted in RPMI 1640 broth (GIBCO^{TM}) to obtain the following final concentrations: ketoconazole (16 µg/mL-0.007 µg/mL) (Janssen Beerse), itraconazole (16 µg/mL-0.007 µg/mL) (Janssen Beerse), clotrimazole (64 µg/mL-0.125 µg/mL) (Bayer), voriconazole (16µg/mL-0.007 µg/mL) (Pfizer), miconazole (64 µg/mL-0.125 µg/mL) (Labware), nystatin (64 µg/mL- 0.125 µg/mL) (Bristol-Myers), amphotericin B (16 µg/mL-0.007 µg/mL) (Bristol-Myers) and fluconazole (64 µg/mL-0.125µg/mL) (Pfizer). Inocula were obtained from 48-h pure Dixon agar cultures and consisted of

microorganism suspensions in sterile saline (0.85%) plus Triton X-100 (0.05%) (Merck), whose turbidity was adjusted to 0.5 on the McFarland scale. Inocula were diluted 1:50 in sterile distilled water and then at 1:20 in RPMI 1640 broth. For each isolate, microdilution plates containing 10 µLof antifungals in RPMI 1640 diluted in different concentrations were inoculated with 10 µLof the standardized ino-cula. As positive control, the standardized inocula were cultured alone; the negative control was the antifungal alone diluted in RPMI 1640 broth. Culture plates were incubated at 37 °C for 48 h. Minimum inhibitory concentrations (MICs) were recorded following the M27-A3 protocol (CLSI, 2007). All tests were performed in duplicate. The Mann-Whitney test was used to compare the two groups of isolates to determine whether they had similar susceptibility patterns to the antifungal agents tested.

The lowest MICs were observed with ketoconazole, itraconazole and voriconazole. Voriconazole showed the smallest variations of MICs with the MICs for G1 isolates ranging from 0.01-0.25µg/mL, while the MICs for G2 isolates ranged from 0.01 to 0.125 µg/mL.

Cafarchia *et al.* (2014) evaluated the efficacy of a **killer decapeptide (KP)** *in vitro* and *in vivo*. Sixteen dogs with naturally occurring ***M. pachydermatis*** otitis externa were enrolled, and the *in vitro* fungicidal activity of KP was evaluated using yeasts recovered from these animals. The therapeutic activity was evaluated in four groups of four animals each. The dogs were topically treated with KP (150 µl, 2 mg/ml) three times per week (group A) or every day (group B), treated with a scramble peptide every day (group C), or left untreated (group D). Assessment of clinical signs (pruritus, erythema, and lichenification and/or hyperpigmentation), expressed as mean of the total clinical index score (mTCIS), the population size of *M. pachydermatis* at the cytological examination (mean number of yeast cells at 40× magnification [mYC]), and culture testing (mean number of $\log_{10}$CFU/swab [mCFU]), were conducted daily from the first day of treatment (T0) until two consecutive negative cultures (mCFU ≤ 2). KP showed an *in vitro* fungicidal effect against *M. pachydermatis* isolates, with an MFC_{90}value of 1 µg/ml. The mTCIS, mYC and mCFU were negative only in animals in group B after T8. Daily administration of KP for 8 days was safe and effective in controlling both clinical signs and the population size of *M. pachydermatis* causing otitis externa, thus offering an alternative to the currently available therapeutic or prophylactic protocols for recurrent cases of *Malassezia* otitis in dogs. This study compared the susceptibility of *M. pachydermatis* isolates from sick (G1) and healthy (G2) animals to azole and polyene antifungals using the M27-A3 protocol. Isolates from G1 animals were less sensitive to amphotericin B, nystatin, fluconazole, clotrimazole and miconazole.

3.9. Zoonotic potential Malassezia pachydermatis

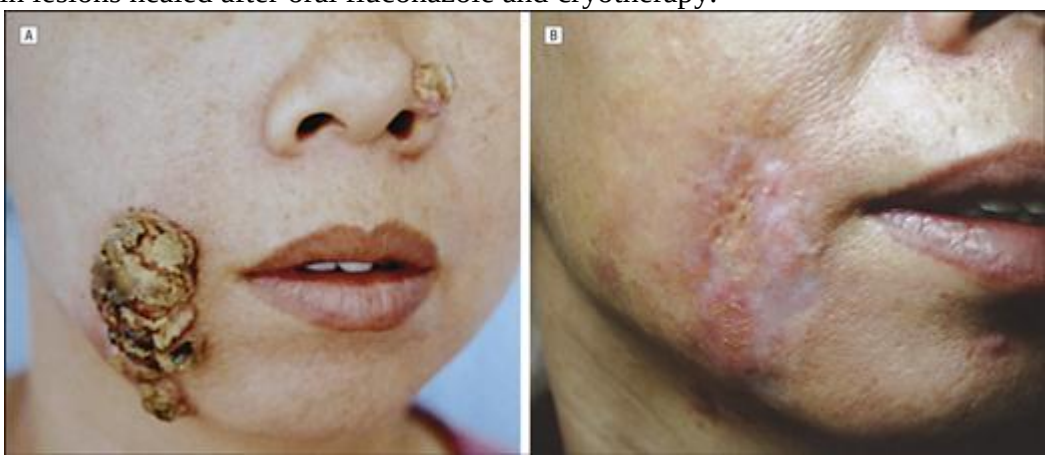
It was identified recently in a human intensive care nursery. The yeast was thought to have been transmitted to 15 infants from the contaminated hands of dog-owning healthcare workers.

Reports:

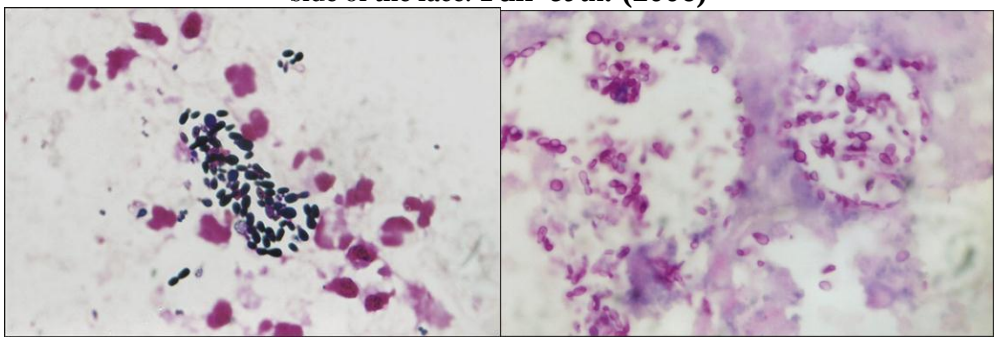
Huan *et al*. (1998) reported an outbreak of ***Malassezia pachydermatis*** in an Intensive Care Nursery, eight patients with bloodstream infections, two with urinary tract

infections, one with meningitis, and four with asymptomatic colonization. In a point-prevalence survey, 9 additional infants, 1 health care worker, and 12 of the health care workers' pet dogs had positive cultures for *M. pachydermatis.* The isolates from all case patients, the 9 additional colonized infants, 1 health care worker, and 3 of the 12 dogs had identical patterns of restriction-fragment–length polymorphisms. In this outbreak, it was likely that *M. pachydermatis* was introduced into the intensive care nursery on health care workers' hands after being colonized from pet dogs at home. The organism persisted in the nursery through patient-to-patient transmission.

Fan *et al.* (2006) isolated *Malassezia pachydermatis* from the facial granuloma of a healthy woman and her dog's skin scrapings and cerumen. The yeast identity was established by standard methods and scanning electron microscopy. A skin biopsy specimen showed chronic inflammatory granuloma, numerous purple-red round or ovoid spores in the superficial necrotic tissue, and sparse red spores in the dermis. The skin lesions healed after oral fluconazole and cryotherapy.



Patient before and after treatment. A, A verrucous plaque on the right side of the face and a hemispheroid nodule on the left ala nasi. B, After treatment, hypopigmented scar on the right side of the face. **Fan *et al.* (2006)**



Left:Secretion smear showing numerous Gram-positive, yeastlike polymorphous spores (Gram stain; original magnification ×1000).Right:Biopsy specimen showing purple-red round or ovoid spores in the superficial necrotic tissue (periodic acid–Schiff stain; original magnification ×1000). **Fan *et al.* (2006)**

Hernández-Escareño (2012) collected samples from 125 dogs from the external canal of the left and right ear (n=250 ears), of which 180 were positive to ***Malassezia pachydermatis***, representing 72% (180/250) and 70 negatives or 28% (70/250). Parameters considered for this study were: kind of ear, length of hair and size of the animal. Samples were taken using sterile cotton swaps. Samples were cultured in potato dextrose agar media with cycloheximide and chloramphenicol. **PCR** tests

amplifying regions D1 and D2, coding for LSU rRNA were also performed and were compared with the reference strain CBS 1879NT of M. pachydermatis. Amplified product yielded a 600 bp product characteristic for Malassezia. Most predominant dog breeds for external otitis were Cocker Spaniel, French Poodle and Criolla. In Mexico, as well as in the state of Nuevo Leon, no documented evidence exists about the presence of this yeast in dogs with external otitis. The objective was to analyze dogs with this pathology and to inform the presence of M. pachydermatis by means of culture and amplification of D1 and D2 regions of gen 26S rRNA.

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4. Trichosporonosis in dogs and cats

Doster *et al.* (1987) diagnosed an infection with Trichosporon spp in 2 cats. In one cat, infection consisted of a granulomatous dermatitis and was concurrent with disseminated lymphoblastic lymphosarcoma. In another cat, urinary cystitis caused by ***T beigelii*** was diagnosed**.**

Jairam and Das (2005) reported a .case of trichosporonosis in an 8-month-old German Shepherd . Red, erythematous, alopecic patches with powdery deposits were observed. A skin scraping sample was collected for fungal culture. Direct microscopic examination (10% KOH mount) was inconclusive. No evidence of dermatophyte infection or ectoparasitic infestation was observed. Bilobed yeast cells with true mycelium, some fragmenting into arthrospores of rectangular shape, were observed at various microscopic fields. Skin scrapings with hair were seeded onto Sabouraud's-potato dextrose agar with or without cycloheximide and chloramphenicol and incubated at 250°C for 4 days. Growth of yeast-like, dull white colonies showing budding yeast cells after 4 days was observed. The isolate was non-fermentative and urease-positive. The development of mycelia with arthrospores was observed by 2 to 3 weeks. The isolate was identified as *Trichosporon* spp.

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C.Fungal diseases of cats and dogs caused by moulds

1. Aspergillosis in dogs and cats

1.1. Introduction

Aspergillosis is an infection caused by the *Aspergillus* fungus, which is commonly found in the environment. There are two types of *Aspergillus* infection, nasal and disseminated. Both types can occur in cats and dogs, but they occur more frequently in dogs. Young adult dogs with a long head and nose (known as *dolichocephalic* breeds= A long head, usually very narrow like a **greyhound)** and dogs with a medium length head and nose (known as mesatcephalic breeds) are also more susceptible to the nasal form of aspergillosis. The disseminated version of the disease seems to be more common in German Shepherds**.** No particular breed in cats is more prone than another, **Persians** show a slightly higher incidence.



German Shepherd, www.dogchannel.com,Greyhound dog, at, animaltheory.blogspot.com

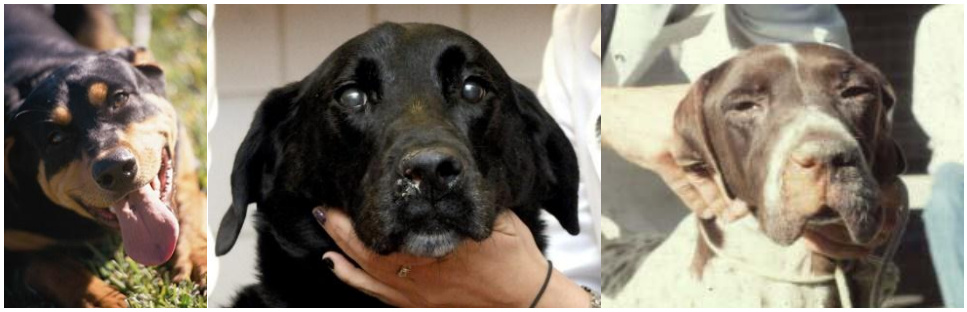
www.pinterest.com .Persian c

1.2. Aspergillosis in dogs

1.2.1. Sinonasal aspergillosis

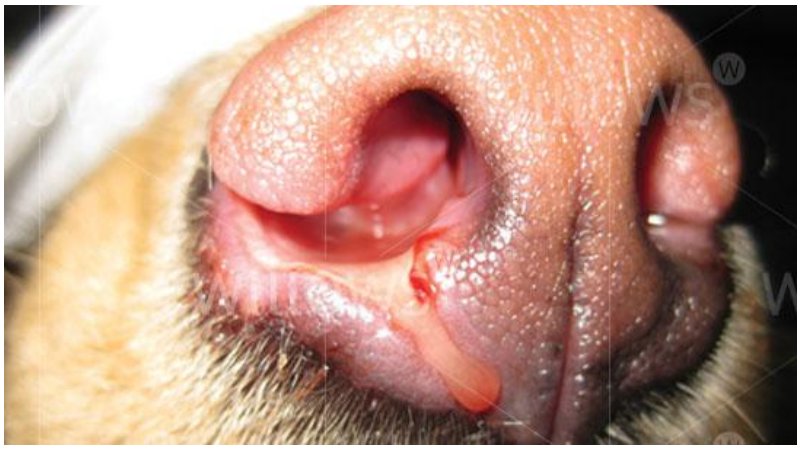
- Sinonasal aspergillosis occurs in apparently immunocompetent dogs
- Sinonasal aspergillosis is caused predominantly by *Aspergillus fumigatus* , although infection can occur with other species including *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus nidulans*.
- Sinonasal aspergillosis occurs mostly in young to middle aged, with a mean age of 4.4 years (range = 1.5 to 8 years).
- Sinonasal aspergillosis is associated with uni- or bilateral profuse purulent to mucopurulent nasal discharge, facial discomfort, depigmentation or ulceration of the nares, sneezing, epistaxis, decreased appetite, lethargy, stertor, stridor, and open-mouth breathing. Ocular discharge and exophthalmos may be seen, and, destruction of the cribriform plate may result in signs of forebrain dysfunction
- Sinonasal aspergillosis leads mostly to marked destruction of turbinate bones and mucosa. In severe cases, destruction of the frontal bones with invasion into the periorbital soft tissues and penetration through the cribriform plate into the central nervous system may occur.

www.pethealthnetworkwww.germanshepherds.comwww.springerrescue.org www.njmoldinspection

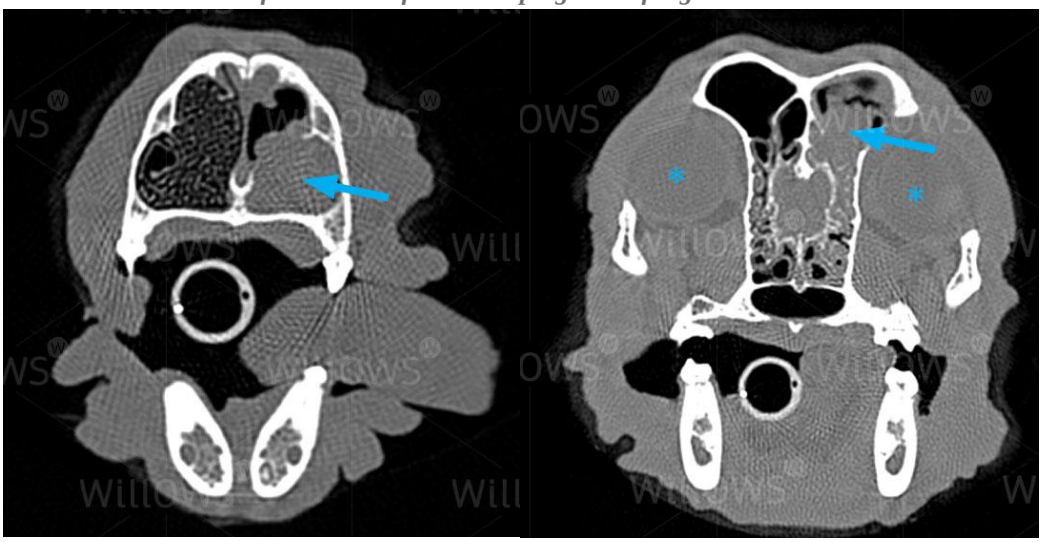


VCA Animal Hospitals www.njmoldinspection.com

Nasal aspergillosis due to ***A. fumigatus*** Nasal infection by *Aspergillus terreus*.Dr. R. Mallik, Sydney, Australia



front of a dog's nose (the nasal planum) showing some blood-tinged creamy discharge from the patient's right nostril and ulceration, ***http://www.willows.uk.net/specialist-services/pet-health-information/soft-tissue/aspergillosis-fungal-rhinitis***

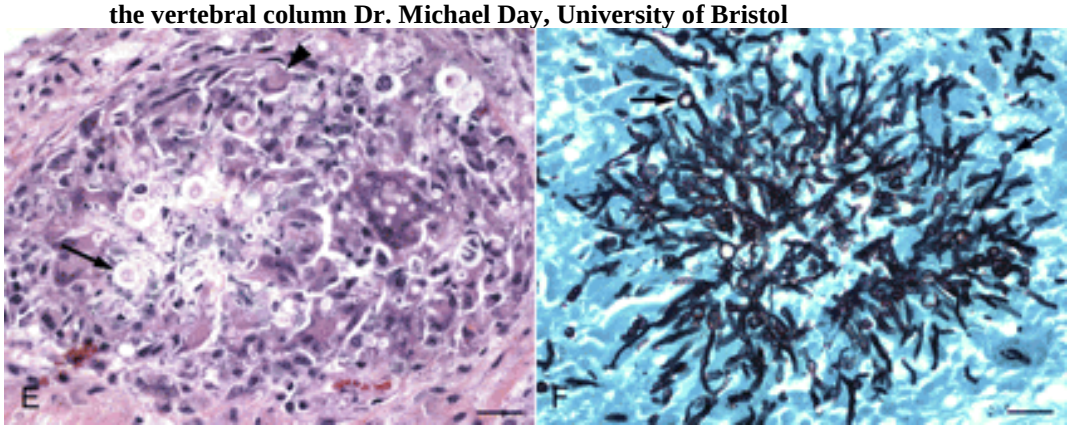


A computed tomography (CT) image of a cross section of a dog's nose. The right side (seen on the left in the picture) has a fine lace-like appearance representing the scrolls of bone (turbinates) that are normally present. The arrow is pointing to the left side of the nasal cavity which has suffered from destruction of these turbinates and accumulation of discharge that appears the same shade of grey as the soft tissues of the dog's head. These changes are strongly suggestive of fungal rhinitis (aspergillosis) and would be very difficult to identify on normal X-rays. CT images such as this are always displayed as if the head was facing you and therefore the animal's left appears on the right of the image. ***http://www.willows.uk.net/specialist-services/pet-health-information/soft-tissue/aspergillosis-fungal-rhinitis***

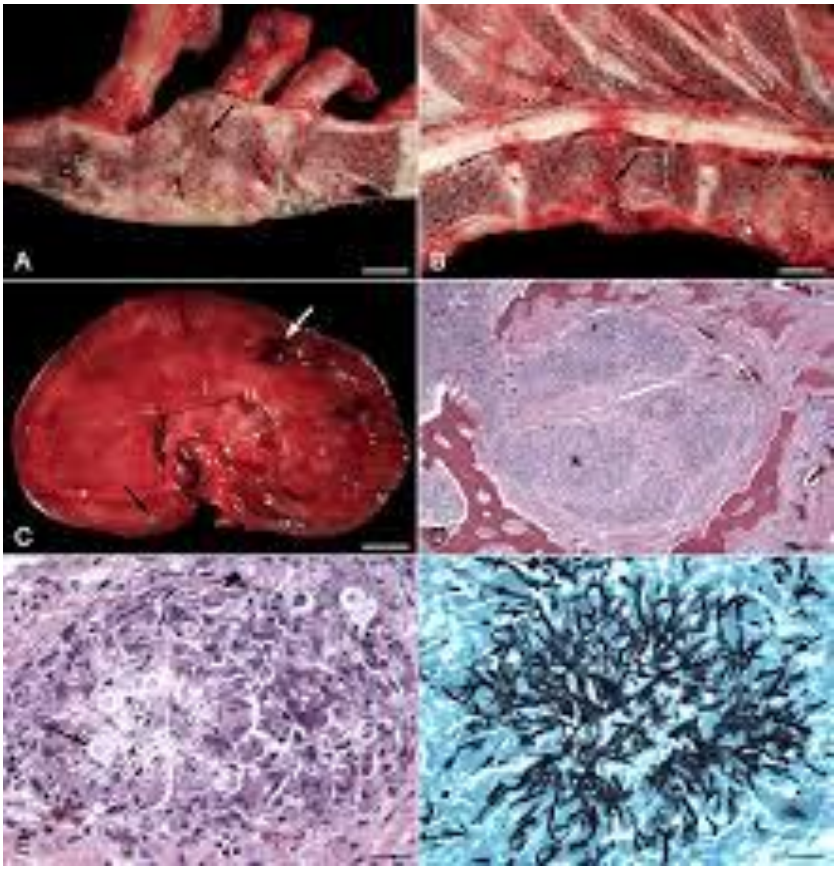
1.2.2. Disseminated spergillosis

- Disseminated aspergillosis is relatively rare in dogs compared with the sinonasal form.
- Infection occur mostly through the respiratory tract with subsequent hematogenous spread to other sites, including intervertebral disks, kidneys, and irises as well as other organs, muscles, and long bones.
- *Aspergillus terreus* and *Aspergillus deflectus* are the predominant
- Many affected dogs have underlying immunocompromise, such as diabetes mellitus or bacterial infections, or are receiving immunosuppressive medications, such as glucocorticoids or chemotherapeutics.
- Genetic factors may also play a role, as German shepherds are substantially predisposed to this disease.
- Clinical signs of disseminated disease depend on the organ systems involved,
- Nonspecific signs such as anorexia, lethargy, and fever are common
- Diskospondylitis is commonly noted, associated with vertebral pain, paraparesis, paraplegia, or lameness.

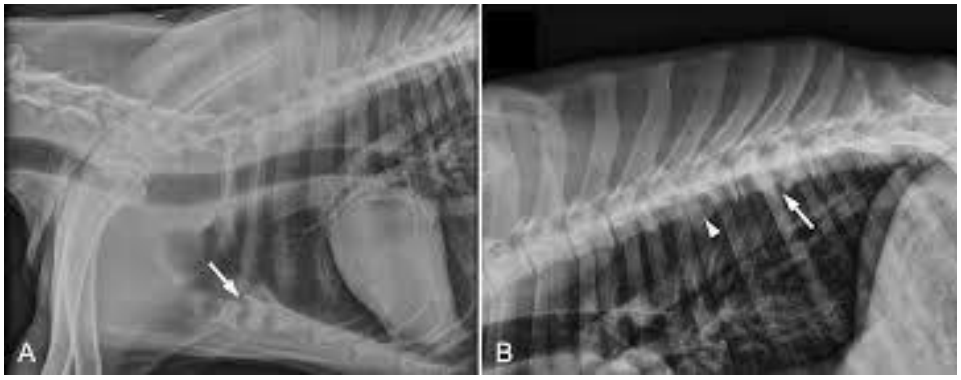
Disseminated aspergillosis due to *A. terreus*, Saggital section of kidney ,**Saggital section of the vertebral column Dr. Michael Day, University of Bristol**



Higher magnification showing marked granulomatous inflammation with giant cell formation (arrowhead) surrounding septate fungal hyphae and bulbous spore-like structures (arrow)m Grocott's methenamine silver-stained section demonstrating prominent fungal hyphae and terminal conidiophores (arrows).. **Dr. Michael Day, Bristol Univ**



(A) Right sagittal section of sternum; the first sternebra is on the left. The second and third sternebrae are collapsed, and areas of bony proliferation obscure the joint space. An area of necrotizing osteomyelitis partially separates the two sternebrae (arrow). Bar, 1 cm. (B) Left sagittal section of thoracic vertebrae; the cranial end is to the right. The intervertebral disk at T9-T10 is missing (arrow). The end plates are eroded, and a wedge-shaped piece of tissue compresses the spinal cord dorsally. Bar, 1 cm. (C) Sagittal section of left kidney. Small white areas are scattered throughout the cortex and medulla (black arrow). The pelvis is dilated, and the renal crest is ulcerated. Areas of hemorrhage (white arrow) are visible in the cortex. Bar, 1 cm. (D) Photomicrograph of second sternebra showing areas of inflammation () and surrounding fibrous tissue invading and replacing the marrow cavity. Bar, 250 m. (E) Higher magnification of sternebra showing marked granulomatous inflammation with giant cell formation (arrowhead) surrounding septate fungal hyphae and bulbous spore-like structures (arrow). Bar, 25m. (F) Grocott's methenamine silver-stained section of sternebra taken from same area as previous image, demonstrating prominent fungal hyphae and terminal conidiophores (arrows). Bar, 25 m, **Canine Disseminated Aspergillosis jcm.asm.org**



(A) Lateral radiograph of the thorax. There is lysis of the first four sternebrae and marked shortening of the second and third sternebrae, which have irregular margins and loss of the end plates (arrow). (B) Lateral radiograph of the thoracic vertebral column. There is end plate lysis of the 9th (T9) and 10th (T10) thoracic vertebrae that is centered on the intervertebral space (arrow), with spondylosis

deformans ventrally. There is also narrowing, end plate sclerosis, and spondylosis deformans between the seventh (T7) and eighth (T8) vertebrae (arrowhead) **Canine Disseminated Aspergillosis jcm.asm.org**.

1.2.3. Other forms of aspergillosis in dogs

- **Bronchopulmonary aspergillosis**
- **Aspergillosis of the CNS**
- **Aspergillosis of the ears (otomycosis)**
- **Aspergillosis of the eye (oculomycosis)**

1.3. Aspergillosis in cats

- Aspergillosis in cats is a sporadic mycosis that leads to a usually chronic, only rarely acute disease that mainly affects the nasal cavity and sinuses.
- *Aspergillus* spp. infections are commonly associated with predisposing local or systemic factors. Local disease can spread and involve the central nervous system or the lungs. Some Aspergillus spp. can also disseminate, causing systemic infections.
- Aspergillosis is rare in cats, but considered an emerging infection in e.g. Australia.
- There are two clinical forms of aspergillosis in cats, the sinonasal (characterized by signs of chronic nasal infection) and the newly emerging more invasive sinoorbital form (characterized by signs of orbital and surrounding tissue invasion).
- feline aspergillosis has been described in North America, the United Kingdom, Switzerland, Germany, Japan, and Italy.
- No age or sex predisposition has been detected.
- A predisposition was found in brachycephalic breeds, especially Persian and Himalayan cats.
- **Aspergillosis occurs in two main forms in cats:**

1.3.1. Sinonasal aspergillosis (SNA)

- **SNA** is characterized by more local signs of chronic nasal infection, such as sneezing, uni- or bilateral serous to mucopurulent nasal discharge, and sometimes epistaxis. Sterterous breathing, granuloma formation, soft tissue masses protruding from the narines, and bone lysis are less frequent abnormalities.

1.3.2. Sinoorbital aspergillosis (SOA).

- **SOA** is the more invasive form.
- **SOA** probably represents an extension of SNA to orbital and subcutaneous tissues,

- **SOA** is caused by invasive *Aspergillus* spp.
- Most cats with SOA have a history of nasal discharge, and nasal lesions have been identified at necropsy as well as lysis of the orbital lamina using imaging techniques.
- SOA is characterized by signs of orbital and surrounding tissue invasion, including
    - unilateral exophtalmus,
    - third eyelid prolapse,
    - conjunctival hyperaemia, and
    - keratitis
    - ulceration of the hard palate
    - extension and destruction of the nasal cavity
    - CNS can be involved leading to neurological signs, peripheral vestibular signs, and blindness, and regional lymphadenopathy and fever can occur.

Exophthalmos of the left eye in a cat with a left retrobulbar fungal granuloma (sino-orbital aspergillosis). There is prolapse of the third eyelid. A partial lateral tarsorrhaphy was performed to prevent exposure keratitis Right exophthalmos, third eyelid prolapse and oedema and swelling of the right side of the face in a cat with a right retrobulbar and paranasal fungal granuloma (courtesy of **Vanessa Barrs, University Veterinary Teaching Hospital, Sydney, Australia**).

Ventral expansion of retrobulbar fungal granulomas causes a mass effect in the pteryoplatine fossa as seen in this cat with a right retrobulbar fungal granuloma (arrow) (courtesy of **Vanessa Barrs, University Veterinary Teaching Hospital, Sydney, Australia**).

Thoracic radiographs (latero-lateral view) of a cat with pulmonary aspergillosis, diagnosed at necropsy (courtesy of **Katrin Hartmann, Medizinische Kleintierklinik, Ludwig-Maximilians-Universität München, Germany**).

1.4. Aspergillus species isolated from dogs

- ***Aspergillus fumigatus*** (Khan *et al.,* 1984, Kulendra *et al*., 2010, Adamama-Moraitou *et al*. (2011 and Ferreira *et al*., 2011)
- **Aspergillus terreus** (Wood *et al.* 1978, Kabay *et al*., 1985, Day *et al.*, 1985 and 1986. Day and Penhale, 1988 and 1991, Dallman *et al.*, 1992, Bruchim *et al*, 2006, Elad et *al*., 2008 and Schultz *et al*., 2008).
- ***Aspergillus niger*** (Kim *et al*. 2003).
- ***Aspergillus deflectus*** Robinson (2000), Schultz *et al.* (2008),
- ***Aspergillus ochraceus*** (Ghibaudo and Peano, 2010)
- ***Aspergillus alabamensis*** Burrough *et al*. (2012
- ***Aspergillus versicolor*** Zhang *et al* (2012).
- ***Aspergillus felis*** Barrs *et al.* (2013)

1.5. Aspergillus species isolated from cats

- ***Aspergillus fumigatus*** (Barachetti *et al.*, 2009, Giordano *et al.*, 2010, Hazell *et al.*, 2011, Barrs *et al.*, 2014 , Barrs and Talbot, 2014).
- ***Aspergillus niger*** **(**Barrs and Talbot 2014),
- ***Aspergillus wyomingensis*** (Kano *et al*., 2008),
- ***Aspergillus udagawae*** (Kano *et al.*,2008 and Kano *et al.*, 2013),
- ***Aspergillus viridinutans*** (Kano *et al.* (2013) and
- ***Aspergillus fischeri*** (Kano et *al.*, 2015)
- ***Aspergillus felis*** Barrs *et al.* (2013)

1.6. Description of commonly isolated Aspergillus species from dogs and cats

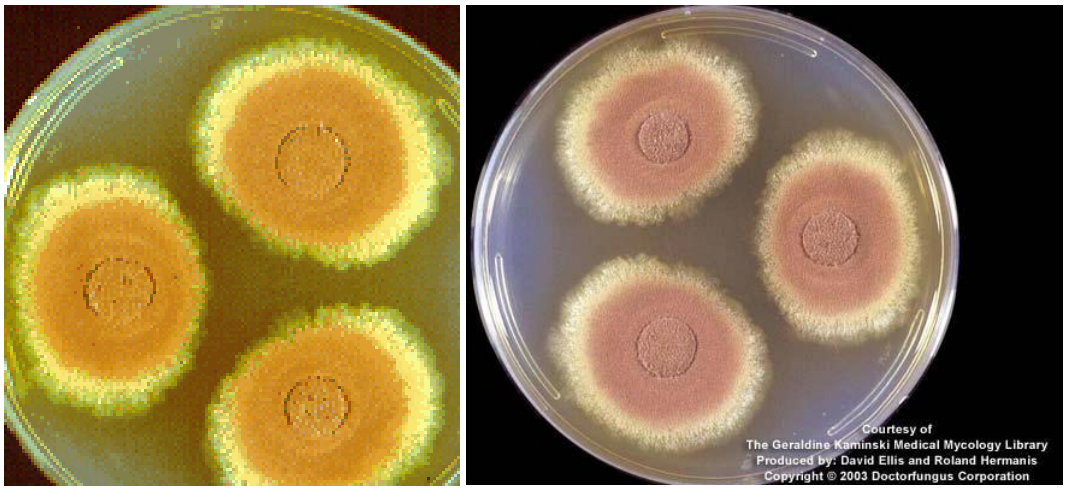
1.6.1. *Aspergillus fumigatus* Fresenius, 1863.

Colony diam (7 d): CYA25: 21-67 mm; MEA25: 25-69 mm; YES25: 48-74 mm; OA25: 34-62 mm, CYA37: 60-75 mm, CREA: poor growth, no or very weak acid production. Colour: greyish turquoise or dark turquoise to dark green to dull green. Reverse colour (CYA): creamy, yellow to orange. Colony texture: velutinous, st. floccose. Conidial head: columnar. Conidiation: abundant, rarely less abundant. Stipe: 50-350 × 3.5-10 µm. Vesicle diam, shape: 10-26 µm, pyriform to subclavate, sometimes subglobose, but rarely globose. Conidia length, shape, surface texture: 2-3.5(-6) µm, globose to ellipsoidal, smooth to finely rough

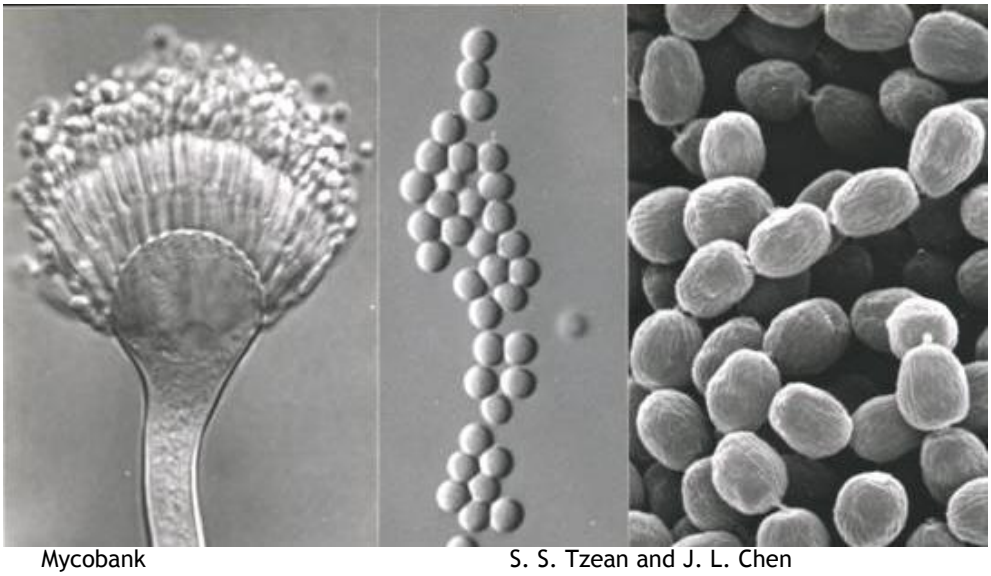
Aspergillus fumigatus, Mycoba

1.6.2. *Aspergillus terreus* Thom, (1918)

Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with conidial production. Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae.



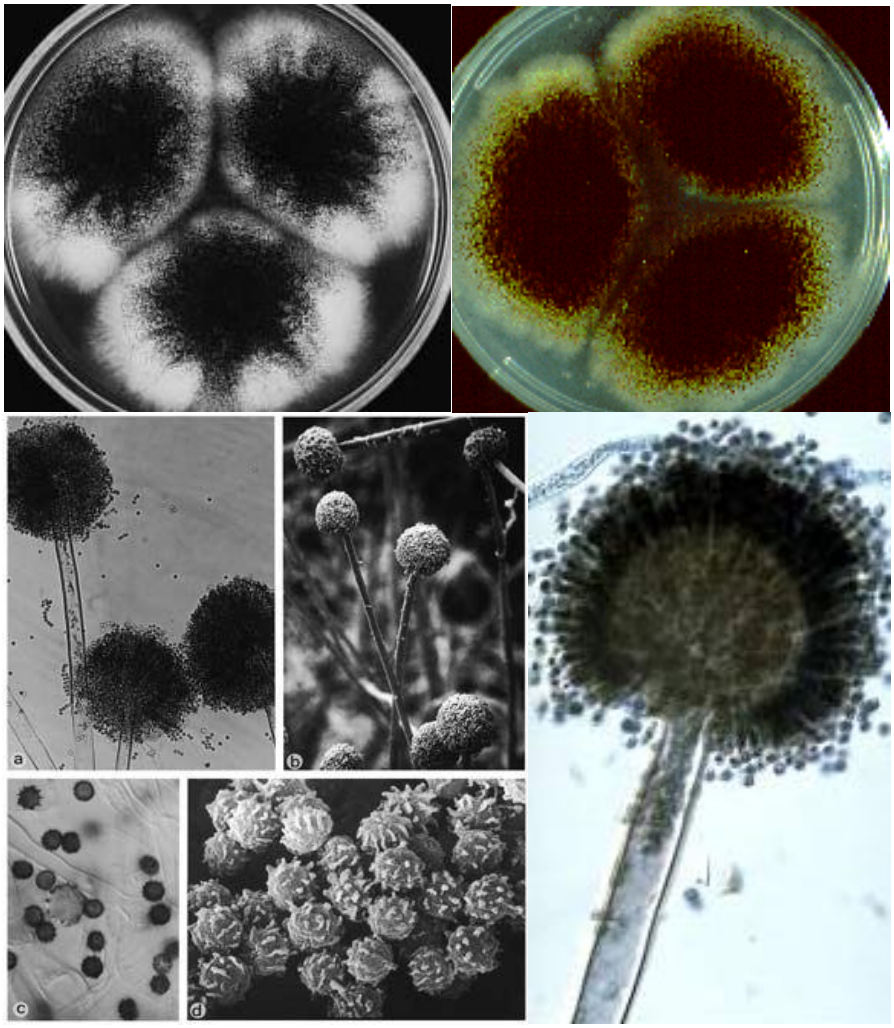
Aspergillus terreus mycology.adelaide.edu.au www.mold.ph



Mycobank S. S. Tzean and J. L. Chen

1.6.3. *Aspergillus niger* van Tieghem 1867

On Czapek dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm x 15-20 um in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled.

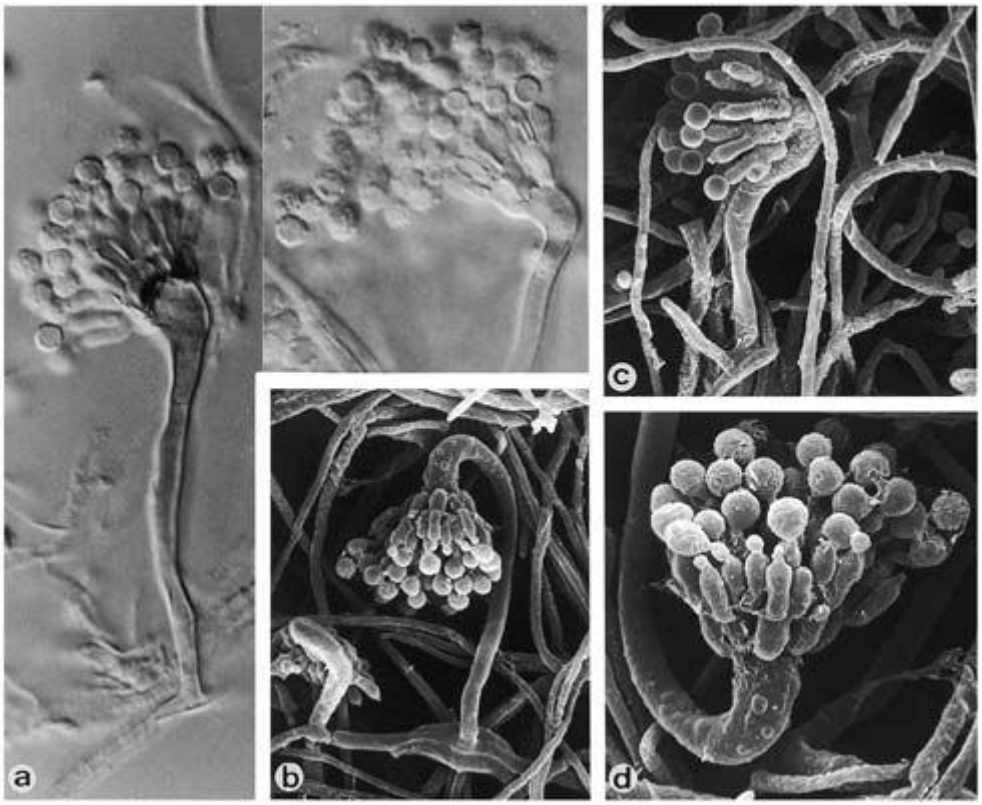


Varga *et al*., 2011 Mycobank

1.6.4. ***Aspergillus deflectus*** Fennell & Raper, Mycologia 47: 82 (1955)

Colonies on Czapek's agar growing restrictedly, attaining a diameter of 1.5 to 2.0 cm. in 10 days to 2 weeks at room temperature (24-26°C), very compact, consisting of a close-felted, tough, basal mycelium, slightly zonate, somewhat radially furrowed; margins abrupt near congo pink from admixture of vegetative mycelium; conidial structures abundantly produced; central colony area mouse gray to deep mouse gray; exudate fairly abundant but not conspicuous, clear, embedded in the mycelial mass as small droplets; reverse and agar in dull orange-red shades near terra cotta, becoming brown in age; odor fairly pronounced, moldy. Conidial heads evenly distributed, typically broadly columnar and mostly 25 to 30 µm in diameter but in some strains tending to be radiate, borne on short conidiophores from the aerial felt; conidiophores sinuous, mostly 40 to 50 µm long in some strains but up to 125 µm in others, 2.5 to 3.5 µm in diameter, smooth walled, reddish brown with pigmentation extending into the vesicles and sterigmata; vesicles rounded, flask shaped, 5.5 to 6.5 µm in diameter,

characteristically borne at, or nearly at, right angles to the main axes of the conidiophores, bearing sterigmata on the uppermost surfaces only; sterigmata in two series, primaries 4.5 to 5.5 µm by 2.8 to 3.3 µm, secondaries 4.5 to 5.5 µm by 1.8 to 2.2 µm, occasionally abortive and failing to produce conidia; conidia globose to subglobose 3.0 to 3.5 µm, with variable ornamentation, ranging from almost smooth when young to irregularly roughened at maturity.

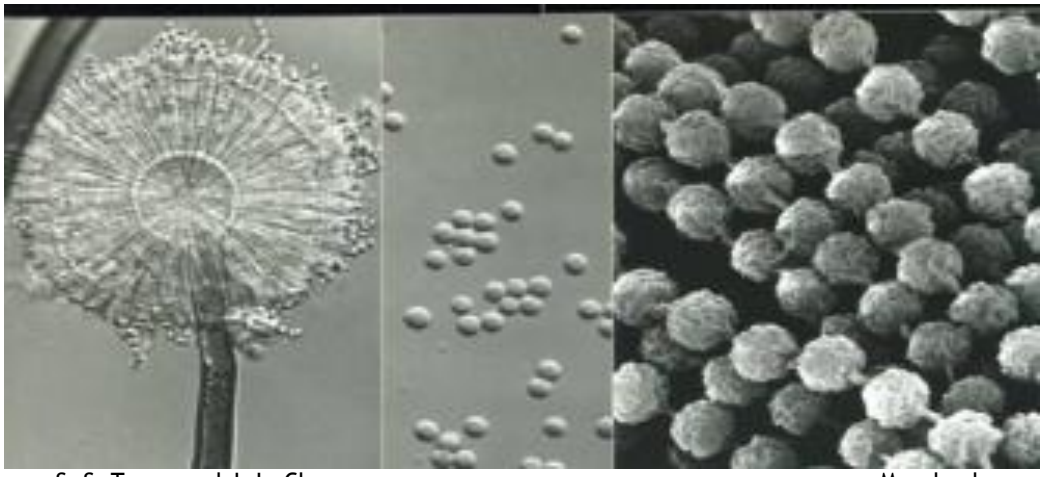


Mycobank

1.6.5. *Aspergillus ochraceus* K. Wilh., (1877)

Colony diameters on Czapek's Agar 3.0-3.5 cm in 10 days at 25°C, wrinkled; conidial heads spherical, or splitting into compact divergent columns, cream color, pinkish buff or near dark olive-buff; mycelium white, inconspicuously floccose to floccose; exudate uncolored; reverse dull yellow brown to victoria lake; soluble pigment, pale capucine buff ; stipes 360-1390 × 4.0-16.0 µm, pale yellow to light yellow brown, slightly to coarsely roughened; vesicles spherical to subspherical, 8.8-46.0 µm in diameter. Aspergilla biseriate, metulae covering the entire surface of the vesicle, 4.8-33.3 × 2.4-10.3 µm; phialides 5.6-143.0 × 1.8-4.8 µm. Conidia spherical to subspherical, smooth to irregular rough, 2.0-3.8 . Sclerotia produced by the same isolate, purple, up 1000 µm in diameter. Colony diameters on Malt Extract Agar 5.0-5.5 cm in 10 days at 25°C, more or less floccose; conidial heads globose or splitting into a few columns, near antimony yellow to ochraceous-buff; mycelium white, reverse dull yellow brown to pale auburn.

lookfordiagnosis.com Show.wnmu.edu1155 × 1148 www.drthrasher.org Mold Library



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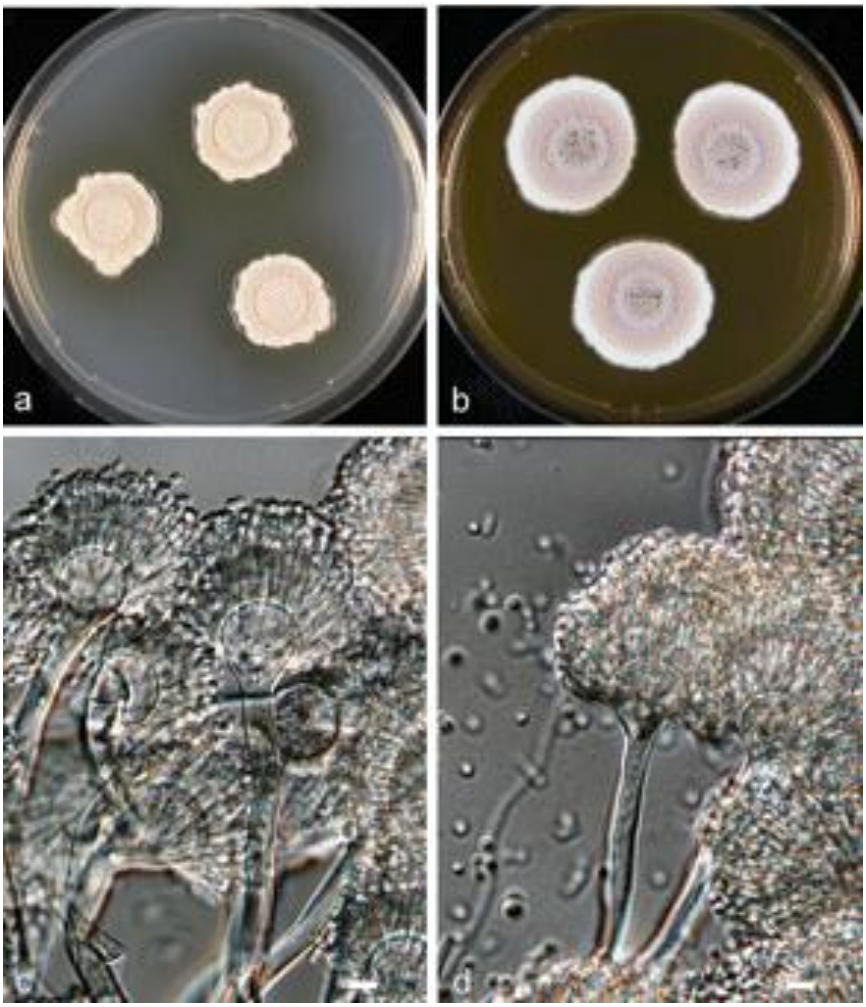
1.6.6. *Aspergillus alabamensis* Balajee, Baddley, Frisvad & Samson, sp. nov.

Colonies on Czapek yeast extract and MEA are yellowish-brown to cinnamon-brown, often consisting of a dense felt of conidiophores but also showing floccose growth. Conidial heads are densely columnar. Conidial heads are long, columnar, 30 to 50 µm in diameter, and 150 to 500 µm or more in length at maturity; conidiophores are biseriate, smooth, colorless, and 100 to 250 µm by 4.5 to 6.0 µm. Vesicles are subglobose and 10 to 16 µm in diameter. Phialides are 5.0 to 7.0 µm by 2.0 to 2.5 µm. Metulae are closely packed and 5.5 to 7.5 µm by 1.5 to 2.0 µm. Conidia are globose to slightly elliptical, smooth, and 1.8 to 2.4 µm in diameter.



Aspergillus alabamensis sp. nov. UAB 20T. Shown are colonies on MEA after 7 days at 25°C on CYA (a) and on MEA (b), conidial heads (c and d), Balajee **et al.,2009**

1.6.7. ***Aspergillus versicolor*** (Vuill.) Tirab., (1908)

Colonies on CYA 16-25 mm diam, plane or lightly sulcate, low to moderately deep, dense; mycelium white to buff or orange; conidial heads sparse to quite densely packed, greyish green; pink to wine red exudate sometimes produced; reverse orange or reddish brown. Colonies on MEA 12-25 mm diam, low, plane, and dense, usually velutinous; mycelium white to buff; conidial heads numerous, radiate, dull or grey green; reverse yellow brown to orange brown. Colonies on G25N 10-18 mm diam, plane or umbonate, dense, of white, buff or yellow mycelium; reverse pale, yellow brown or orange brown. No growth at 5°C. Usually no growth at 37°C, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 300-600µm long, with heavy yellow walls, vesicles variable, the largest nearly spherical, 12-16µm diam, fertile over the upper half to two-thirds, the smallest scarcely swollen at all and fertile only at the tips, bearing closely packed metulae and phialides, both 5-8µm long; conidia mostly spherical, very small, 2.0-2.5µm diam, with walls finely to distinctly roughened or spinose, borne in radiate heads.

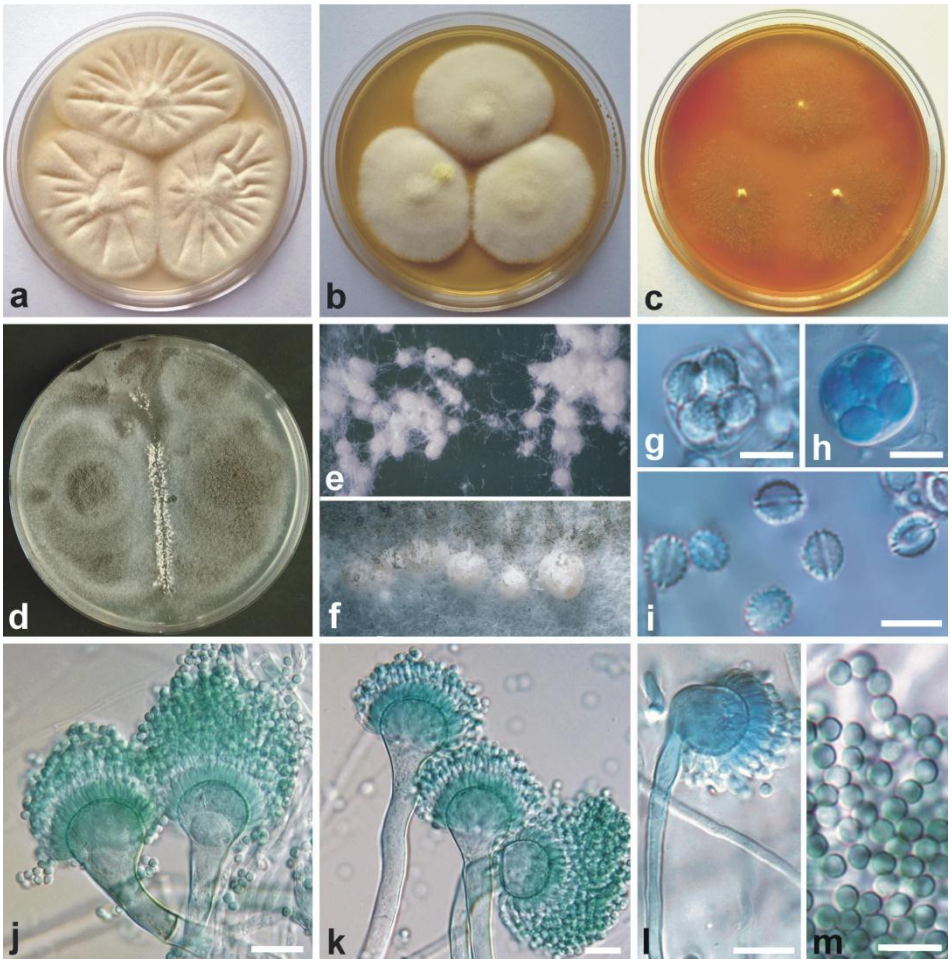
Jurjevic Z, Peterson SW, Horn BW - IMA Fungus (2012)

Aspergillus versicolor, www.tamagawa.ac.jp

1.6.8. ***Aspergillus wyomingensis*** A. Nováková, Dudová & Hubka, Fungal Diversity 64: 270 (2014)

Colonies on CYA in 52–58 mm in diam at 25 °C in 7 days, velutinous, wrinkled, yellowish white with poor sporulation on the colony margin (pale yellow green) after 14 days, no exudate or soluble pigment production, reverse pale yellow. Colonies at 37 °C 65–70 mm, floccose to lanose, wrinkled, yellowish white (No. 92), reverse pale yellow. Colonies on MEA 43–44 mm, floccose, plane, yellowish white, no exudate, soluble pigment present after 14 days—brilliant greenish yellow to vivid greenish yellow, reverse light yellow with moderate yellow parts, brilliant greenish yellow to vivid greenish yellow. Colonies on YES (60−)68−70 mm in diam, velutine to floccose, wrinkled, yellowish white, no exudate, no soluble pigment, reverse strong yellow (No. 84) to deep yellow). Colonies on CZA 38–42 mm in diam, plane, whitish yellow. Colonies on CREA 46–50 mm in diam, poor mycelial growth, acid production strong or only under the colony. Ehrlich test negative. Only some isolates were able to grow restrictedly (up to 16 mm) at 45 °C, all grew at 42 °C. Conidial heads columnar. Conidiophores arising from aerial hyphae, smooth, up to 275.0×(3−)4−6.5(−7) µm, nodding heads occasionally present. Conidial heads uniseriate, vesicles subglobose to globose, pigmented, 11−19(−24) µm, two-thirds covered by ampuliform phialides. Conidia subglobose, delicately rough, 1.7−2.8(−3.3) µm, light green in mass. Heterothallic species; the ascomata visible after 3 weeks of incubation on OA at 25, 30 and less abundant at 37 °C, mature ascospores present after 4–5 weeks. Cleistothecia white, globose or subglobose 180−500(−600) µm in diameter, covered by a dense felt of white hyphae; asci eight-spored, globose to subglobose 10–12×10–11 µm; ascospores lenticular, spore bodies (3.2−)3.6−5 µmin longer axis, equatorial crests absent or are very low and difficultly distinguishable by light microscopy, shallow equatorial

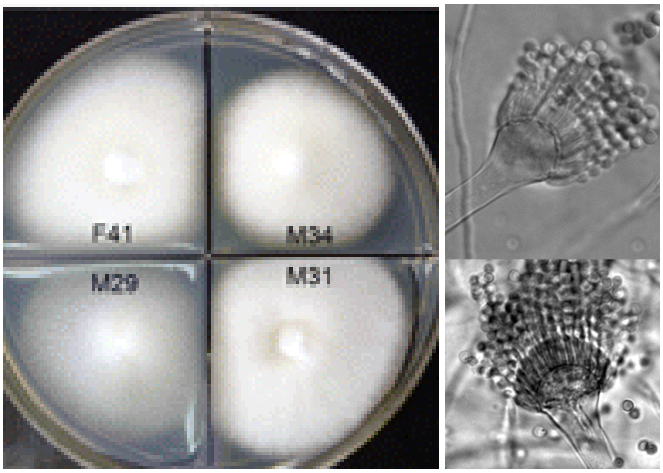
furrow is present, short ribs, rough tubercles and echines are present on the convex surface and clearly visible using light microscopy, a part of ascospores lack ornamentation as well as equatorial crests and furrow.



***Aspergillus wyomingensis*, Mycobank**

1.6.9. ***Aspergillus udagawae*** (Kano *et al.*,2008)

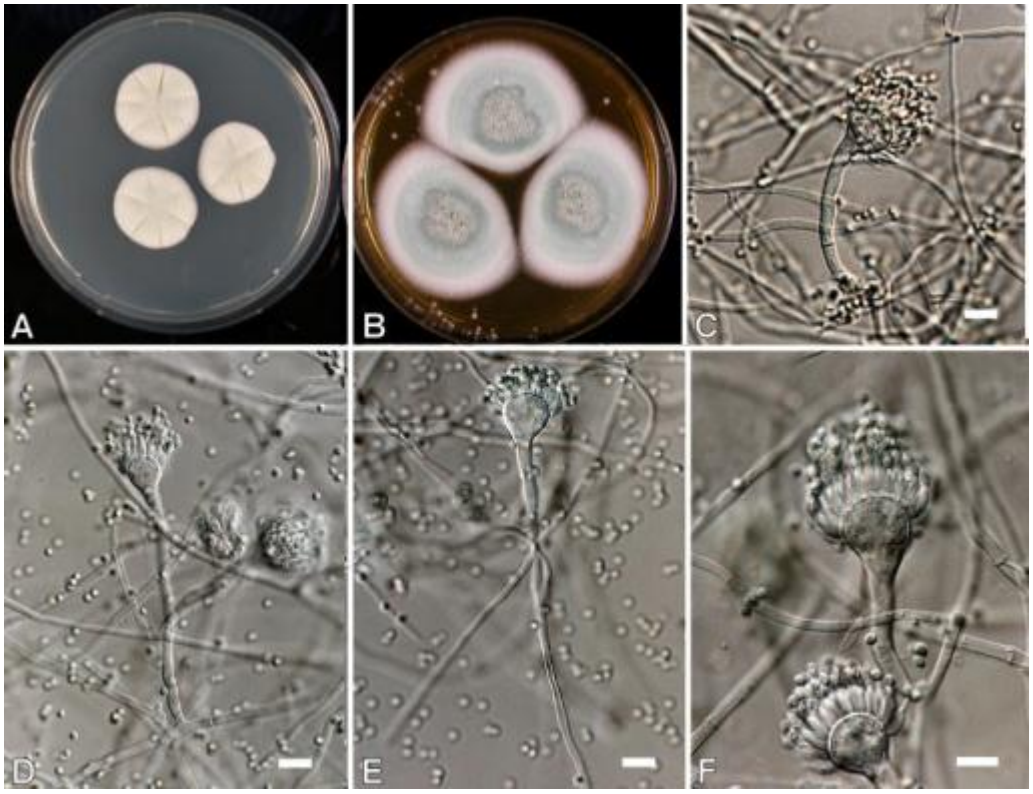
A. udagawae, a heterothallic fungus, has an anamorph that is morphologically indistinguishable from Aspergillus fumigatus. Because of the similarity in their conidial morphology, clinical isolates of N. udagawae are frequently identified as A. fumigatus on phenotypic characteristics alone. Growth of the A. udagawae strains is considerably slower than A. fumigatus at temperatures between 30°-37°C. While A, fumigatus grows at 55°C but fails to grow at l0°C, A. udagawae fails to grow at the temperatures >42Â°C but formed colony at 10°C.



A. udagawae clinical strains M29, M31, M34, and F41. (C to F) Growth characteristics on Czapek-Dox agar after 5 days at 37°C., Sugui et al., 2014

1.6.10. *Aspergillus viridinutans* Ducker & Thrower, Australian Journal of Botany 2 (3): 355 (1954)

Colonies on Czapek's agar attaining a diameter of 3.5 to 4.0 cm. in 14 days at 25°C, with centers raised, thinning in a marginal region about 1 cm. wide which is white flecked with green; conidial heads limited in number, in shades near sage green to pois green; reverse colorless or with the green shades showing through the thin white margins; no odor; no exudate. Colonies on malt agar reaching cm. in diameter in 14 days at 25°C, with surface velvety, slightly zonate, in colors near Niagara green, from abundant conidial heads except in a narrow white margin; reverse yellowish green to light brownish olive. Conidial heads mostly columnar, up to 50 µm by 30 µm when borne from the substratum, narrower when borne on branches from aerial hyphae; conidiophores arising from the substrate 50 µm or more in length, from trailing hyphae 20 to 35 µm, 3.3 to 4.4 µm in diameter, sinuous, thin walled, smooth and often septate; vesicles flask shaped to subglobose, 7.5 to 12 µm (average 9 µm) in width but ranging up to 15 µm wide, usually set at an angle on the conidiophore to present a "nodding" appearance but with upright heads often seen; sterigmata in a single series, borne on the uppermost surface of the vesicle only, comparatively few in number, 5.5 to 7.5 µm by 2.0 to 2.5 µm; conidia globose, described as smooth but delicately roughened, pale green, 2.0 to 2.8 µm.



Aspergillus viridinutans. A-B. Colonies 7 d 25 °C. A. CYA. B. MEA. C-F. Conidiophores. **Samson et al., 2007**

1.6.11. *Aspergillus fischeri* Wehmer, Centralbl. Bakteriol., 2 Abth.: 390 (1907)

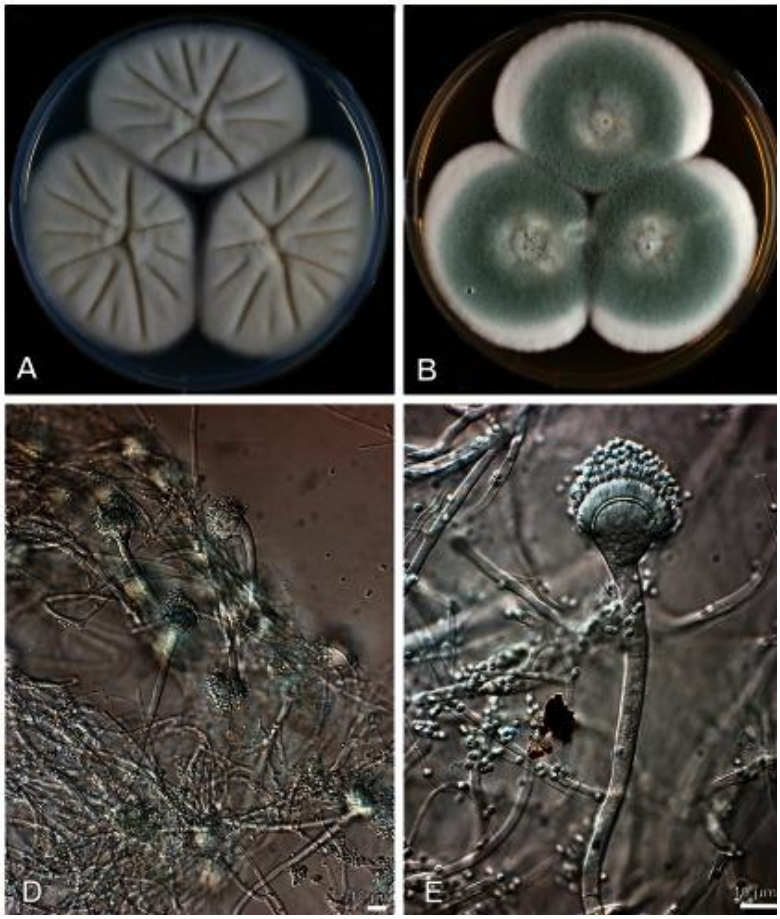
Colonies on Czapek's solution agar growing rapidly at room temperature (25±°C) and at 37°C, attaining diameters of to 6 cm. in 2 weeks; at the lower temperature characterized by abundant cleistothecia, with limited conidial heads in pale blue-green shades; at 37°C typically producing fewer cleistothecia and abundant conidial heads in shades near deep olive-gray borne from the substrate and from a thin aerial mycelium; no exudate; reverse colorless to flesh colored. Conidial heads columnar but with some spore chains divergent and with terminal areas somewhat expanded, up to 150 µm in length by 20 to 35 µm in diameter; conidiophores variable in length, in some strains exceeding 1 mm., in others mostly 300 to 500 µm by 4.0 to 7.0 µm in diameter; vesicles usually flask shaped, mostly 12 to 18 µm in diameter, faintly to definitely colored in gray-green shades, bearing sterigmata over the upper one-half to three-fourths; sterigmata in a single series, crowded, usually in pale to dull greenish shades, 5.5 to 7.0 µm by 2.0 to 2.5 µm; conidia typically subglobose but in different strains ranging from nearly globose to elliptical, delicately roughened, faintly pigmented, 2.0 to 2.5 µm in diameter when globose and up to 3.0 µm in long axis when elliptical. Cleistothecia borne singly or in small clusters within a loose hyphal envelope, typically globose, commonly up to 400 µm in diameter, with walls thin, fragile, consisting of 2 to 3 layers of irregularly flattened cells; asci maturing rap-idly and filling the cleistothecium within a few days, eight-spored, 8 to 10 µm by 10 to 12 µm breaking down quickly; ascospores biconvex, uncolored, usually 7.0 by 4.0 µm consisting of a central body 5.0 by 4.0 µm with two ruffled equatorial bands about 1.0

µm wide and convex surfaces bearing anastomosing ridges, which may also approach 1.0 µm

Aspergillus fischeri Mycota

1.6.13. Aspergillus felis Barrs, van Doorn, Varga & Samson, **sp. nov.**

Colonies grow rapidly on CYA agar attaining a diameter of 5.0 to 5.5 cm in 7 days at 25°C and on MEA reach 5.5 cm in diameter in 7 days at 25°C. On CYA the colony texture is mostly floccose; colonies are usually white and often sporulate poorly. On MEA colonies are more or less velvety with abundant greenish sporulation occurring after 5 to 7 days. In reverse, colonies are cream to light green. Conidiophores are uniseriate with greenish stipes and subclavate, "nodding" heads. Vesicles are subclavate with a diameter of 15–16.5 µm. Conidia are green, globose to subglobose, finely roughened and 1.5–2.5 µm in dimensions. Cleistothecia are white to creamish, 100–230 µm. Asci are globose, 8-spored, 12–16 µm in diameter. Ascospores are lenticular with two prominent equatorial crests and with short echinulate convex surfaces 5.0–7.0×3.5–5.0 µm



***Aspergillus felis*.** Colonies growing 7 days at 25°C on CYA (A) and MEA (B); Conidiophores and conidia**(**D, E) **Barrs et al., 2013**

1.7. Diagnosis:

1.7.1. **Serology.** Tests that can detect serum antibodies against *Aspergillus* species include Agar gel immunodiffusion (AGID), complement fixation, and ELISA techniques.

1.7.2. **Agar gel immunodiffusion (AGID)**,

- It is used for detection of antibodies to *A. fumigatus*, *A. niger*, and *A. flavus*.
- It is widely available through veterinary diagnostic laboratories
- It is probably the most commonly performed fungal serologic test at this time.
- Its sensitivity is only 67% in dogs with sinonasal aspergillosis

1.7.3. **Imaging studies.**

- Radiographs and CT may reveal lesions associated with diskospondylitis (collapsed disk spaces, proliferative bony changes adjacent to the intervertebral disk spaces, sclerosis) or lysis and destruction of long bones. Ultrasonography may reveal changes in affected organs
- Radiographs of the nasal cavity and frontal sinus can be diagnostically useful.,
- The patient must be anesthetized during the radiographic examination to permit proper positioning

- Lateral, ventrodorsal (both open- and closed-mouth) and rostrocaudal views should be obtained.
- Common radiographic changes associated with aspergillosis are areas of increased radiolucency, which suggest turbinate destruction.
- Opacification of the nasal cavities and frontal sinuses may also be noted.
- The diagnostic sensitivity of radiography is limited by superimposition of bony structures and the complexity of the nasal turbinates.
- Magnetic resonance imaging (MRI) and computed tomography (CT) are superior

1.7.4. Rhinoscopy and sinuscopy.

- Rhinoscopy allows visualization of the nasal cavity and guided collection of biopsy samples a
- It is routinely performed after imaging studies.
- The nasopharynx can be viewed by using a small retroflexed endoscope or a dental mirror with a rigid endoscope.
- Biopsy samples can be collected through the endoscope or by adjacent placement of a rigid device.

1.7.5. Cytology and histology.

- can provide direct evidence of fungal hyphae
- have high sensitivity
- findings include mucosal ulceration and inflammation, with a predominance of lymphocytes and plasma cells.
- Cytologic identification of *Aspergillus* species in urine, blood, synovial fluid, lymph node, bone or intervertebral disk material etc.

1.7.6. Isolation of fungi.

Most Aspergillus sp. grow relatively rapidly (typically within 48 hr) and on most microbiology media including both mycological media such as Sabouraud's agar and blood agars used for general bacteriological culture.Identification of cultures of most species Aspergillus is generally straightforward by colony and microscopic morphology.

1.7.7. Molecuar identification of aspergilli

Sequencing of genes, such as actin, calmodulin, ITS, rodlet A (rodA) and/or β-tubulin (βtub), has been used to distinguish *A. fumigatus* from related species. Multilocus sequence typing can alternatively be used for the identification of those related species, which is a strategy that also involves sequencing of several gene fragments. A few other techniques, such as random amplified polymorphic DNA, restriction fragment length polymorphisms and a new proposed microsphere-based Luminex assay, may enable molecular identification of *A. fumigatus* without sequencing.

1.8. Treatment

- **Topical antifungals.**

- Topical antifungal medications are regarded as the treatment of choice if the cribriform plate is intact.
- enilconazole and clotrimazole are more effective in the treatment of sinonasal aspergillosis than oral antifungal agents are.
- The topical azoles have poor solubility and minimal intestinal absorption and are fungicidal (rather than fungistatic) at higher concentrations.
- enilconazole instillation twice a day for one or two weeks. Or
- one infusion via both nares of either clotrimazole or enilconazole, under general anesthesia.
- topical clotrimazole was shown to resolve clinical disease in 65% of dogs after one treatment and in 87% of dogs after two treatments.
- topical enilconazole in dogs resolved 57% of clinical disease after one treatment and 94% after one to three treatments.

- **Systemic antifungals.**
  - systemic therapy is recommended in case of fungal invasion of extranasal structures.
  - Several azole drugs have been used to treat dogs with sinonasal aspergillosis, but the success rates are moderate at best.
  - Successful treatment of a dog with sinonasal aspergillosis was reported using itraconazole alone
  - Anorexia, vomiting, and hepatotoxicosis have been reported with long-term use of azoles

1.9. Reports on aspergillosis in dogs:

1.9.1. Nasal aspergillosis

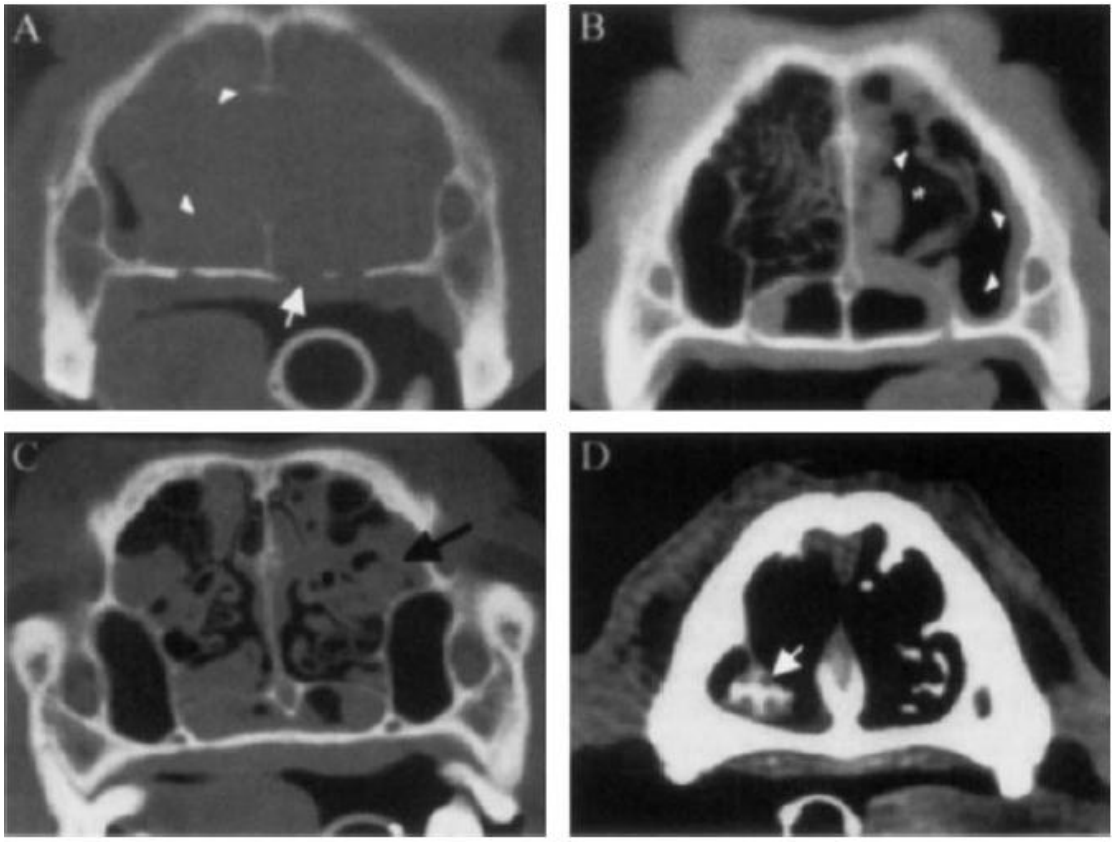
Khan *et al.* (1984) considered ELISA to be a less reliable method than counter immune electrophoresis for the diagnosis of **nasal aspergillosis** in the dog. False-positive or false-negative results were recorded for anti-***A. fumigatus*** IgG in nine animals with aspergillosis and in 27 disease-free dogs although this problem could be reduced with careful selection of antigen.

Mortellaro *et al.* (1989) reported 29 cases of **nasal aspergillosis** out of 150 dogs with nasal discharge in dogs based on cultural, serologic, radiologic, endoscopic and histopathologic studies. In all of the 29 cases the thermotolerant, rhinotropic ***Aspergillus fumigatus*** was cultured from the nasal lesions. The fungal infection mostly occurred in male **German shephard** dogs, the most common breed of companion dogs, living in apartments.

Codner *et al.* (1993) evaluated **computed tomography** as a non-invasive technique for the diagnosis of chronic nasal disease in dogs. Computed tomographic images, radiographs, and histopathologic findings were compared in 11 dogs with chronic nasal disease. Definitive diagnosis was made following traumatic nasal flush, exploratory surgery, or necropsy. The study included 8 dogs with intranasal tumors, 2 dogs with bacterial rhinitis (Pasteurella sp), and 1dog with **mycotic rhinitis** (Aspergillus sp). Computed tomography was superior to radiography in defining the extent of the disease process and in differentiating infectious rhinitis from nasal

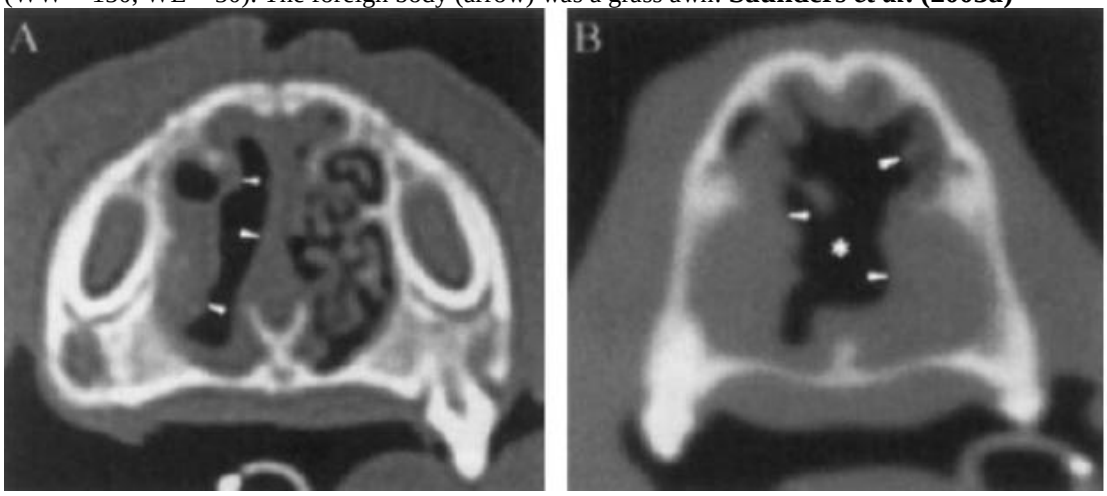
neoplasms. It defined lesions in the palate, nasopharyngeal meatus, maxillary sinus, caudal ethmoturbinates, and periorbital tissues that were difficult to demonstrate by use of conventional radiography. Tumors appeared as space-occupying lesions that obliterated the turbinates, caused deviation of the nasal septum, and eroded bone. Rhinitis appeared as a cavitating lesion that spared the paranasal sinuses, thickened and distorted the turbinates, and widened the meatus.

Saunders *et al*. (2003a) reviewed computed tomographic (CT) studies of 80 dogs with chronic nasal disease (nasal neoplasia (n = 19), **nasal aspergillosis** (n = 46), nonspecific rhinitis (n = 11), and foreign body rhinitis (n = 4)) retrospectively by two independent observers. Each observer filled out a custom-designed list to record his or her interpretation of the CT signs and selected a diagnosis. Accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the diagnosis of each disease. The agreement between observers was evaluated. The CT signs corresponded to those previously described in the literature. CT had an accuracy greater than 90% for each observer in all disease processes. The sensitivity, specificity, PPV, and NPV were greater than 80% in all dogs with the exception of the PPV of foreign body rhinitis (80% for observer A and 44% for observer B). There was a substantial, to almost perfect, agreement between the two observers regarding the CT signs and diagnosis. This study indicated a high accuracy of CT for diagnosis of dogs with chronic nasal disease. The differentiation between nasal aspergillosisrestricted to the nasal passages and foreign body rhinitis may be difficult when the foreign body is not visible.



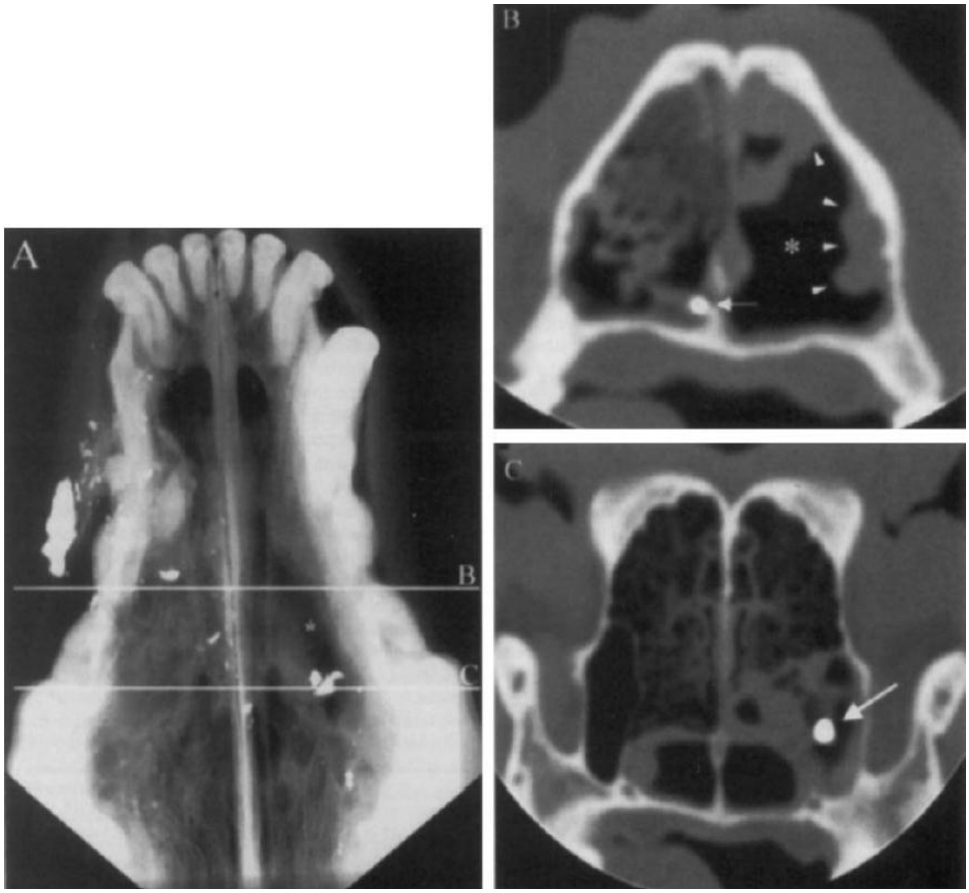
Transverse CT images of the nasal cavities from four dogs. Classification of the process as mass-like for nasal neoplasia (A), cavitated-like for nasal aspergillosis (B), nondestructive for nonspecific rhinitis (C) and, when a foreign body could be visualized as foreign body rhinitis (D) permitted acorrect

diagnosis to be made in 93-95% of the dogs. **(A)** Eleven-year-old Bobtail with a nasal adenocarcinoma (window width (WW) = 3500, window level (WL) = 500). Both nasal cavities are completely tilled with a soft tissue density. Some deformed turbinates are visible (arrowheads), There is also lysis of the palatine bone (arrow). (B) Five-year-old Golden Retriever with nasal aspergillosis (WW = 3500, WL = 500). Severe turbinate destruction creates an increased air space in the left nasal cavity (asterisk). There is also mucosal thickening (arrowheads). (C) Four-year-old German Shepherd Dog with a diagnosis of nonspecific rhinitis (WW = 3500, WL = 500). There is a severe bilateral fluid/epithelial edema (arrow). The integrity of the turbinates is conserved. (D) Eight-year-old Poodle with a foreign body rhinitis (WW = 150, WL = 50). The foreign body (arrow) was a grass awn. **Saunders *et al*. (2003a)**

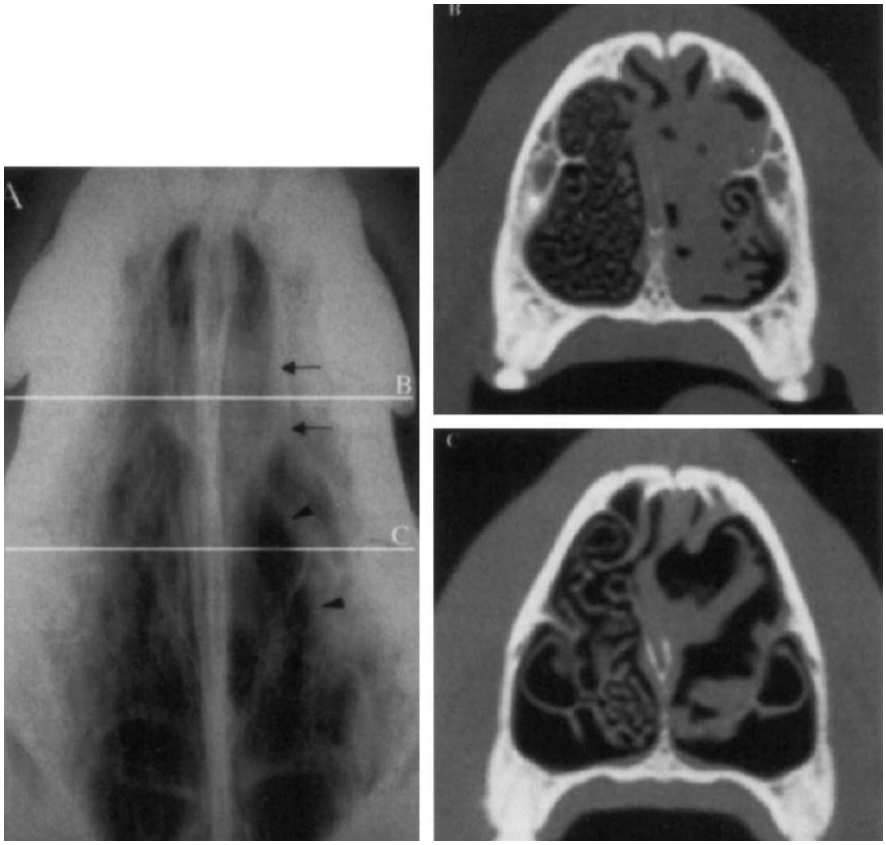


Transverse CT images (window width = 3500; window level = 500) showing the similarity of CT features in nasal aspergillosis and foreign body rhinitis. **(A)** Six-month-old Cairn Terrier with foreign body rhinitis. There is unilateral turbinate destruction and mucosal thickening (arrowheads) in the right nasal cavity. The foreign body (small plant part) could not be visualized. (B) Six-year-old Teckel with nasal aspergillosis. There is bilateral turbinate destruction (asterisk) and mucosal thickening (arrowheads). **Saunders *et al*. (2003a)**

Saunders *et al*. (2003b) performed a study to compare the radiographic and computed tomographic (CT) findings and to evaluate the sensitivity of radiography and CT for diagnosis of **nasal aspergillosis** in dogs, the radiographic and CT studies of 48 dogs with chronic nasal disease were reviewed separately. The radiographic and CT findings were recorded, and a diagnosis was made. The results obtained in the dogs with nasal aspergillosis (n = 25) were used. Based on definite aspergillosis as diagnosis, CT had a sensitivity of 88% and radiography of 72%. Considering definite and probable aspergillosis as equivalent, CT had a sensitivity of 92% and radiography of 84%. The sensitivity was higher in dogs with lesions affecting the entire nasal cavity and frontal sinus on at least one side (n = 20) with a sensitivity of 100% for CT and 90-95% for radiography than in dogs with lesions restricted to the nasal cavities (n = 5) where CT had a sensitivity of 60-80% and radiography of 0-40%. CT was superior to radiography for evaluation of the nasal cavities (mucosal thickening along the nasal bones, surrounding bone hyperostosis/lysis), frontal sinuses (mucosal thickening along the frontal bone, fluid/soft tissue, frontal bone hyperostosis/lysis), and differentiation between a cavitated-like or a mass-like process. This study suggests that CT is more sensitive than radiography for diagnosis of nasal aspergillosis in the dog because of a better demonstration of some changes suggestive of nasal aspergillosis. A diagnosis of a nasal aspergillosis restricted to the nasal cavities or associated with an FB is challenging, even with the use of CT.



Radiographic and CT examinations of the nasal cavities of a 5-year-old Labrador retriever. **A)** DV (intra-oral) projection showing an area of lucency (asterisk) extending from the canine tooth to the maxillary recess in the left nasal cavity. Multiple metallic foreign bodies (FB) are visible as well as fragments of the right canine tooth. The radiographic diagnosis was probable FB rhinitis. B) Transverse CT image (window width (WW) = 3500, window level (WL) = 500) at the level of the rostra1 aspect of the third premolary tooth. There is a complete turbinate destruction (asterisk) in the left nahal cavity. The mucosa is thickened (arrowheads) along this destructive area. One rounded metallic FB is visible in the right nasal cavity against the vomer hone (arrow). C) Transverse CT image (WW = 3500, WL = 500) at the level of the maxillary recesses. There is a rounded metallic FB (arrow) located in the left nasal cavity at the level of orbital lamina of the maxillary recess and surrounded by abnormal soft tissue. CT diagnosis was definite nasal aspergillosis associated with an intranasal FB. **Saunders *et al.* (2003b)**

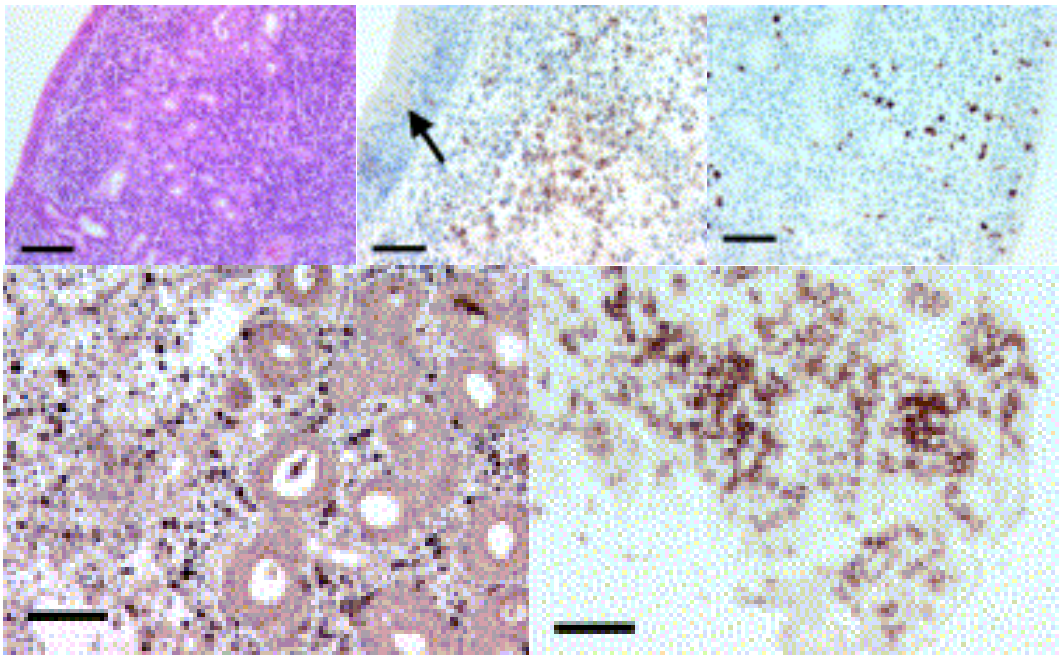


Radiographic and CT examinations of the nasal cavities of a 3-year-old Bull Terrier. A) DV (intra-oral) radiographic projection. There is an increased opacity in the rostral third (arrows) and an increased lucency (arrowheads) in the middle third on the left nasal cavity. The radiographic diagnosis was probable nasal aspergillosis. B) Transverse CT image (window width [WW] = 3,500, window level [WL] = 500) at the level of the first premolar. There is abnormal fluid/soft tissue, thickening of the mucosa, and areas of turbinate destruction (difficult to quantify) in the left nasal cavity. **A** small amount of abnormal soft tissue is present in the right nasal cavity. C) Transverse CT image (WW = 3,500, WL = 500) at the level of the third premolar. There is turbinate destruction and thickening of the mucosa along the nasal cavity and resting turbinates. **A** CT diagnosis of probable nasal aspergillosis was made. **Saunders *et al*. (2003b)**

Saunders *et al.* (2004) conducted a study to determine radiographic, magnetic resonance imaging (MRI), computed tomography (CT), and rhinoscopic features of **nasal aspergillosis** in 15 client-owned dogs. All dogs had clinical signs of chronic nasal disease; the diagnosis of nasal aspergillosis was made on the basis of positive results for at least 2 diagnostic tests (serology, cytology, histology, or fungal culture) and detection of typical intrasinusal and intranasal fungal colonies and turbinate destruction via rhinoscopy. Radiography, MRI, and CT were performed under general anesthesia. Rhinoscopy was repeated to evaluate lesions and initiate treatment. Findings of radiography, MRI, CT, and rhinoscopy were compared. MRI and CT revealed lesions suggestive of nasal aspergillosis more frequently than did radiography. Computed tomography was the best technique for detection of cortical bone lesions; the nature of abnormal soft tissue, however, could not be identified. Magnetic resonance imaging allowed evaluation of lesions of the frontal bone and was especially useful for differentiating between a thickened mucosa and secretions or fungal colonies; however, fungal colonies could not be differentiated from secretions. Rhinoscopy allowed identification of the nature of intranasal and

intrasinusal soft tissue but was not as useful as CT and MRI for defining the extent of lesions and provided no information regarding bone lesions.

Peeters *et al.* (2005) used histochemistry and immunohistochemistry to characterize the phenotype and distribution of leucocytes in the distal nasal mucosa of 15 dogs with **nasal aspergillosis**. The most consistent histopathological finding was a severe, predominantly lymphoplasmacytic, inflammatory infiltration of the lamina propria. Fungal hyphae were not observed to invade the mucosa but were found at the mucosal surface and within material collected from the nasal cavity. The main immunohistochemical findings were (1) a predominance of IgG(+) plasma cells over IgA(+) and IgM(+) plasma cells, (2) significant numbers of macrophages and dendritic cells expressing MHC class II molecules, (3) macrophages and neutrophils expressing L1 antigen and (4) a mixture of CD4(+) and CD8(+) T cells. These findings were consistent with a dominant Th1-regulated cell-mediated immune response. The nature of the inflammatory infiltrate and the lack of invasiveness of the mucosa by the fungus, together with the clinical course of the disease and the apparent immunocompetence of the affected dogs, suggest that canine nasal aspergillosis resembles the chronic erosive non-invasive fungal sinusitis described in human patients.

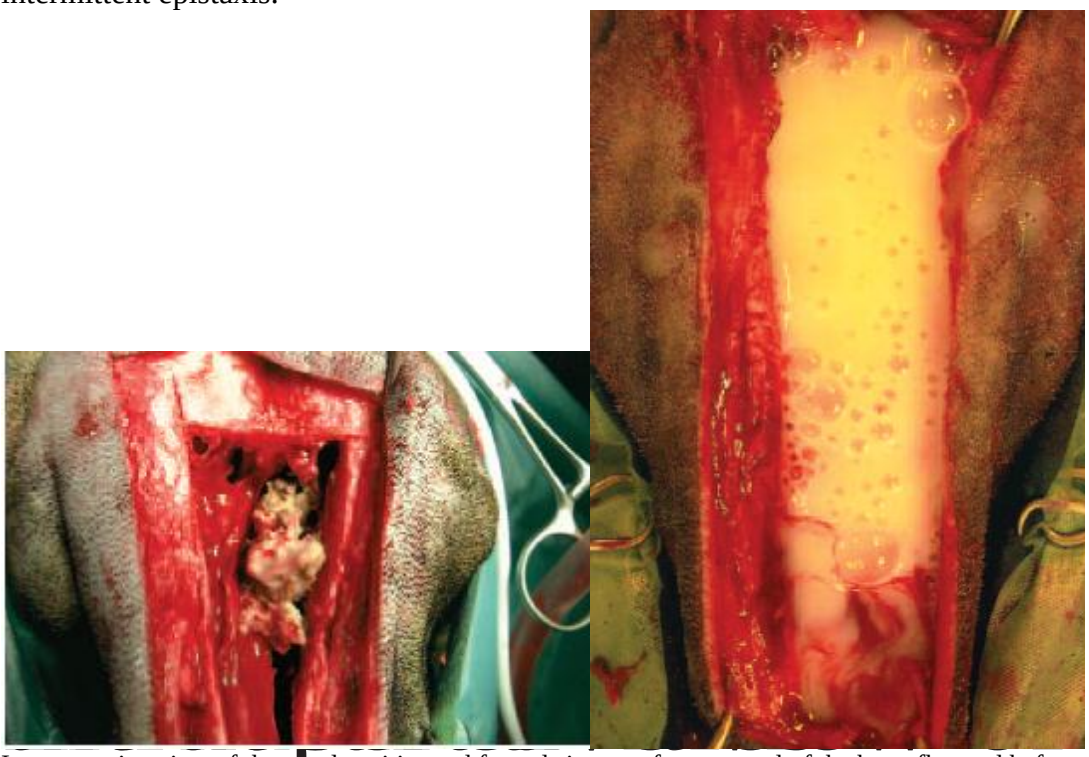


Peeters *et al.* (2005)

Benitah (2006) reported that chronic nasal discharge is a common clinical sign of disease in dogs. Canine sinonasal aspergillosis is a relatively common disease in dogs. The three hallmarks of **canine nasal aspergillosis** are a profuse mucoid to hemorrhagic chronic nasal discharge that may alternate with periods of epistaxis, ulceration of the external nares with crusting, and pain or discomfort in the facial region. Diagnostic imaging (preferably computed tomography, CT) of the nasal cavity and paranasal sinuses is an important component of the evaluation of dogs with signs of nasal disease. Rhinoscopy is an important part of both the diagnosis and the therapy for nasal aspergillosis. Therapeutic recommendations for

sinonasalaspergillosis have included surgery and the use of several systemic and topical antifungal drugs.

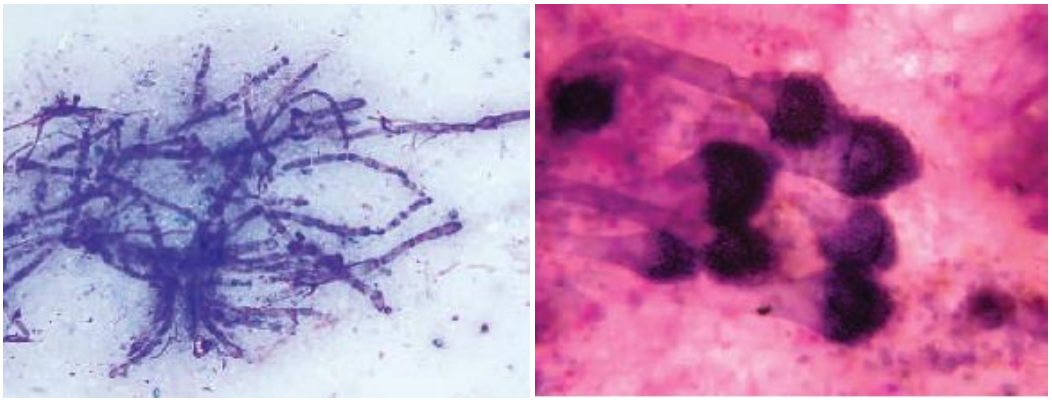
Claeys *et al, (2006)* evaluated the effectiveness of rhinotomy and surgical debridement associated with topical administration of 2 per cent enilconazole and oral itraconazole in dogs with severe or recurrent **sinonasal aspergillosis**. A standard rhinotomy was performed on seven dogs. In the initial study, the bone flap was left attached cranially and replaced at the end of the procedure. In the main study group, the bone flap was discarded. Nasal passages were debrided and irrigated with enilconazole solution for one hour. Oral itraconazole was administered to four dogs for one month postoperatively. Follow-up rhinoscopy was performed in all dogs. All three dogs in the initial study had recurrence of the disease and two dogs had a second surgery to remove the flap. The main study group included four dogs in which the flap was initially removed, and the two dogs from the initial study that required a second surgery. At follow-up rhinoscopy, five dogs were free of aspergillus but had bacterial or inflammatory rhinitis and one dog had a small aspergilloma but was subsequently asymptomatic. Telephone follow-up revealed that four dogs were asymptomatic, one dog had intermittent sneezing and serous nasal discharge, and one dog had intermittent epistaxis.



Intraoperative view of the nasal cavities and frontal sinuses after removal of the bone flap and before debridement. Note the heavy fungal plaques in the left frontal sinus. ***Claeys* et al, (2006)**

De Lorenzi *et al.* (2006) compared the efficacy and **diagnostic** value of four different sample collection techniques for cytological identification of **nasal aspergillosis-penicilliosis** in dogs. Fifteen dogs with a history of persistent nasal discharge and clinical and radiographic findings suggestive of aspergillosis were evaluated using four different cytological sampling techniques. These were a direct smear from the nasal discharge, blind swab collection under general anaesthesia, brushing from suspect lesions under direct endoscopic visualisation and a squash technique of mucosal biopsies from suspect lesions obtained under direct endoscopic visualisation.

Direct smear collection and blind swab collection detected fungal hyphae in 13.3 and 20 per cent of examined cases, respectively; brush samples detected fungal hyphae in 93.3 per cent and fungal spores in the 45 per cent of examined cases and squash samples detected fungal hyphae in 100 per cent and fungal spores in 36 per cent of examined cases. This study confirmed the high accuracy of cytology samples in the diagnosis of nasal aspergillosis-penicilliosis when collected under direct endoscopic visualisation and showed the poor value of samples that were collected by blind swabs or prepared from samples of nasal discharge.



Squash preparation from an endonasal biopsy (case 5). Note the large (4 to 6 *m*m in diameter), dark blue septate hyphae with parallel sides that branch dichotomously at a 45º angle. Giemsa, _650
Squash preparation from an endonasal biopsy (case 11). Conidial heads are only rarely seen in cytological samples from fungal rhinitis. Aspergillus fumigatus conidial heads can be seen here. Giemsa, _1000, **De Lorenzi *et al.* (2006)**

Johnson *et al*. (2006) reviewed medical records of 46 dogs with **nasal aspergillosis** for information on computed tomographic findings; rhinoscopic findings, including whether fungal plaques were seen in the nasal cavity; results of frontal sinus trephination and sinuscopy, including whether fungal plaques were seen in the frontal sinus; and results of histologic examination of biopsy specimens. In 38 (83%) dogs, fungal plaques were seen in the nasal cavity during rhinoscopy, whereas in the remaining 8 (17%), fungal plaques were not seen in the nasal cavity but were seen in the frontal sinus. Duration of clinical signs, proportions of dogs in which the referring veterinarian had performed a nasal examination prior to referral, proportions of dogs with computed tomographic evidence of nasal cavity cavitation or sinus involvement, and proportions of dogs with rhinoscopic evidence of destructive rhinitis were not significantly different between dogs with nasal fungal plaques and dogs with fungal plaques only in the frontal sinus. Results confirm that frontal sinus involvement is common in dogs with nasal aspergillosis and suggest that frontal sinus trephination and sinuscopy may aid in the diagnosis of aspergillosis in dogs, particularly dogs with rhinoscopic evidence of destructive rhinitis and computed tomographic evidence of sinus involvement that lack detectable fungal plaques in the nasal cavity

Peeters and Clercx (2007) stated that **sinonasal aspergillosis** is a frequent cause of nasal discharge that occurs in otherwise healthy, young to middle-aged dogs. A local immune dysfunction is suspected in affected animals, and the role of increased interleukin-10 mRNA expression in the nasal mucosa of affected dogs is currently

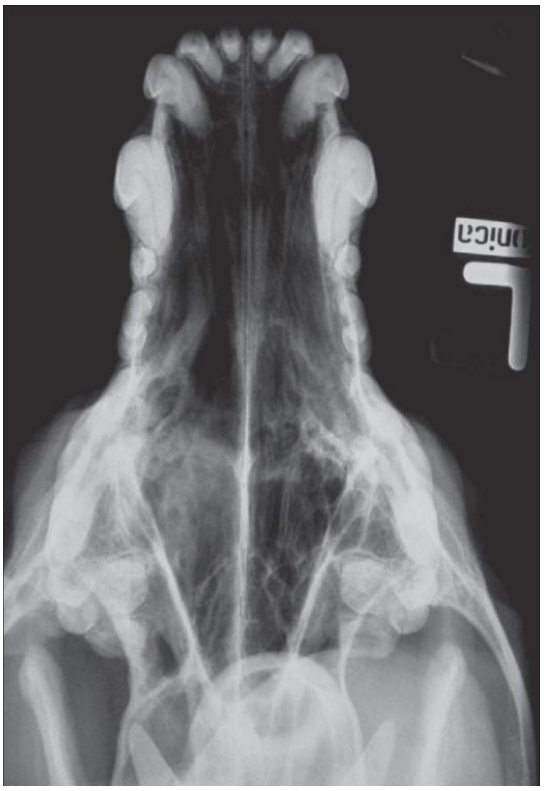
under investigation. Despite recent advances in imaging techniques, the "gold standard" for diagnosing the disease is direct visualization of fungal plaques during endoscopy or observation of fungal elements on cytology or histopathologic examination. **Treatment** can be challenging; however, the use of topical enilconazole or clotrimazole through noninvasive techniques has increased the success of treatment and decreased the morbidity and duration of hospitalization.

Pomrantz *et al.* (2007) carried out a study to compare the sensitivity and specificity of serologic evaluation and fungal culture of tissue for **diagnosis** of **nasal aspergillosis** in 58 dogs with nasal discharge and 26 healthy dogs. Dogs with nasal discharge were anesthetized and underwent computed tomography and rhinoscopy; nasal tissues were collected for histologic examination and fungal culture. Sera were assessed for antibodies against Aspergillus spp (healthy dog sera were used as negative control specimens). Nasal aspergillosis was diagnosed in dogs that had at least 2 of the following findings: computed tomographic characteristics consistent with aspergillosis, fungal plaques detected during rhinoscopy, and histologically detectable fungal hyphae in nasal tissue. Histologic characteristics of malignancy were diagnostic for neoplasia. Without evidence of neoplasia or fungal disease, non-fungal rhinitis was diagnosed. Among the 58 dogs, 21 had nasal aspergillosis, 25 had non-fungal rhinitis, and 12 had nasal neoplasia. Fourteen aspergillosis-affected dogs and 1 dog with non-fungal rhinitis had serum antibodies against Aspergillus spp. Fungal culture results were positive for Aspergillus spp only for 17 dogs with aspergillosis. With regard to aspergillosis diagnosis, sensitivity, specificity, and positive and negative predictive values were 67%, 98%, 93%, and 84%, respectively, for serum anti-Aspergillus antibody determination and 81%, 100%, 100%, and 90%, respectively, for fungal culture. Results suggest that seropositivity for Aspergillus spp and identification of Aspergillus spp in cultures of nasal tissue are highly suggestive of nasal aspergillosis in dogs; however, negative test results do not rule out nasal aspergillosis.

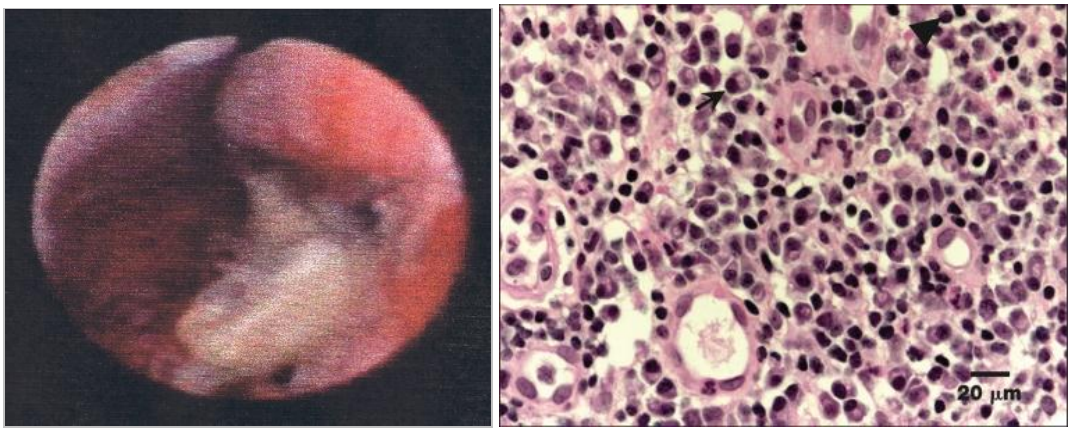
Schuller and Clercx (2007) evaluated long-term outcomes (mean 38+/-17 months) in 27 dogs with **sinonasal aspergillosis** after successful medical **treatment** using intranasal infusions of 1% or 2% enilconazole (1%, n=15; 2%, n=12). Long-term outcomes with both treatment protocols were good, with half of the dogs being asymptomatic throughout the follow-up period. The remaining dogs showed mild clinical signs compatible with chronic rhinitis/sinusitis. These clinical signs were interpreted as chronic lymphoplasmacytic rhinitis/sinusitis and episodes of bacterial rather than fungal infection. Three dogs had confirmed reinfection or relapse 2 to 36 months after clinical resolution.

Meler *et al.* (2008) conducted a retrospective study to determine the percentage of cases for which the etiology was determined in hospital population. Medical records from 80 dogs met the criteria of inclusion in the study. Non-specific **rhinitis** was identified in 23.7% of cases. Other diagnoses were neoplasia (15.0%), fungal infection (nasal aspergillosis) (8.7%), cleft palate (8.7%), periodontal disease (4.0%), parasites (1.3%), foreign body (1.3%), and primary bacterial disease (1.3%). A definitive diagnosis could not be established in 36.3% of cases. Dogs with neoplastic and mycotic diseases often presented with severe radiographic and rhinoscopic lesions. Despite a systematic approach, numerous cases went undiagnosed. The use of

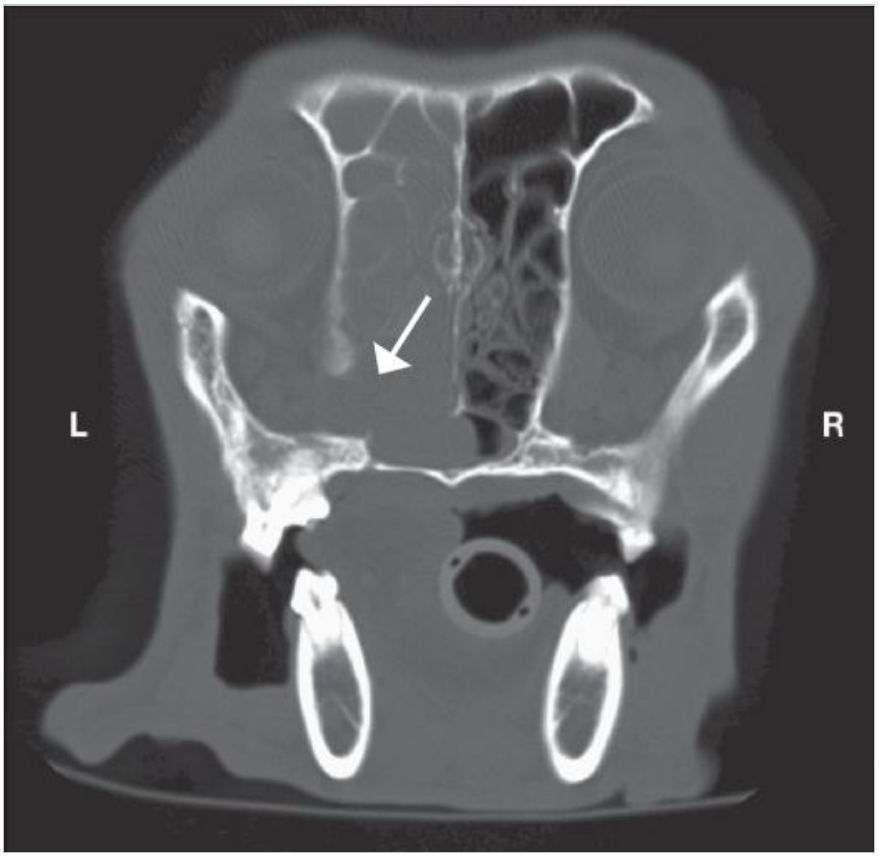
advanced imaging should increase our ability to obtain an etiologic diagnosis in canine nasal disease.



Dorsoventral intraoral radiograph of a dog with nasal adenocarcinoma. This intraoral nasal radiograph shows a unilateral, localized, increased density associated with the disappearance of the ethmoid turbinate pattern in the caudal portion of the right nasal cavity. The dog presented for a unilateral right-sided mucopurulent nasal discharge. Nasal adenocarcinoma was confirmed by biopsy **Meler *et al.* (2008)**

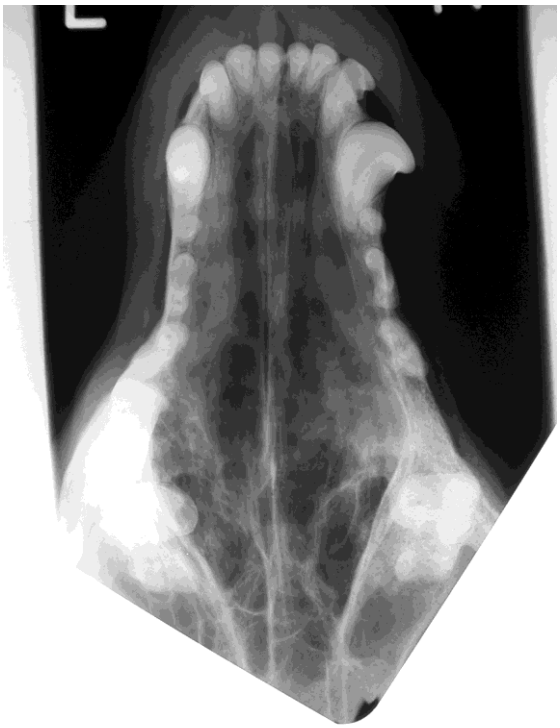


Left:Endoscopic examination of a dog with nasal aspergillosis. Endoscopic view of the caudal portion of the ventral meatus of the nasal cavity of a dog with nasal aspergillosis. Greyish plaques characteristic of aspergillosis are visible. Note the erosion of the mucous membranes and the atrophy of nasal conchae.Right: Photomicrograph of the nasal mucosa of a dog with lymphoplasmacytic rhinitis. Endoscopic biopsies of this dog showed marked lymphocytic (black arrowhead) and plasmacytic (black arrow) infiltration of the nasal mucosa. No other etiology could be found in this dog to explain the chronic bilateral rhinitis. The dog was diagnosed with lymphoplasmacytic rhinitis. Hematoxylin-Eosin-Safran. Bar = 20 µm. **Meler *et al.* (2008)**

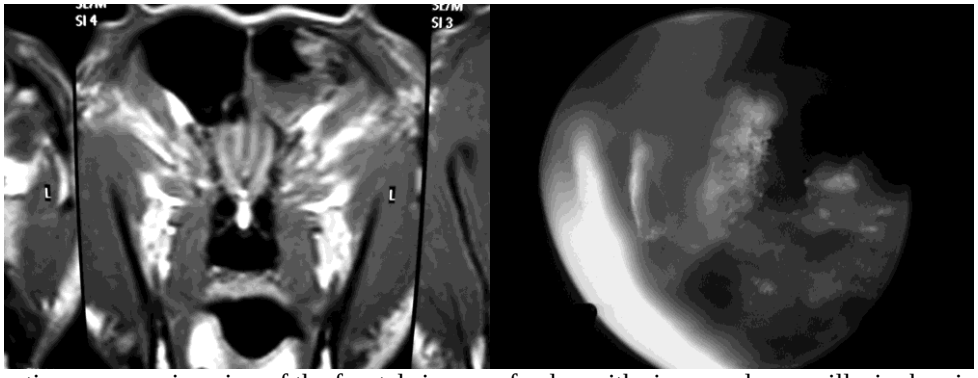


Computed tomography of a dog with nasal adenocarcinoma. A caudal transverse view shows a soft tissue density invading the left nasal cavity. The nasal septum is eroded and invasion of the ventral portion of the left orbit through the frontal bone and nasopharyngeal area is observed (white arrow). A biopsy confirmed the presence of a nasal adenocarcinoma. **Meler *et al.* (2008)**

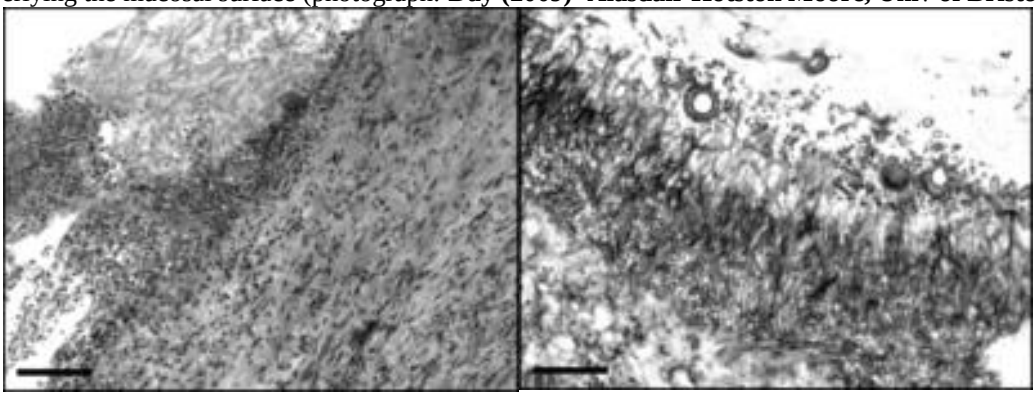
Day (2009) stated that canine **sino-nasal** aspergillosis (SNA) is characterized by the formation of a superficial mucosal fungal plaque within the nasal cavity and/or frontal sinus of systemically healthy dogs. The most common causative agent is Aspergillus fumigatus. The fungus does not invade beneath the level of mucosal epithelium but incites a severe chronic inflammatory response that leads to local destruction of nasal bone. These clinicopathological features are equivalent to those of human chronic erosive non-invasive fungal sinusitis. The clinical diagnosis of canine SNA relies on multiple modalities but local instillation of anti-fungal agents is an effective therapy with high cure-rate. Recent studies have investigated the immunopathogenesis of canine SNA. The mucosal inflammatory infiltrate involves a mixture of CD4+ and CD8+ T lymphocytes, IgG+ plasma cells and activated macrophages and dendritic cells expressing class II molecules of the major histocompatibility complex. There is active recruitment of blood monocytes and neutrophils. Real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis of mucosal tissue samples has revealed up-regulation of Th1 (IL-12, IL-18 and IFN-gamma), Th17-related (IL-23) and pro-inflammatory (IL-6, TNF-alpha) cytokine mRNA with evidence of expression of genes encoding monocyte chemoattractant proteins 1-4. Additionally, there is significant transcription of the IL-10 gene consistent with local immunosuppression that prevents secondary immune-mediated sequelae whilst permitting chronicity of the infection. The source of this IL-10 may be a T regulatory population or a Th1 population that switches phenotype during the course of disease. This understanding of the immunopathogenesis of canine SNA establishes this disorder as a valuable model for the equivalent human pathology.



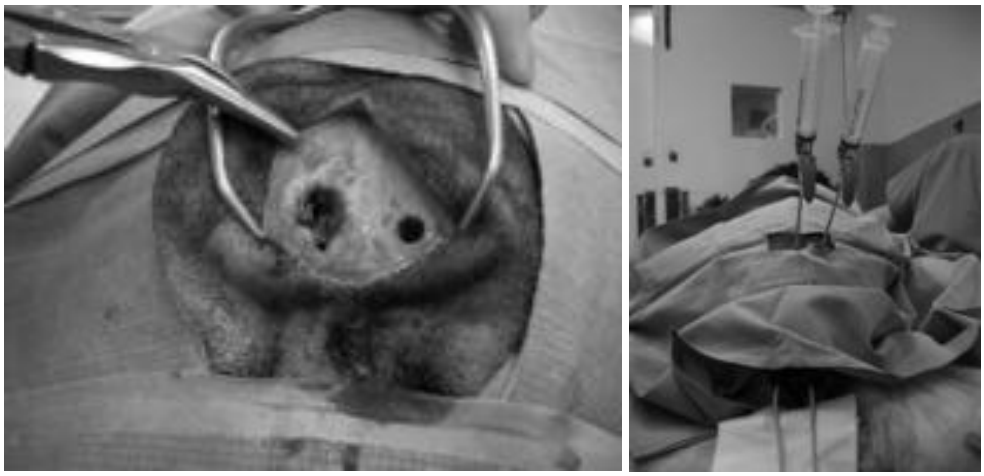
Skull radiograph of a dog with sino-nasal aspergillosis showing destructive bony lesions (photograph courtesy **Day (2009) ,Alasdair Hotston Moore, University of Bristol**).



Magnetic resonance imaging of the frontal sinuses of a dog with sino-nasal aspergillosis showing the presence of a fungal plaque overlying the mucosal surface. Rhinoscopic image of upper respiratory tract mucosa of a dog with sino-nasal aspergillosis. A 'fluffy' white fungal plaque is readily observed overlying the mucosal surface (photograph: **Day (2009) Alasdair Hotston Moore, Univ of Bristol**).

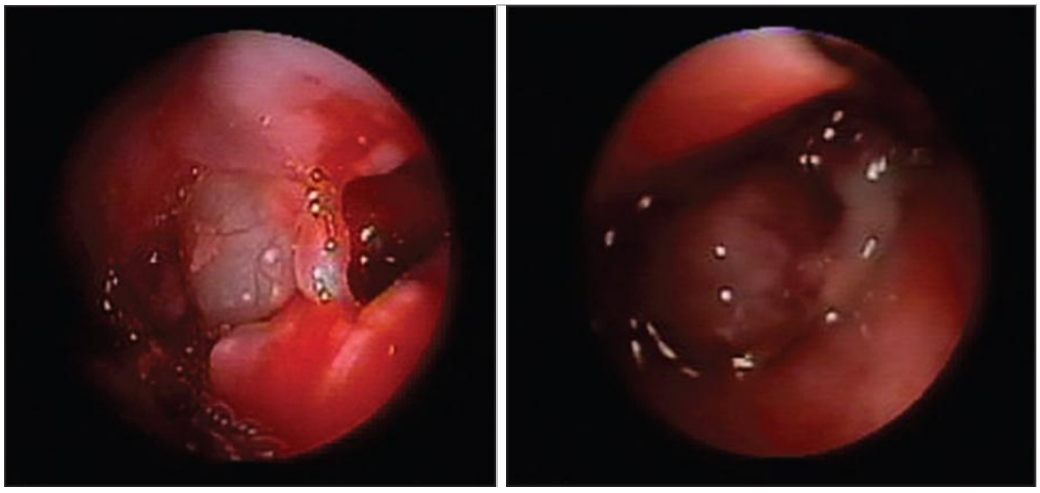


Biopsy taken from a fungal plaque in the right frontal sinus of a 4-year-old, male Jack Russell Terrier with sino-nasal aspergillosis. A mycelial mat overlies a central zone of necrosis, haemorrhage and fibrinocellular exudation, and deep to this is a bed of fibrovascular granulation tissue. Biopsy taken from a fungal plaque in a dog with sino-nasal aspergillosis. Grocott hexamine silver**, Day (2009)**
Alasdair Hotston Moore, University of Bristol



Trephination of the frontal sinuses of a dog with sino-nasal aspergillosis. Note the fungal debris in the larger trephine hole**,** Instillation of clotrimazole into the frontal sinuses of a dog with sino-nasal aspergillosis. The dog is anaesthetized and placed in sternal recumbency. The two large syringes have been used to inject infusate into the sinuses via the rigid catheters. The two Foley catheters at the base of the image exit the nares. These Foley catheters have inflated balloons that occlude the nares and minimize leakage of infusate cranially. **Day (2009) Alasdair Hotston Moore, Univ of Bristol**

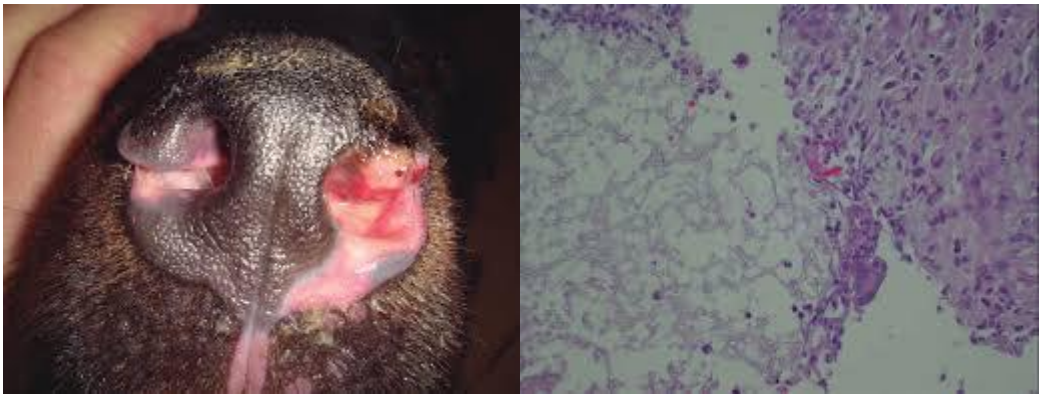
Greci *et al.* (2009) diagnosed 3 dogs (a 7-year-old female German shepherd, a 3-year-old male Rottweiler and a 6-year-old male German shepherd) with aspergillosis that developed **sinonasal tumors** several months after successful treatment with topical clotrimazole solution. Chronic rhinosinusitis was also detected in all cases prior to diagnosis of sinonasal tumors. The inflammatory response to Aspergillus, clotrimazole treatment, and chronic inflammation after treatment were discussed as possible neoplastic promoting factors.



Presence of blood, edema of the mucosa and mucosal blebs at the opening of the left frontal sinus. Endoscopic findings at 30 months post treatment (case 3): presence of a whitish vascularized new growth of tissue occluding the left nasal cavity. **Greci *et al.* (2009)**

Ferreira *et al*. (2011) reported an 18-months old, male, Rottweiler breed dog with purulent **nasal** discharge, variably bloody, and sneezing of approximately 6 months duration. During this period, the dog was treated with various antibiotics with no success and lost 10 kg of corporal mass. The alterations found in the physical exam were bilateral sanguine-purulent nasal discharge, depigmentation of nose and paranasal region, as well as subnutrition. The dog was anesthetized and sinus and chest x-rays were performed (latero-lateral and ventrodorsal positions). In the radiographic analysis, it was verified the lessening of radiolucency on the left nostril, indicating the destruction of the nasal concha. The chest radiographies did not show alterations. A rhinoscopy was carried out showing destruction in the endoturbinate, purulent discharge and presence of a dark color mass in the frontal sinus, which was collected for histopathological and microbiological culture exams. Histopathologic examination revealed the presence of hyaline, branching septate hyphae, consistent with Aspergillus spp. and inflammatory cells. Culture identified ***Aspergillus fumigatus***. Bacteriological culture was negative. Antibodies to Aspergillus fumigatus were detected by counter electrosyneresis. The haemogram showed lymphocytosis and monocytosis. The dog was treated with itraconazole (5 mg/kg of body weight, orally, twice a day for 30 days). After this period, nasal discharge decreased and a good repigmentation was observed with the dog showing improvement of his appetite and energy level. Discussion: The presence of antibodies to Aspergillus spp. does not always confirm canine nasal aspergillosis. Serological tests can yield 5% to 15% false positive results in dogs. Therefore, it is necessary to perform complementary exams such as radiography, rhinoscopy, histopathology and fungal culture in order to confirm the diagnosis. For many years, aspergillosis was considered as an incurable

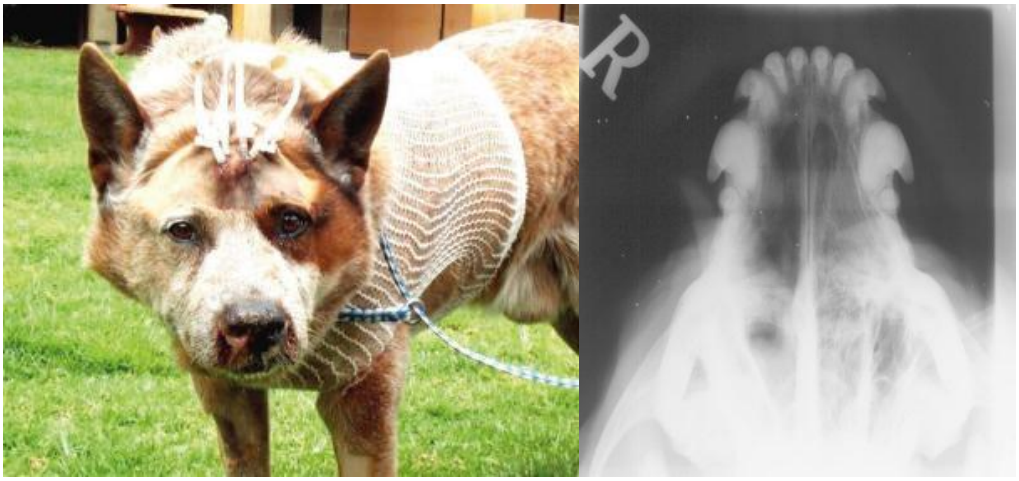
chronic rhinitis characterized by the turbinate destruction, nasal discharge and intermittent epistaxis.



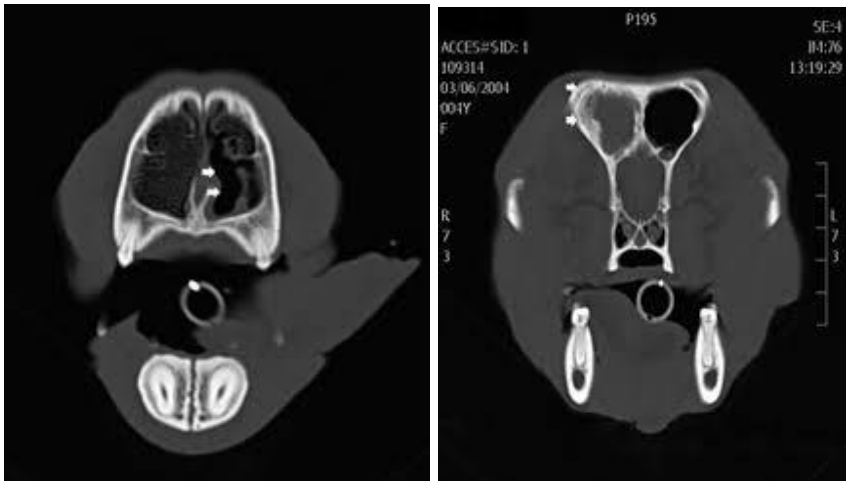
18-months old, male, Rottweiler breed dog with purulent nasal discharge, depigmentation of nose and skin. histopathological picture of a dark color mass in the frontal sinus revealing the presence of Aspergillus hyphae and inflammatory cells., **Ferreira *et al.* (2011)** www.ufrgs.br

Burrow *et al.* (2012) performed a study with the objective to assess whether the **frontal sinuses** in dogs with aspergillosis and of breeds typically affected by this condition were deeper at a more caudal location. CT scans of the head performed at the Small Animal Teaching Hospital, University of Liverpool, between April 2007 and March 2009 for dogs diagnosed with aspergillosis (group 1) and unaffected dogs of similar breeds (group 2) were selected for study. Sinus depth was measured at four standardised locations from reconstructed images of these CT scans. Data were compared for differences in sinus depth between groups and between landmarks. No significant difference was found between measurements within individual dogs or for each of the various landmarks between groups. Difference in depth of the sinuses between landmarks was significant ($P<0.001$). Sinus depth was significantly greater at the more caudal landmarks and was shallowest at the previously recommended landmark for sinus entry. In 54 per cent of dogs, the frontal sinus depth measured less than or equal to 2 cm at one or more of the landmarks. Sinus entry at the deepest point will reduce the risk of accidentally damaging underlying structures. This may be approximately 1 cm caudal, in breeds of dog that typically develop aspergillosis, to a previously suggested landmark

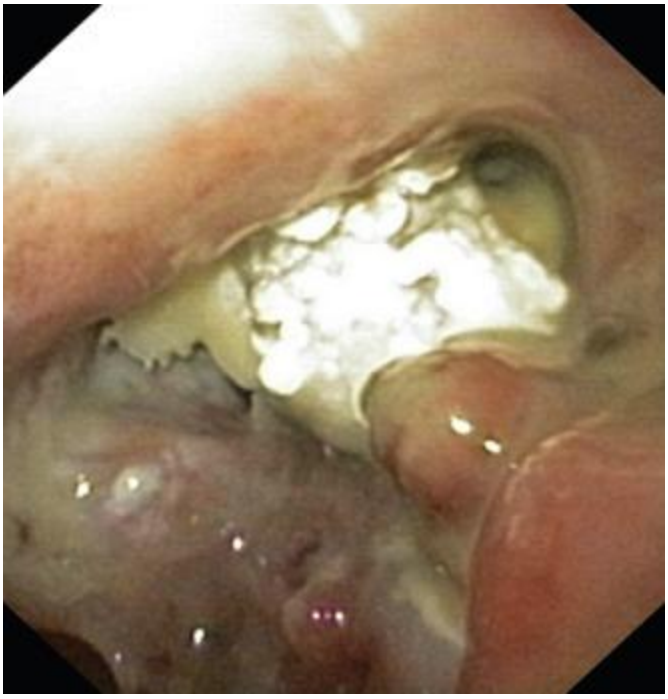
Sharman and Mansfield (2012) in a **review** on **sinonasal aspergillosis** mentioned that it is an uncommon, yet debilitating and often frustrating condition to treat in dogs despite years of research evaluating pathogenesis, diagnosis and treatment. The disease is most commonly caused by non-invasive fungal infection, thought to be secondary to altered innate and/or adaptive immune responses. Attempts to confirm this have however failed. A variety of conflicting opinions regarding the diagnosis and treatment of sinonasal aspergillosis exist. Often the use of a particular treatment protocol is based upon personal or regional preference. Evaluation of the veterinary literature demonstrates that the evidence base in support of individual treatment recommendations is weak. A number of recent publications have helped to expand the current knowledge base and therefore our understanding of important practicalities for both diagnostic options and treatment protocols. The following review examines the current evidence for the pathogenesis of sinonasal aspergillosis in dogs, as well as the various diagnostic options. The available evidence for frequently utilised -therapeutic options and their likely outcomes is also explored.



Mucopurulent nasal discharge and nasal depigmentation in a dog receiving treatment for sinonasal, www.researchgate.net, Open mouth, ventrodorsal projection of the left and right nasal cavities showing a patchy increase in opacity within the mid to caudal left and right nasal cavities, **Sharman and Mansfield (2012)**.



Destruction of the nasal turbinates identified on computed tomography (CT) of the nasal cavity.Marked hyperostosis of the frontal bone associated with severe involvement of the right frontal sinus, as identified using computed tomography (CT). Image courtesy of Murdoch University Veterinary Hospital, Western Australia, **Sharman and Mansfield (2012).onlinelibrary.wiley.com**

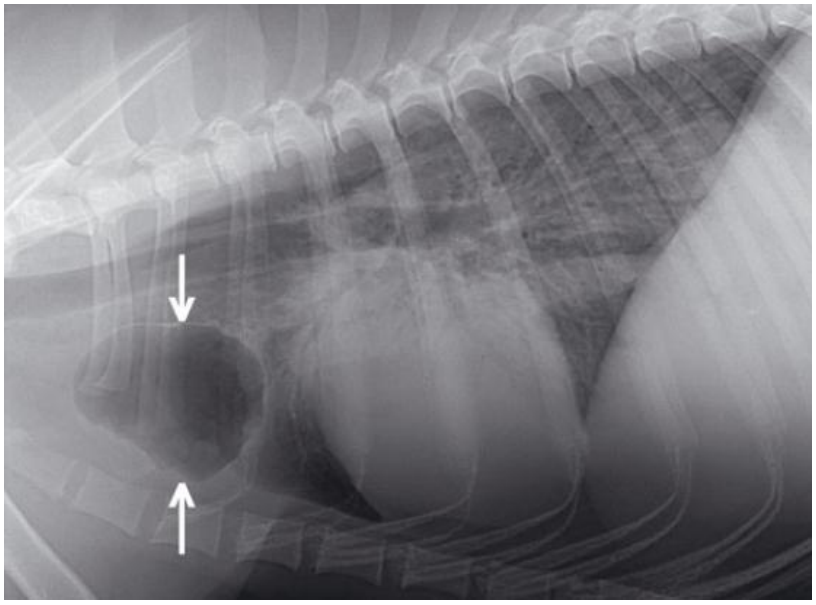


Severe nasal turbinate destruction and fungal plaques within the nasal cavity of a dog affected by sinonasal aspergillosis. Image courtesy of Associate Professor Vanessa Barrs, University of Sydney Veterinary Teaching Hospital, New South Wales, Australia, **Sharman and Mansfield (2012)**.**onlinelibrary.wiley.com**

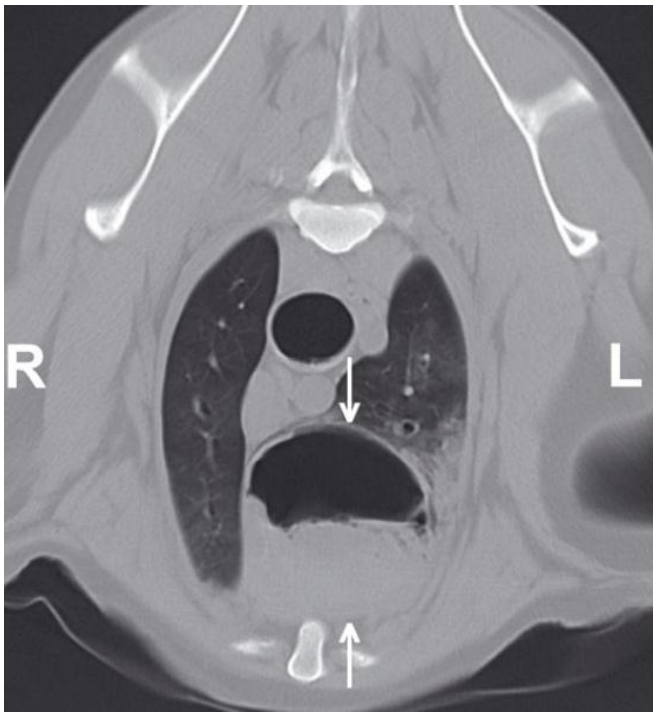
1.9.2. Bronchopulmonary aspergillosis:

Kim *et al*. (2003) presented a 6-month-old male golden retriever with fever, bloody-watery diarrhea and mild cough. Parvovirus and Isospora canis infection was confirmed and successfully treated. Two weeks later, the dog had severe cough and mucopurulent nasal discharge. ***Aspergillus niger*** was cultured from endotracheal washings on blood agar at 37 degrees C. Treatment with itraconazole for about 10 weeks resolved the clinical signs.

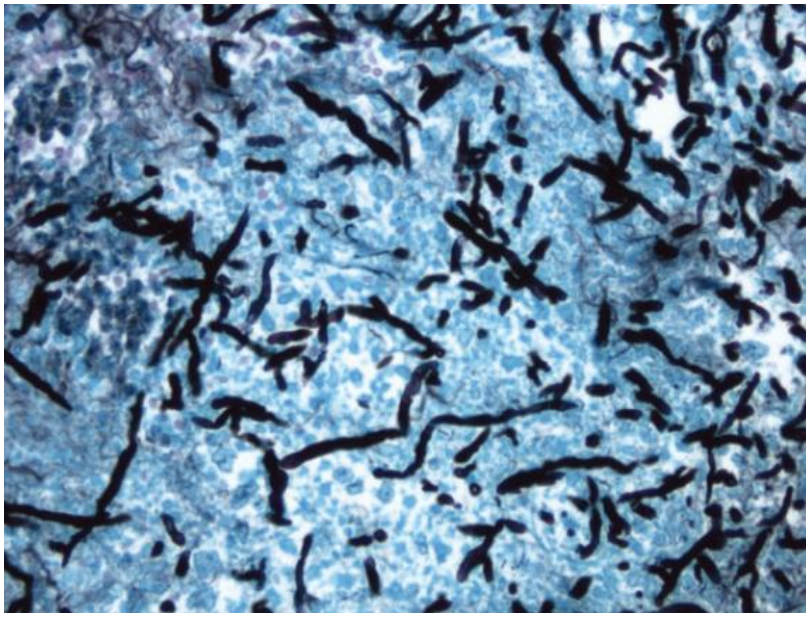
Kulendra *et al*. (2010) presented a two-year-old female German shepherd dog with chronic cough and haemoptysis. Thoracic radiographs revealed a thin-walled cavitary lesion within a consolidated left cranial lung lobe. Bronchoalveolar lavage confirmed a concurrent bacterial infection; however, despite antibiotic and anthelmintic therapy the clinical signs failed to resolve. A left cranial lung lobectomy was performed. Histopathology and fungal culture confirmed the presence of Aspergillus fumigatus. The necrotic cavity had features compatible with a bronchial origin, possibly a form of **cystic bronchiectasis**, arising either as a congenital anomaly or acquired secondary to infection. Surgery provided resolution of clinical signs for just over a year before the dog deteriorated again and was subsequently euthanised. Necropsy was declined by the owners. This case report presents a unique presentation in which the predominant clinical sign was coughing due to pulmonary involvement. ***Aspergillus fumigatus*** was isolated from the left cranial lung lobe.



Right lateral thoracic radiograph. A thin-walled cavitary lesion outlined by the white arrows can be seen within a consolidated left crania lung lobe. **Kulendra *et al*. (2010)**



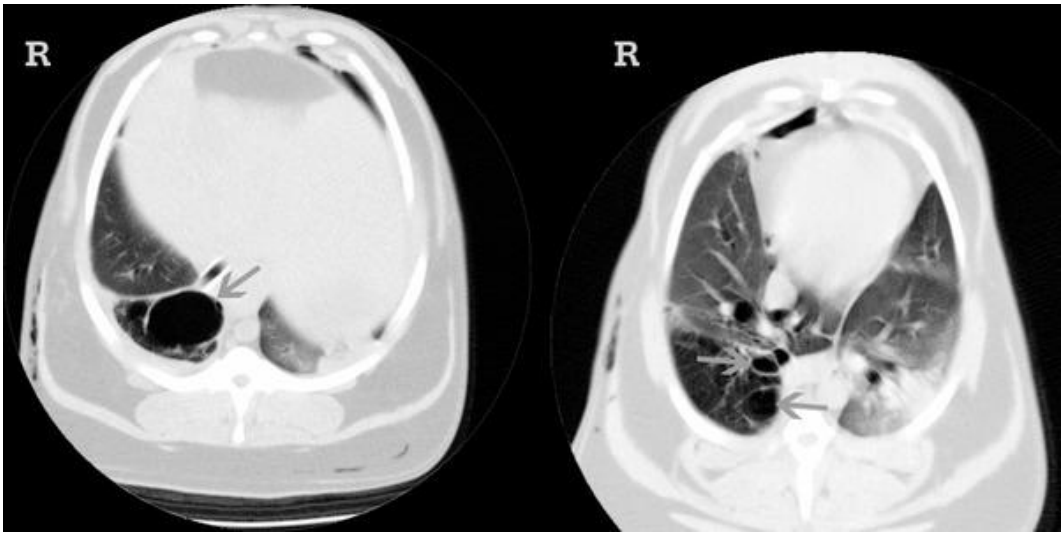
Transverse CT image of the thorax. Thin walled cavitary lesion in the left cranial lung lobe. The outline of the cavitary lesion (white arrows), a fluid gas interface within the cavitary lesion and adjacent consolidated lung are visible. **Kulendra *et al*. (2010)**



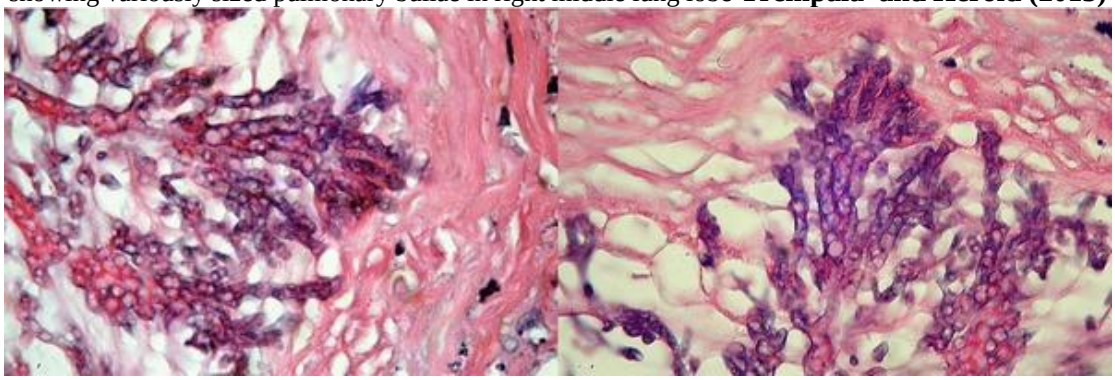
Low power magnification ×40. Groccot stained tissue reveals numerous fungal hyphae lining the inside of the cavitary lesion. **Kulendra *et al*. (2010)**

Adamama-Moraitou *et al*. (2011) described a 3 yr old intact female Hellenic shepherd dog with depression, partial anorexia, fever, and a mild productive cough of 2 m duration. Thoracic radiographs showed increased opacity of all of the left lung lobes. Upon bronchoscopy, a sanguineous, purulent discharge was detected in the tracheal lumen with hyperplastic tissue narrowing the left main stem bronchus. Cultures were positive for bacteria (Bacillus spp. and Clostridium spp.) but negative for fungi. Due to the severity of the lesions, a complete left lung pneumonectomy was performed. Histopathological examination of the excised lung tissues revealed a severe **granulomatous bronchopneumonia** with numerous alveolar macrophages laden with structures stained positively by periodic acid-Schiff and Grocott stain that had morphology consistent with fungi. PCR and sequencing of internal transcribed spacer regions 1 and 2 from genetic material extracted from paraffin-embedded pulmonary tissue confirmed the presence of ***Aspergillus fumigatus***. **Itraconazole** was administrated for 5.5 mo and the dog was clinically normal 26 mo after surgery.

Trempala and Herold (2013) described a case of **pulmonary aspergillosis** in a previously healthy dog that manifested as a spontaneous pneumothorax. A 3-year-old neutered male mixed-breed dog was presented with inappetence and respiratory distress. Thoracic radiography revealed a right-sided pneumothorax. Following stabilization, thoracic computed tomography found 1 large and many small pulmonary blebs in the right caudal lung lobe. The dog underwent a right lateral thoracotomy, identifying numerous emphysematous regions in the right middle lung lobe, and a right middle lung lobectomy was performed. Histopathologic examination of the resected lung lobe revealed severe, diffuse bronchopneumonia with necrotizing pleuritis and the presence of fungal organisms strongly suggestive of **Aspergillus sp.** Surgical removal of the affected lung lobe and continued medical treatment with itraconazole resolved the dog's clinical signs.



Thoracic CT images of a dog with spontaneous pneumothorax secondary to pulmonary aspergillosis showing variously sized pulmonary bullae in right middle lung lobe **Trempala and Herold (2013)**


Hematoxylin and eosin stain (50× magnification) of resected lung tissue from a dog with pulmonary aspergillosis showing septate and branching fungal hyphae consistent with Aspergillus sp **Trempala and Herold (2013)** www.researchgate.net

1.9.3. Disseminated aspergillosis:

Wood *et al.* (1978) reported a dog with **disseminated *Aspergillus terreus*** infection. The dog died after a protracted course of hospitalization. Treatment with amphotericin B methyl ester was without effect. The causative organism was found in bone, myocardium, spleen, kidneys, liver, thymus, lymph nodes, and both eyes. Treatment with antimicrobials and corticosteroids prior to hospitalization may have contributed to dissemination of the fungus.

Richardson *et al*. (1982) detected ***Aspergillus fumigatus*** precipitins in dog sera by counter immune electrophoresis (CIE). The procedure gave results within 90 minutes compared with 72 to 96 hours required in agar-gel double diffusion. Culture-filtrate antigens from two-week-old cultures of A fumigatus and two-day-old mycelial antigens produced the strongest reactions in CIE tests and the former antigen also revealed high titres in tests with serum from seven of 14 dogs with A fumigatus precipitins. Serum from these 14 dogs also reacted in CIE tests with a number of Penicillium species antigens.

Day *et al.* (1985) reported eight cases of **disseminated canine aspergillosis (*A. terreus*)** in German Shepherd dogs. Immunoglobulin determination revealed depression of serum IgA (cases 1 and 5) and IgM (case 2) levels and elevated levels of IgG in all cases. Total complement activity (CH50) and complement components tests, (C3, C4) were present in normal amounts in all cases. Using agar gel diffusion, serum antibody to A. terreus was found in only one case and aspergillus antigenaemia in two of the remainder. Lectin transformation of lymphocytes in two dogs was found to be depressed relative to normal controls in case 1 and initially in case 2. Two dogs failed to respond to the intradermal injection of A. terreus antigen.

Kabay *et al*. (1985) diagnosed **disseminated *Aspergillus terreus*** infection in 10 previously healthy adult dogs--nine German shepherds and one dalmatian. The disease was characterized by the presence of multiple granulomas and infarcts in a wide range of organs. The kidney, spleen, and skeletal system were most commonly and severely affected. Fungal hyphae were demonstrated in large numbers within granulomas and thrombi, and ***A. terreus*** was readily isolated by culture. This disseminated mycosis appears unique; in this series of cases there was no apparent predisposing factor, portal of entry, or primary focus for dissemination of the infection.

Day *et al.* (1986) reported clinical and pathological findings from a series of 12 cases of **disseminated aspergillosis (*A. terreus*)** in 11 German Shepherd dogs and one Dalmatian referred to Murdoch University Veterinary Hospital (MUVH) over the period 1980 to 1984. A preliminary study of humoral and cell mediated immune components and complement levels revealed no consistent abnormality in 9 dogs tested apart from raised IgG levels. Serum IgA levels were depressed in 30% of cases. Serial data from one extensively monitored case is presented. The unusual epidemiological and pathogenetic features of the disease were discussed.

Jang *et al.* (1986) presented 4 of **disseminated aspergillosis** caused by ***Aspergillus deflectus*** in German Shepherds. Three of the cases, which involved multiple organs, terminated in euthanasia. One case, with bony involvement of the limbs and skull, lived. The unique morphological characteristic of the conidial head resembling a briar pipe led to the identification of A. deflectus.

Day and Penhale (1988) studied aspects of humoral immunity in 17 dogs with **disseminated aspergillosis** (16 cases ***Aspergillus terreus***, 1 case *Aspergillus flavipes*). Alldogs had markedly raised serum IgG levels by single radial immunodiffusion (range 1500-6000 mg dl-1). Despite this, serum antibody to A. terreus was demonstrated in only 7/16 cases by agar gel diffusion, 9/16 cases by counter immunoelectrophoresis, 10/16 by ELISA and 11/16 by an indirect immunofluorescence assay. Serum antibody was also detected in 2/5 clinically normal relatives of 2 cases, indicating previous exposure or subclinical infection.

Day and Penhale (1991) conducted an immunohistochemical study of 25 lesions from 7 dogs with **disseminated aspergillosis (*Aspergillus terreus*)**. All had multiple fungal granulomas in many viscera, with centres of necrotic tissue and hyphal elements surrounded by a mixed infiltrate of predominantly mononuclear cells. Within these lesions, hyphae coated with immunoglobulin (IgG, IgM, IgA) and complement (C3, C4) were identified, together with peri-lesional mononuclear cells that reacted with antisera directed towards either IgG, IgM, IgA or a T lymphocyte

marker (MUII). A conspicuous feature was the prominent hyphal fluorescence seen with IgA and C3 antisera. The IgA reagent also marked large numbers of mononuclear cells both around lesions and scattered throughout interstitial tissue, suggesting an abnormality of IgA production or regulation as a factor predisposing to this condition.

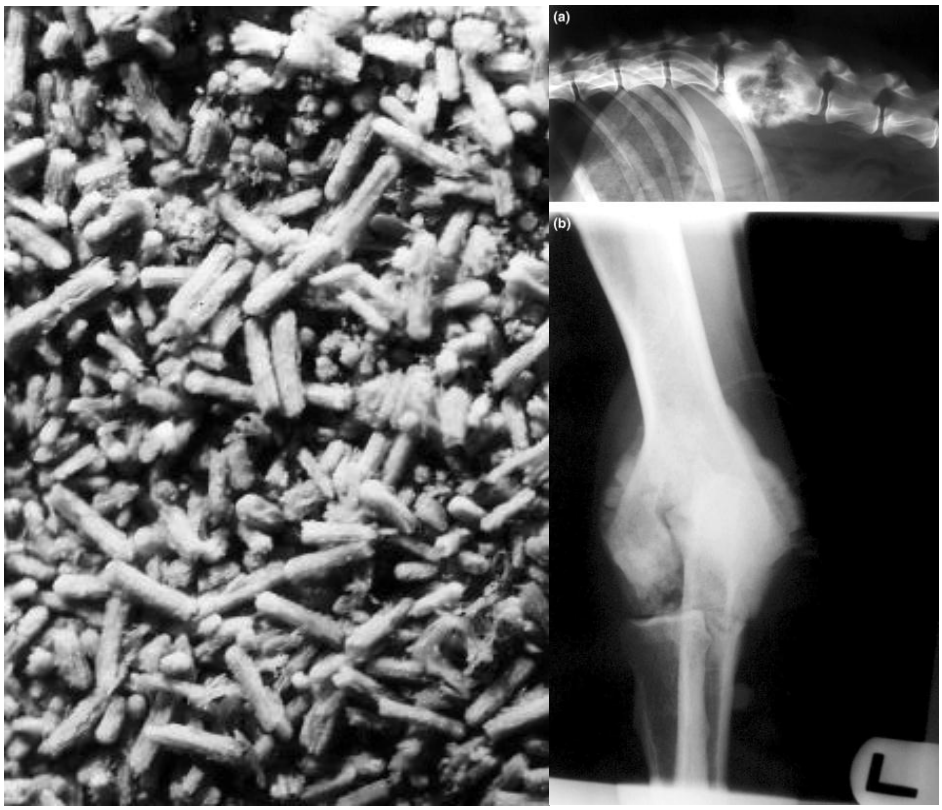
Dallman *et al.* (1992) treated a German shepherd dog initially for signs of urinary tract infection; subsequently, signs of spinal pain and neurologic deficits developed. Fungal hyphae were found in the urine sediment, and spinal radiography revealed changes in the vertebrae and intervertebral disks at the levels of T3 to T8, T12 to T13, L3-4, and L5-6, consistent with diskospondylitis. Fungal cultures of urine and specimens from spinal lesions yielded ***Aspergillus terreus***. Itraconazole (5 mg/kg of body weight, PO, q 24 h) was used to treat this infection, and locomotion improved. Sudden death occurred 4 weeks after treatment was initiated; this was attributed to exsanguination associated with a weakened renal artery. This dog was raised in Florida and resided in central Virginia. The **disseminated aspergillosis** found in this dog was not limited to the hot arid climates that some reports suggest are optimal conditions for growth.

Pastor *et al.* (1993) diagnosed **systemic aspergillosis** in a two-and-a-half-year-old spayed German shepherd dog which had suffered an acute attack of paralysis of the pelvic limbs. The neurological deficits were attributed to the destruction of the seventh vertebral body and the intervertebral disc, with protrusion of necrotic material into the vertebral canal and compression of the spinal cord at this level. Microscopically, fungal invasion and destruction of the body of T-7 was observed and Aspergillus species were identified. Fungal granulomas were also found in the liver, lung, spleen and mesenteric lymph nodes.

Robinson *et al.* (2002) reported a 4-year-old, entire female, German Shepherd Dog with a 3-month history of right foreleg lameness that partially responded to nonsteroidal anti-inflammatory and antimicrobial therapy. The bitch lost weight, was polydipsic and had reduced exercise tolerance. On referral, the animal was in poor condition, pyrexic and exhibited moderate pain on full extension of the right shoulder. The bitch was lymphopaenic, hyperfibrinogenaemic, hyperglobulinaemic, mildly azotaemic, mildly proteinuric and isosthenuric. Branching fungal hyphae were present in the urine. On radiography, the thorax contained a large ventral mediastinal mass and the humeral head had extensive areas of radiolucency. An aspirate from the right humeroscapular joint exhibited branched fungal hyphae and numerous neutrophils and macrophages. A diagnosis of disseminated mycosis was made and euthanasia was performed. At necropsy, numerous caseating granulomas were present, especially in the kidneys, adrenal glands, heart and lymph nodes. Extensive osteomyelitis involved the head of the right humerus, the sternebrae and the fifth intervertebral disc. Fungal hyphae were detected in sections of granulomas in all affected organs and a diagnosis of disseminated fungal granulomatosis was made. ***Aspergillus deflectus*** was readily isolated from affected lymph nodes, but confirming its identity as A deflectus using standard procedures proved difficult. The identity of the fungus was finally confirmed by sequencing part of the 185 rRNA of the isolate.

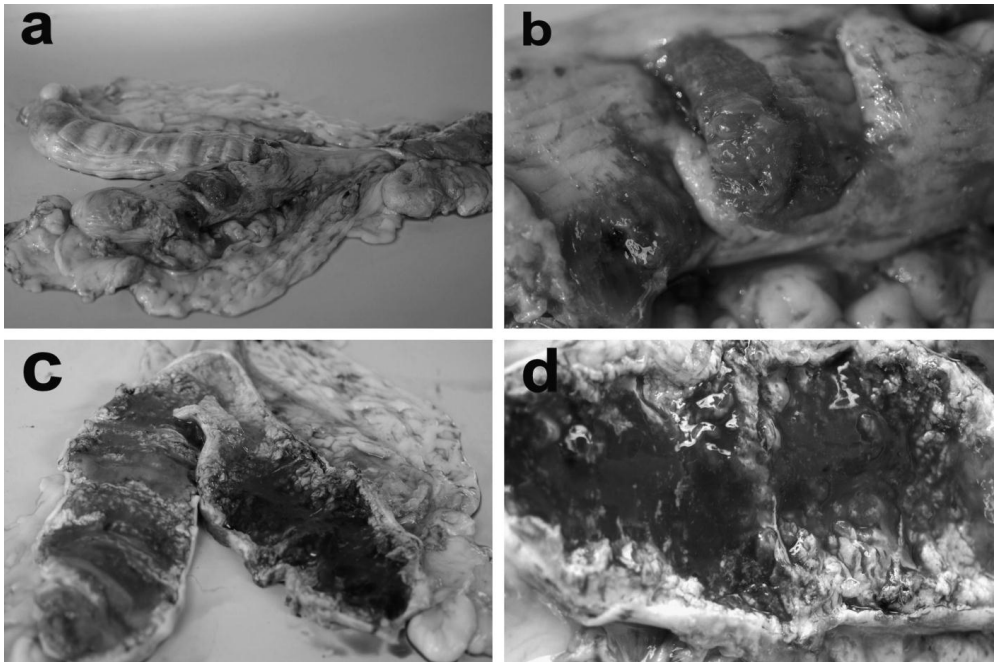
Bruchim *et al.* (2006) described two cases of German shepherd dogs with a history of anorexia and weakness. Case 1 suffered from neurological deficits, paraparesis and lumbar pain whereas case 2 suffered from unilateral uveitis and exophthalmus. Both dogs were treated symptomatically, but deteriorated progressively despite

therapy and were therefore euthanised. Necropsy revealed **disseminated aspergillosis,** and numerous organs had multiple, miliary, white-yellow foci. Microscopically, these were identified as granulomas, containing fungal hyphae. Affected tissue included brain, heart, kidneys, spleen, lymph nodes and bones (case 2). ***Aspergillus terreus*** was isolated from different organs and from urine culture. They suggested that disseminated aspergillosis should be considered as a differential diagnosis in German shepherd dogs presenting with ocular disease, neurological deficits, spinal column pain, urinary system disorders, and radiographic evidence of skeletal and/or respiratory pathology.

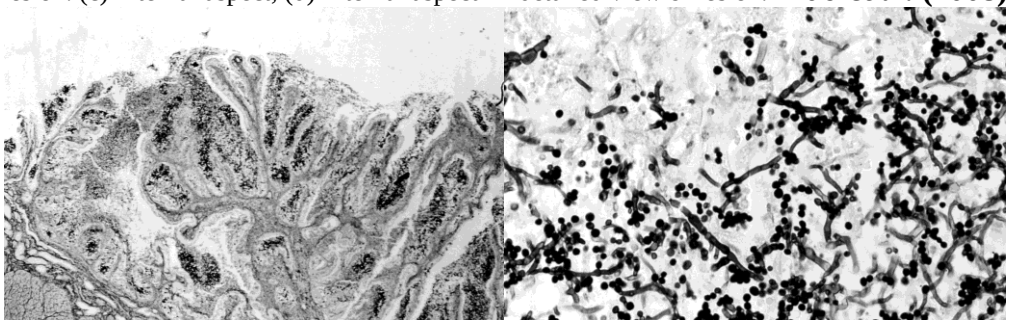


Aspergillus terreus golden-brown colony and compactly columnar heads , (a) Right lateral view of the thoracolumbar vertebrate of discospondylitis at L1-L2. Bone destruction and proliferation. (b) Anteroposterior view of a left elbow. Osteomeyelitis, note the bone proliferation and reaction **Bruchim *et al.* (2006)**

Elad et *al.* (2008) isolated ***Aspergillus terreus*** from the organs of a German Shepherd puppy removed from the bitch by cesarean intervention. In the following days, the bitch developed signs of canine **disseminated aspergillosis** and was euthanized. The fungus was isolated from a necrotic lesion in the uterus and other organs. To the best of our knowledge, this is the first report of the transuterine transmission of A. terreus during a case of canine disseminated aspergillosis.



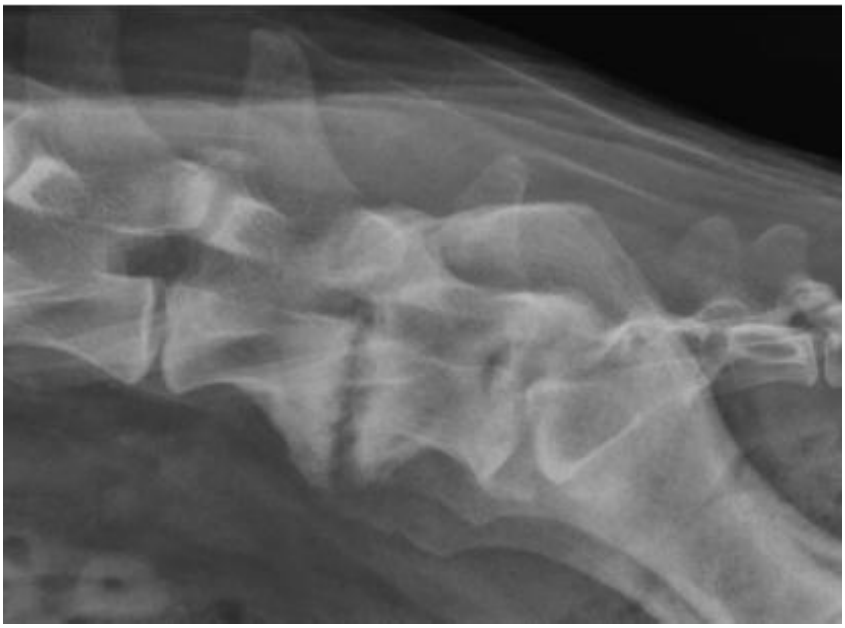
Uterine lesions. (a) External aspect, (b) External aspect — detailed view — note perforation of left lesion. (c) Internal aspect, (d) Internal aspect — detailed view of lesion. **Elad et *al.* (2008)**



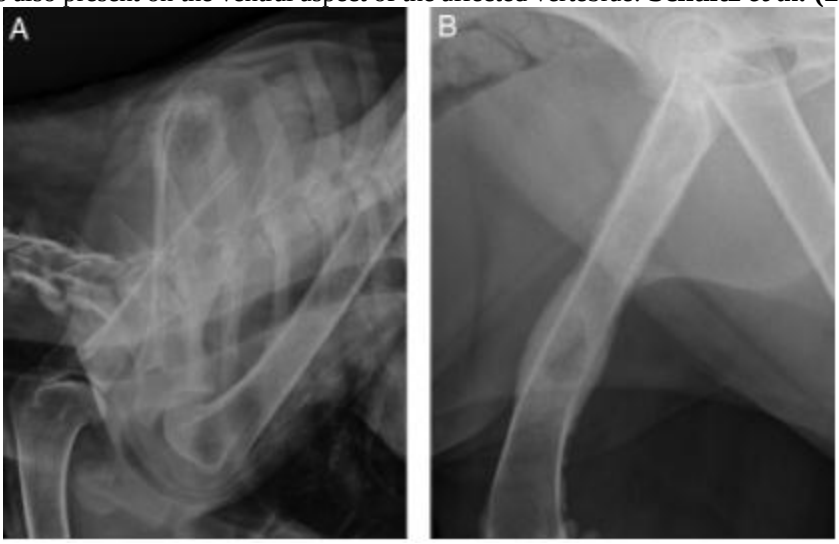
Grocott's modification of Gomori's methenamine silver stain of uterine lesions. (a) Massive fungal (dark brown elements) invasion (bar = 200 µm). (b) Hyphae and large number of globose aleuriospores **Elad et *al.* (2008)**

Schultz *et al.* (2008) reviewed. medical records for signalment, clinical features, and results of clinicopathologic testing and diagnostic imaging of **systemic aspergillosis** in 30 dogs. Diagnosis was confirmed by culture of ***Aspergillus terreus*** (n = 13), ***Aspergillus deflectus*** (n = 11), or other ***Aspergillus spp***. (n = 6). German Shepherd dogs and female dogs were overrepresented (odds ratio [OR] 43, 95% confidence interval [CI] 20-91, P < .0001, and OR 2.9, 95% CI 1.2-6.7, P= .02), respectively, with 20 of the 30 dogs being German Shepherd dogs and 77% (23 of 30) of the dogs being female. The median age was 4.5 years (range 2-8 years). Anemia, leukocytosis, hyperglobulinemia, azotemia, hypercalcemia, and hypoalbuminemia were present in 8, 21, 12, 9, 8, and 6 dogs, respectively. Diskospondylitis, osteomyelitis and thoracic lymphadenomegaly were present in 16, 10, and 5 dogs, respectively. Sonographic findings were enlarged hypoechoic lymph nodes (n = 12), mottled and irregular kidneys with or without masses (n = 12), pyelectasia, and an aggregate of echogenic material in the renal pelvis (n = 9). Thirteen dogs were treated with antifungal drugs, with survival times ranging from 0 to 25 months after diagnosis. It was concluded that systemic aspergillosis typically involves young to middle-age female German Shepherd dogs, and there are characteristic abdominal ultrasound findings with the disease process. Infection with A. deflectus was as

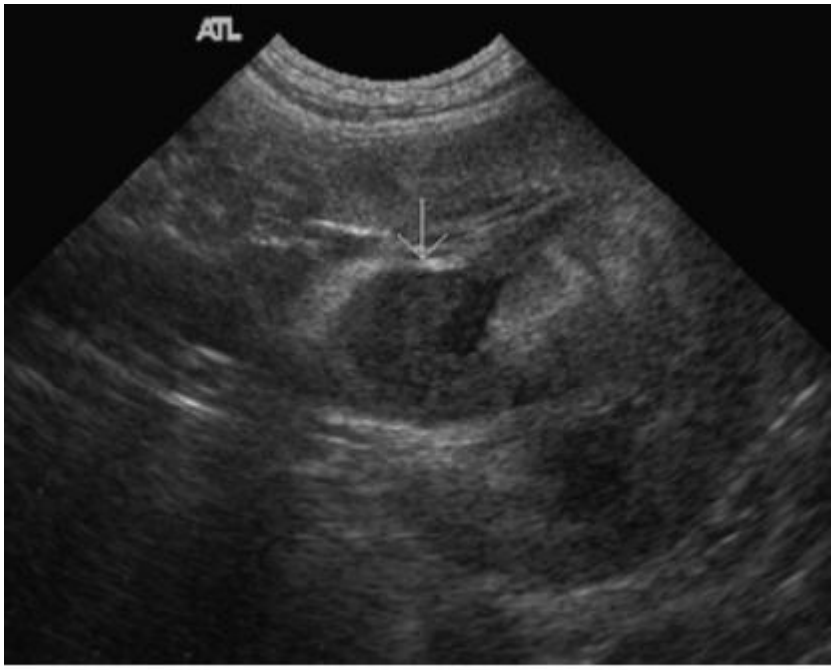
common as A. terreus, and in rare cases, long-term survival was associated with antifungal therapy.



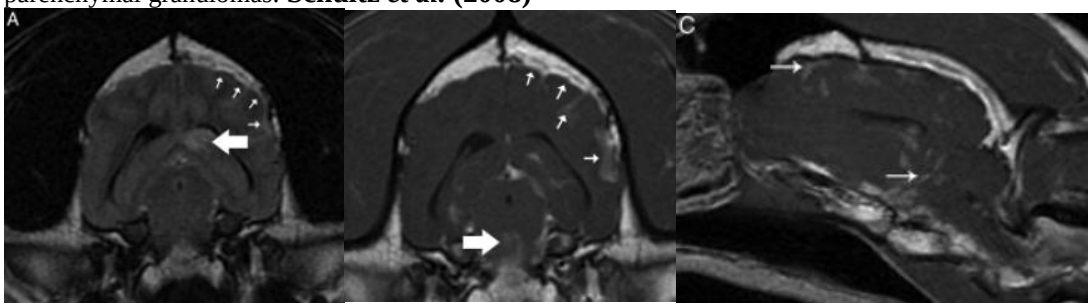
Lateral radiograph of the lumbar spine from a German Shepherd dog with systemic aspergillosis, showing the typical radiographic appearance of Aspergillus spp. diskospondylitis. There is irregular lysis with surrounding sclerosis of the end plates of the 6th and 7th lumbar vertebrae. Spondylosis deformans is also present on the ventral aspect of the affected vertebrae. **Schultz *et al.* (2008)**



Bone survey lateral radiographs from a German Shepherd dog with systemic aspergillosis. (A) An aggressive mixed productive and destructive lesion is present involving the proximal aspect of the body of one of the superimposed scapula. There is also periosteal production and increased medullary opacity of the proximal diaphysis of the caudally positioned humerus. (B) An aggressive mixed productive and destructive lesion is present in the medullary cavity of the femur. In addition, there is an extensive mixed smooth to palisading periosteal reaction along the femoral diaphysis. The histologic diagnosis was granulomatous osteomyelitis.. **Schultz *et al.* (2008)**



Transverse ultrasound image of the left kidney from a 4- year-old female spayed German Shepherd dog with systemic aspergillosis. There is moderate pyelectasia and proximal ureteral dilation. The renal pelvis is filled with an aggregate of echogenic debris (white arrow) and the papilla is blunted. The renal architecture is also distorted and mottled. The histologic diagnosis was fungal pyelonephritis with renal parenchymal granulomas. **Schultz *et al.* (2008)**

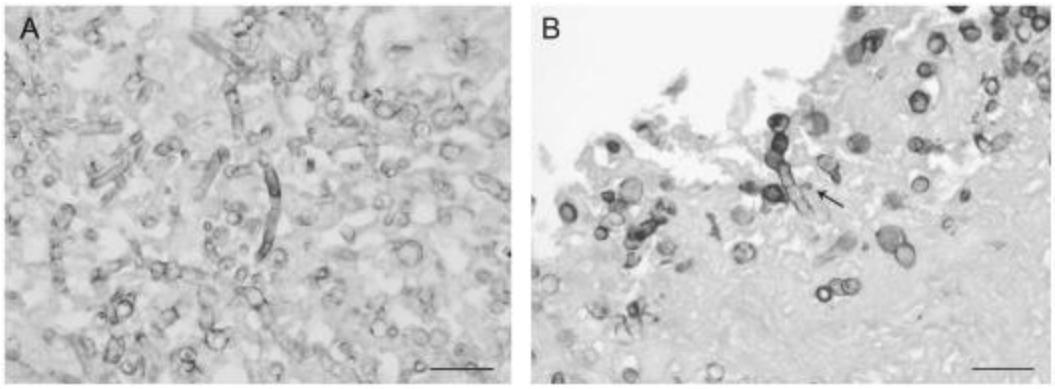


MR images of the brain and brainstem from a German Shepherd dog with systemic aspergillosis. The dog's left is to the right of the transverse images. (A) Transverse FLAIR image of the brain and brainstem reveals asymmetric hyperintensity in the left hippocampus (large white arrow) and meninges (small white arrows). (B) Transverse contrast enhanced T1-weighted image of the brain and brainstem revealing contrast enhancing lesions in the brainstem (large white arrow) and meninges (small white arrows). The plaque-like appearance of the ventral contrast enhancing meningeal lesion is caused by the image being acquired in the plane of a sulcus. (C) Sagittal contrast enhanced T1-weighted image of the cerebrum, cerebellum, and brainstem demonstrating multifocal contrast enhancing lesions involving the meninges (white arrows). The lesions appear deep in the brain parenchyma because the image plane was acquired in plane with the falx cerebri. The histologic diagnosis was fungal meningoencephalitis. **Schultz *et al.* (2008)**

Burrough *et al*. (2012) reported a case of **disseminated** disease in an English springer spaniel from which ***Aspergillus alabamensis*** was recovered by culture and identified by molecular means suggesting a potential role for this agent as a primary pathogen of dogs. A 5-year-old, spayed female, was presented to the Lloyd Veterinary Medical Center of the Iowa State University College of Veterinary Medicine with a 7 days history of vomiting, inappetence, and lethargy. Abdominal radiographs revealed mild generalized hepatomegaly and mild abdominal effusion. Cytology of the effusion revealed 96% neutrophils, and the remaining cells were macrophages and monocytes and a diagnosis of purulent abdominal exudation with associated fungal

hyphae was achieved. At necropsy, there was approximately 400 ml of red-brown effusion in each of the pleural and peritoneal cavities, and approximately 200 ml of a similar effusion within the pericardial space. The liver was diffusely pale and there were numerous multifocal to coalescing raised, tan, pinpoint to 0.3 cm diameter foci along the serosal surface, extending into the cut surface, and throughout the parenchyma. Similar foci were noted in the wall of the gallbladder, along the serosal surface of the stomach, throughout the walls of the heart, within the visceral pleura, and along both the pleural and peritoneal surfaces of the diaphragm. Both kidneys had widely scattered and variably sized pale foci similar to other organs, and there were multiple areas of infarction, most often at the poles. The pancreas was diffusely thickened with multifocal areas of hemorrhage, there were numerous raised black splenic nodules that extended into the cut surface, and there was marked bilateral atrophy of the masseter and temporalis muscles. Histologic evaluation revealed disseminated pyogranulomas in the lung, pleura, heart, thyroid, esophagus, diaphragm, liver, gallbladder, stomach, spleen, small intestine, mesenteric lymph nodes, pancreas, kidney, and adrenal gland. Pyogranulomas often contained numerous moniliform hyphae and fewer short, septate, 4.5–5 μm wide hyphae with parallel walls and occasional laterally branching globose structures consistent with accessory conidia (aleuriospores). Severe segmental necrotizing vasculitis was also identified in the kidney, pancreas, and stomach wall often with evidence of vascular rupture and associated fungal elements of similar morphology to those described in the pyogranulomas.

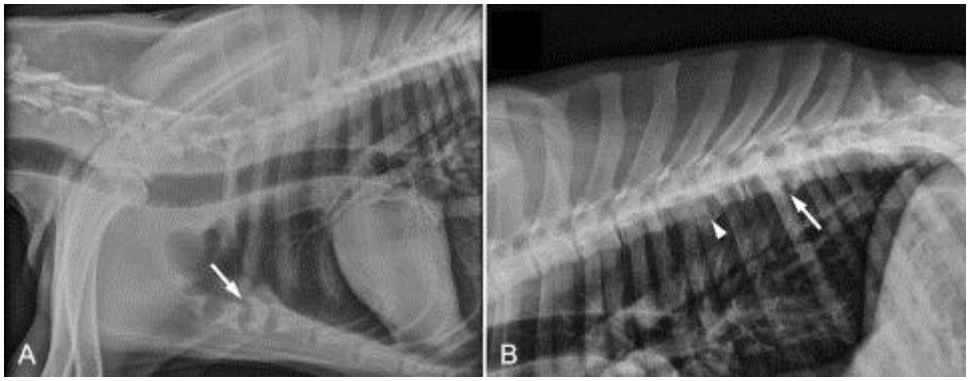
Liver and gallbladder of dog. There are numerous multifocal to coalescing, raised, tan, pinpoint to 0.3 cm diameter foci scattered throughout the hepatic parenchyma and within the thickened and congested gallbladder. Macroscopic colonial morphology of the isolated *A. alabamensis* on potato flakes agar after 16 days of growth at 25 °C revealing the presence of yellow diffusing pigment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) **Burrough *et al*. (2012)**



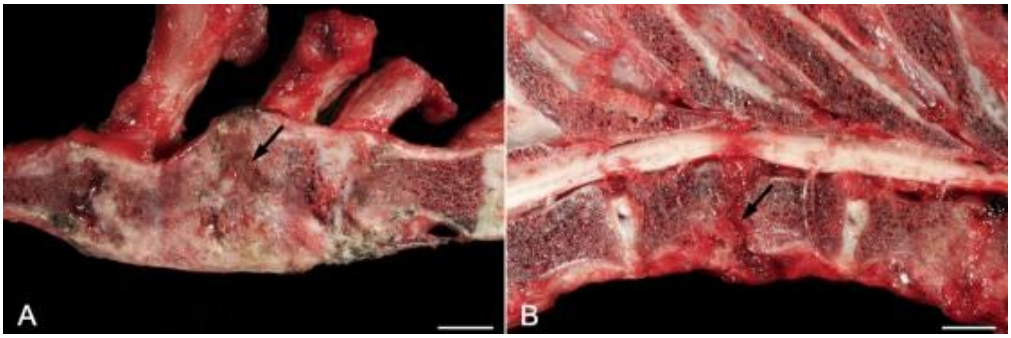
Pancreas of dog. (A) Photomicrograph from the center of a pyogranuloma revealing numerous fungal elements including many cross and tangential sections of short, septate, 4.5–5 µm wide hyphae with parallel walls. Grocott's methenamine silver. Bar=20 µm. (B) Photomicrograph demonstrating a septate fungal hypha with a laterally branching accessory conidium (aleuriospore) (arrow). Grocott's methenamine silver.. **Burrough *et al*. (2012)**

Walker *et al.* (2012) presented an intact bitch with a history of mating with severe lameness and a vulvar discharge. A mixed lytic, proliferative tibial lesion and open pyometra were diagnosed. Bone biopsy and uterine culture revealed **disseminated aspergillosis**. This is the first report of Aspergillus pyometra with dissemination following mating in the dog.

Zhang *et al* (2012) .reported a case of **disseminated *A. versicolor*** infection presenting as diskospondylitis, osteomyelitis, and pyelonephritis. The diagnosis was made based on clinical, radiographic, and pathological findings. The etiologic agent was identified by fungal culture and internal transcribed spacer (ITS) ribosomal DNA (rDNA) sequencing. This is the first description of canine aspergillosis caused by A. versicolor.

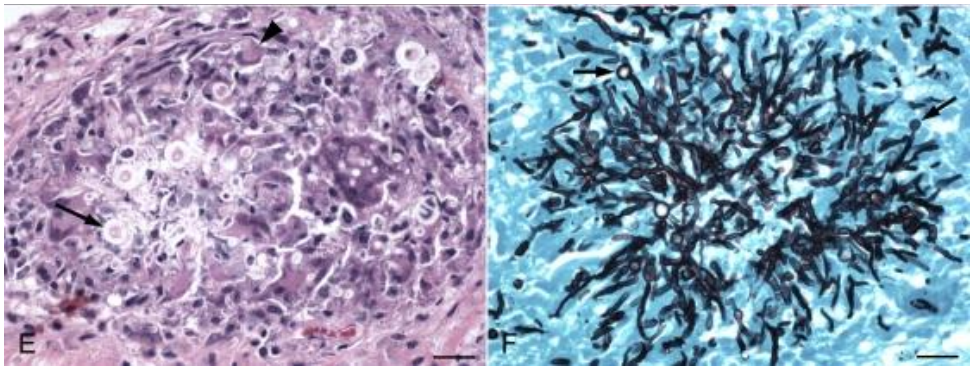


(A) Lateral radiograph of the thorax. There is lysis of the first four sternebrae and marked shortening of the second and third sternebrae, which have irregular margins and loss of the end plates (arrow). (B) Lateral radiograph of the thoracic vertebral column. There is end plate lysis of the 9th (T9) and 10th (T10) thoracic vertebrae that is centered on the intervertebral space (arrow), with spondylosis deformans ventrally. There is also narrowing, end plate sclerosis, and spondylosis deformans between the seventh (T7) and eighth (T8) vertebrae (arrowhead). **Zhang *et al* (2012)**

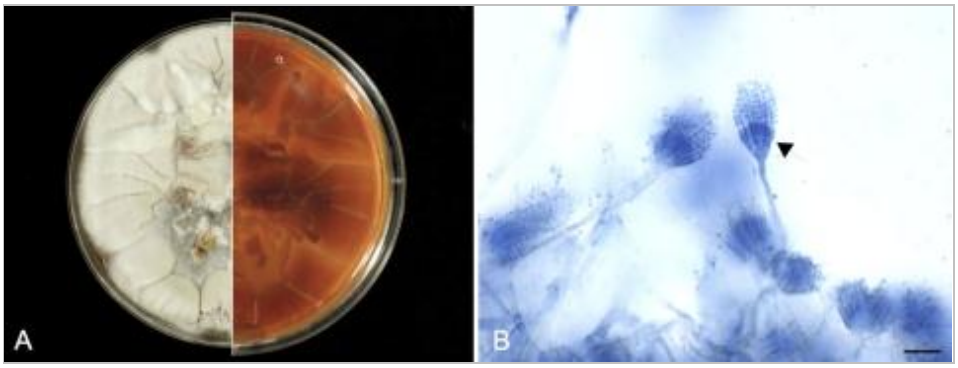


(A) Right sagittal section of sternum; the first sternebra is on the left. The second and third sternebrae are collapsed, and areas of bony proliferation obscure the joint space. An area of necrotizing osteomyelitis partially separates the two sternebrae (arrow (B) Left sagittal section of thoracic vertebrae; the cranial end is to the right. The intervertebral disk at T9-T10 is missing (arrow). The end plates are eroded, and a wedge-shaped piece of tissue compresses the spinal cord dorsally. **Zhang *et al* (2012)**.

(C) Sagittal section of left kidney. Small white areas are scattered throughout the cortex and medulla (black arrow). The pelvis is dilated, and the renal crest is ulcerated. Areas of hemorrhage (white arrow) are visible in the cortex. Bar, 1 cm. (D) Photomicrograph of second sternebra showing areas of inflammation (*) and surrounding fibrous tissue invading and replacing the marrow cavity. **Zhang *et al* (2012)**



(E) Higher magnification of sternebra showing marked granulomatous inflammation with giant cell formation (arrowhead) surrounding septate fungal hyphae and bulbous spore-like structures (arrow). Bar, 25 µm. (F) Grocott's methenamine silver-stained section of sternebra taken from same area as previous image, demonstrating prominent fungal hyphae and terminal conidiophores (arrows). **Zhang *et al* (2012)**

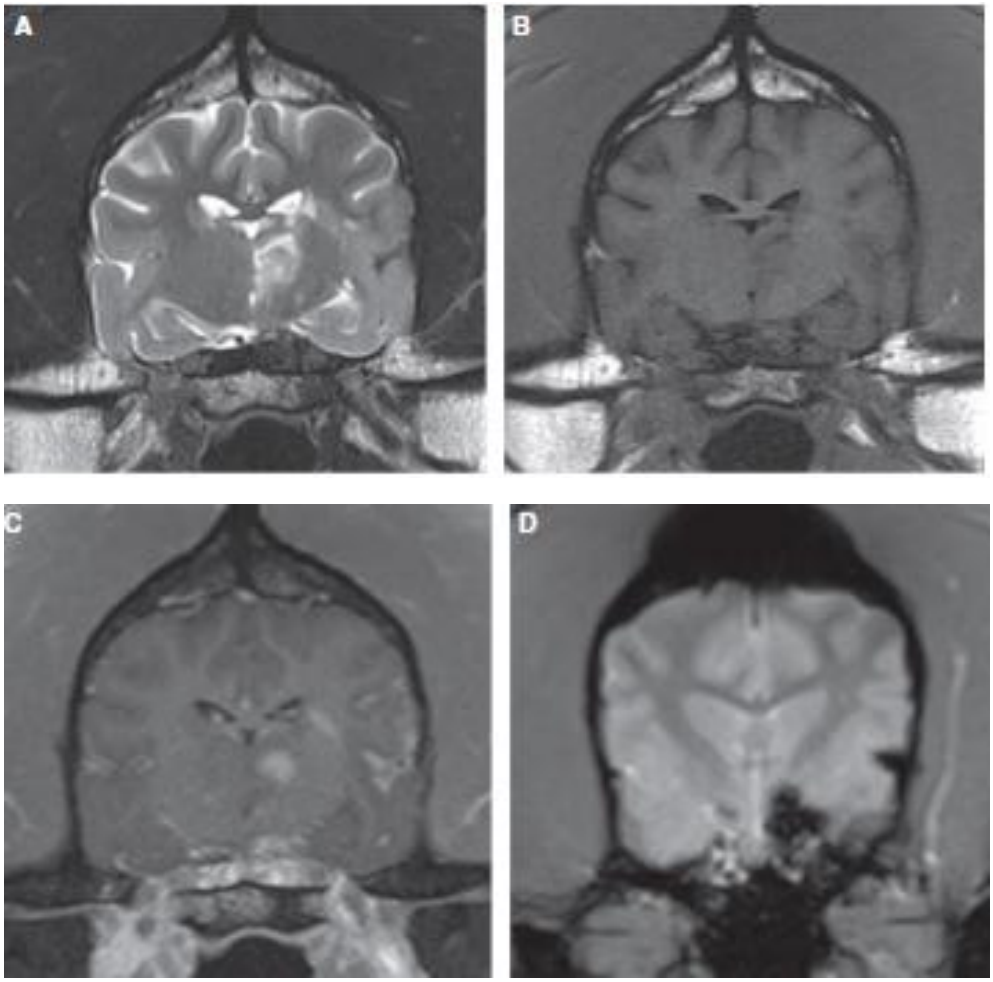


Macroscopic colonial morphology of the *A. versicolor* isolate. The culture was incubated at room temperature for 10 days. The surface of the fungal colonies is white to light tan (left), and the reverse side is yellow to brown (right). (B) Lactophenol blue staining reveals brush-like and radiate conidial heads with round vesicles, biseriate phialides (arrow), and spherical conidia in short chains. **Zhang *et al* (2012)**

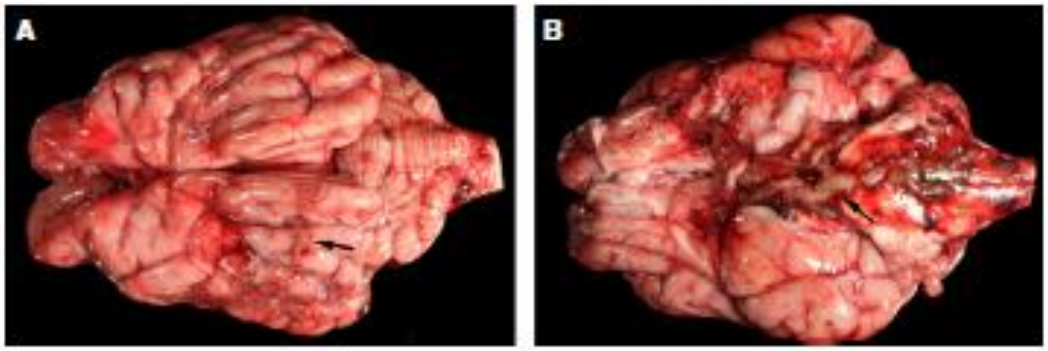
Barrs *et al.* (2013) described *A. felis* (neosartorya-morph) isolated from three host species with invasive aspergillosis including a human patient with chronic invasive pulmonary aspergillosis, domestic cats with invasive fungal rhinosinusitis and **a dog with disseminated invasive aspergillosis.** Disease in all host species was often refractory to aggressive antifungal therapeutic regimens. Four other human isolates previously reported as *A. viridinutans* were identified as *A. felis* on comparative sequence analysis of the partial β-tubulin and/or calmodulin genes.

1.9.4. Aspergillosis of the CNS

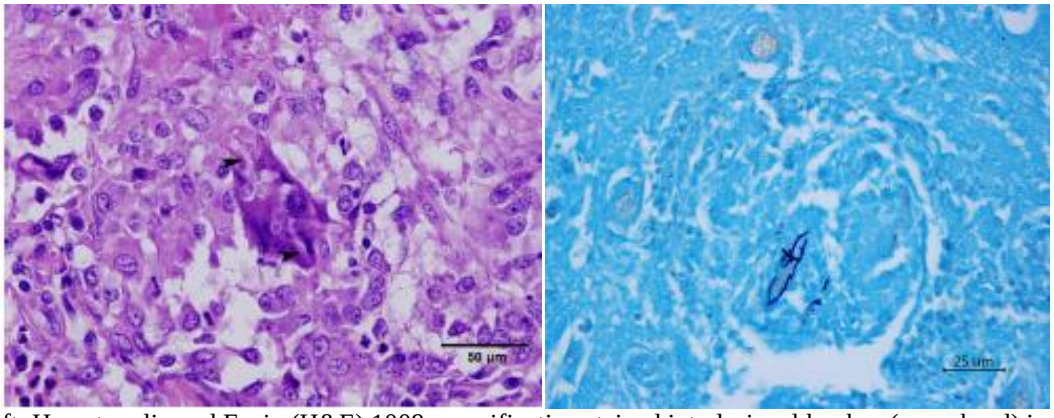
Taylor *et al*. (2015) reviewed archived records from 6 institutions to identify cases with MRI of **CNS aspergillosis** confirmed with serum galactomannan enzyme immunoassay (EIA) testing, culture, or supported by histopathology. Signalment, clinical, MRI, clinicopathologic, histopathologic, and microbiologic findings were recorded and evaluated. Aspergillosis of the CNS was identified in 7 dogs from 3 institutions. The median age was 3 years and six were German Shepherd dogs. Five dogs had signs of vestibular dysfunction as a component of multifocal neurological abnormalities. The MRI findings ranged from normal to abnormal, including hemorrhagic infarction and mass lesions. They document that CNS aspergillosis in dogs, particularly German Shepherd dogs, can be suspected based on neurologic signs, whether MRI findings are normal or abnormal. Confirmatory testing with galactomannan EIA, urine, cerebrospinal fluid (CSF) or tissue culture should be performed in cases where aspergillosis is a differential diagnosis.



MRI images from dog 1. T2-weighted (A), precontrast T1-FLAIR (B), fat-saturated postcontrast T1-FLAIR (C), T2*-weighted (D), **Taylor *et al*. (2015)**



(A) Dorsal aspect of brain of Dog 1 with irregular raised plaques within the leptomeninges overlying the left temporal and parietal lobes (arrow). (B) Ventral aspect of brain of Dog 1 with irregular raised plaques overlying the ventral brainstem (arrow). **Taylor *et al*. (2015)**

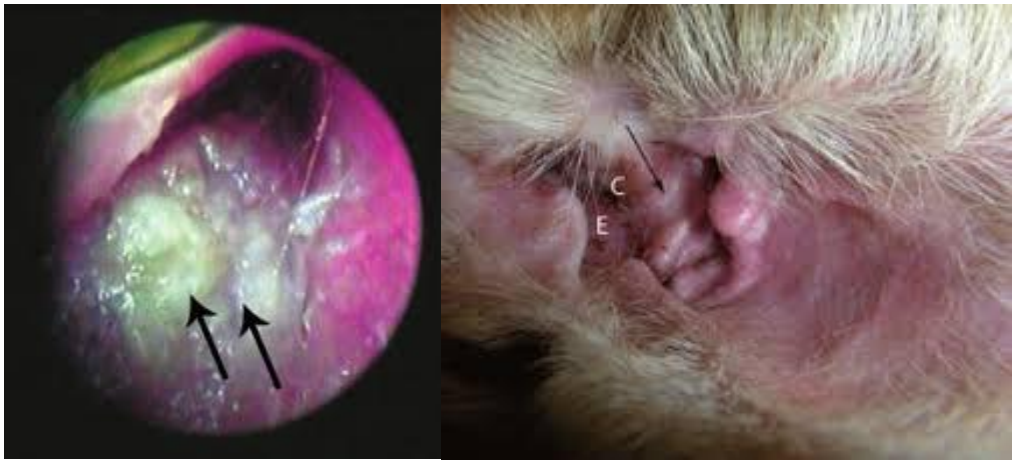


Left: Hematoxylin and Eosin (H&E) 1009 magnification stained intralesional hyphae (arrowhead) in the granulomatous lesions of the neuropil. Right: Grocott's Methenamine Silver (GMS) 409 magnification stained intralesional hyphae in granulomatous lesion of the forebrain. **Taylor *et al*. (2015**)

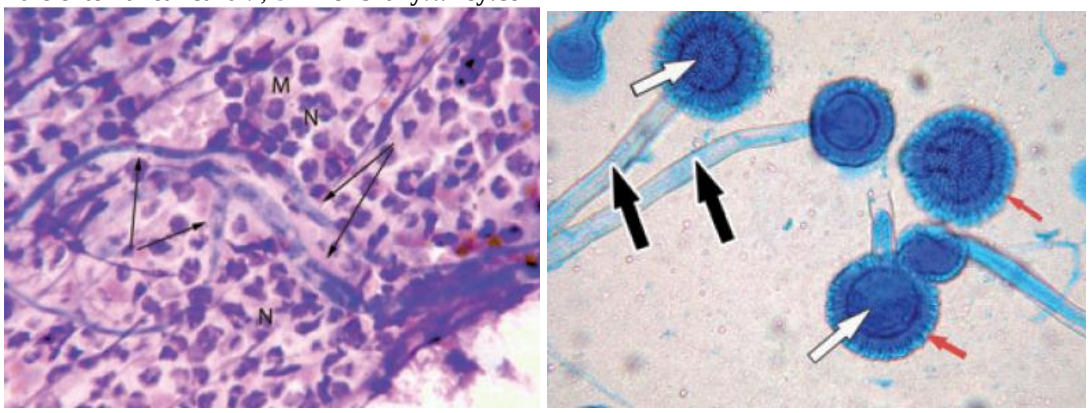
1.9.5. Aspergillosis of the ears (otomycosis)

Coyner (2010) described the clinical findings, clinicopathology and treatment of otomycosis caused by Aspergillus spp. in an atopic dog affected by chronic unilateral purulent otitis externa unresponsive to topical and oral antibiotics and antifungal treatments. Cytology of otic exudate revealed neutrophils and septate fungal hyphae, and otic culture grew Aspergillus spp. and no bacteria. Treatments included allergen-specific immunotherapy, topical and oral antifungal therapy and anti-inflammatory steroid therapy. Final resolution occurred after treatment of the underlying hypersensitivity disorder, administration of topical ketoconazole and debridement of infectious ear exudate. Otomycosis due to filamentous fungi may, as in humans, occur in dogs with ear canals compromised by pre-existing allergic or bacterial otitis, and possibly previous antibiotic therapy. Antifungal medications provided clinical improvement, but the key to successful treatment was the restoration of the normal physiology of the external auditory canal.

Ghibaudo and Peano (2010) reported a 4-year-old male mixed breed dog with pain and discomfort of the left **ear** that began 3 weeks prior to examination. Except for the clinical signs associated with the ear, there were no other complaints. The ear had not responded to several topical antibiotic and anti-inflammatory therapies and cytological examination of otic exudate obtained by a swab from the affected ear revealed numerous fungal hyphae and inflammatory cells (neutrophils and macrophages). Bacteria or other fungi were not seen. The dog was anaesthetized and an otoendoscopic examination was performed in both ears before and after ear flushing. The left horizontal canal showed a whitish lesion mixed with abundant ceruminous debris near the tympanic membrane. No foreign bodies were detected, the tympanic membrane was intact and no signs of otitis media were present upon endoscopic, radiological and cytological examination (performed through myringotomy). Swabs from both ears submitted for bacterial and mycological culture for 4 days at 25 C, cultures on Sabouraud ⁄ gentamicin ⁄ chloramphenicol ⁄ dextrose agar demonstrated numerous fungal colonies with restricted growth in the left ear; these were initially yellowish orange then yellowish-brown. Microscopic examination revealed hyphae and conidial head, typical ***Aspergillus ochraceus***.



Left ear of the dog. Mild erythema (arrow) and ceruminous discharge (C) are present near the entrance (E) of the external ear canal Video-oto-endoscopy (Storz 2,7-mm-diameter 30 endoscope) of the affected ear. Whitish lesion mixed with abundant ceruminous debris (arrows) filling the horizontal part of the external ear canal. , **onlinelibrary.wiley.com**



Cytology of otic exudate from the affected ear showing fungal hyphae, (arrows) degenerate neutrophils (N) and macrophages (M). Modified Wright-Giemsa stain; ·40)., Microscopical preparation. Fungal colonies showing erect unbranched structures (conidiophores) (black arrows) with apical vesicles (white arrows) bearing conidiogenous cells (phialides) (red arrows) that cover the entire surface. (Lactophenol cotton blue stain; ٠ 40).
onlinelibrary.wiley.com

1.9.6. Aspergillosis of the eye (oculomycosis)

Pal and Mehrotra (1986) studied the occurrence and aetiological significance of **Aspergillus fumigatus** have been studied in 93 animals with various ophthalmological problems. Eye swabs collected from 26 dogs were investigated mycologically for the presence of A. fumigatus. The pathogen was isolated in pure and heavy growth from the swabs from two dogs. The fungus was also demonstrated directly in clinical material by the potassium hydroxide technique. The organism was not isolated in pure culture from the conjunctival swabs of 11 apparently healthy Many other saprophytic fungi were recovered in mixed cultures but were considered to be contaminants. The clinical signs and diagnostic criteria of oculomycosis have been discussed.

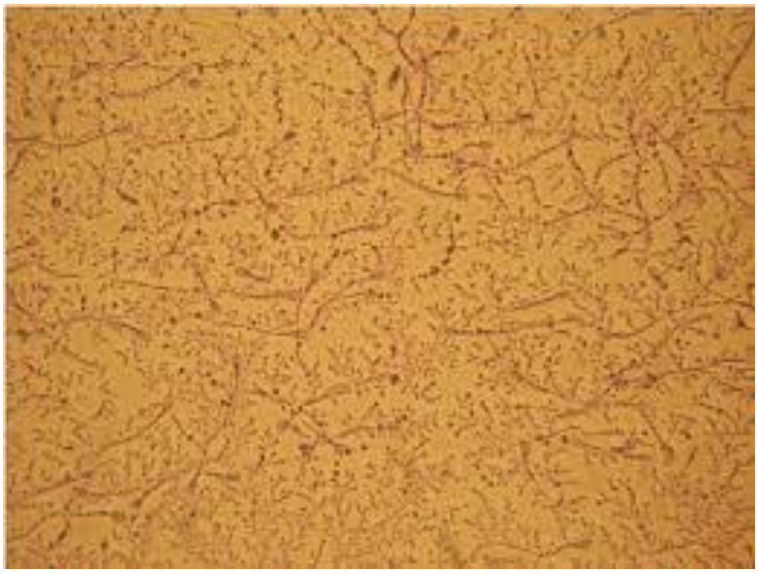
1.9.7. discospondylitis

Butterworth *et al.* (1995) diagnosed multiple **discospondylitis** in a four-year-old, neutered female German shepherd dog which had suffered intermittent pain of the axial skeleton for 10 months, which was followed by the sudden onset of paraplegia associated with the rupture of an affected disc. After surgical and medical management the dog began to improve but then deteriorated as a result of a pathological fracture of the fifth lumbar vertebra. A histological examination revealed fungal hyphae at the sites affected radiographically and they were identified by immunohistochemistry as ***Aspergillus species***. No fungal hyphae were identified in other tissues. This is the first report of canine mycotic discospondylitis in the United Kingdom.

Dallman *et al.* (1992) treated a German shepherd dog initially for signs of urinary tract infection; subsequently, signs of spinal pain and neurologic deficits developed. Fungal hyphae were found in the urine sediment, and spinal radiography revealed changes in the vertebrae and intervertebral disks at the levels of T3 to T8, T12 to T13, L3-4, and L5-6, consistent with **diskospondylitis.** Fungal cultures of urine and specimens from spinal lesions yielded ***Aspergillus terreus***. Itraconazole (5 mg/kg of body weight, PO, q 24 h) was used to treat this infection, and locomotion improved. Sudden death occurred 4 weeks after treatment was initiated; this was attributed to exsanguination associated with a weakened renal artery. This dog was raised in Florida and resided in central Virginia.

1.9.8. Aspergillosis in the genital tract

Siemieniuch *et al.* (2009) reported aspergillosis in the dog **genital tract** with hyphae present in semen. Amoxycillin with clavulonate (Synulox 500mg were administered twice daily orally. Itraconazole was used as an antimycological agent (Orungal, 100mg, twice daily) every other week. In 8th week of therapy no Aspergillus spp. growth was noted, yet slight Penicillium growth was observed. After 12 weeks of treatment, no fungus growth was present. Neither spores or hyphae were seen in the microscopic examination. Three months after the termination of the therapy, the dog mated with two females. In one case, unifetal pregnancy was diagnosed by ultrasound examination on day 42 after mating. Due to purulent discharge on day 45 after mating, the owner decided to terminate the pregnancy. In the other case, severe pyometra appeared 12 days after the second mating and the owner decided to put the female to sleep. The pathogen eradication from the ejaculates may be treated as a serious success, yet the lack of litters after mating calls for an explanation and consequences of Aspergillus spp. infection need to be considered.



Siemieniuch *et al.* (2009), www.researchgate.net

1.10. Molecular studies of aspergillosis in dogs

Mercier *et al*. (2014) performed a study to identify the presence of non-synonymous sino-nasal aspergillosis (SNA) in the coding regions of the **TLR2**, 4 and 9 genes in dogs suffering from SNA, and (2) to investigate the SNP genotypes in dogs with SNA compared with a control population. Direct sequencing of nine dogs of various breeds with SNA revealed two non-synonymous SNPs in the coding region of TLR2, eight in TLR4 and four in TLR9. These non-synonymous SNPs were further evaluated in a case-control study of affected Golden Retrievers, Labrador Retrievers, Rottweilers and Beaucerons. Genotyping was performed using a combination of allele-specific primers and hydrolysis probe assays in 31dogs with SNA and 31 controls. No significant difference in minor allele frequency was identified between these groups, for all studied SNPs, in any of the four breeds.They concluded that, these findings do not support a role for non-synonymous SNPs in the TLR 2, 4 and 9 coding regions in the pathogenesis of canine SNA, but do not exclude a role for innate immunity in the pathogenesis of the disease.

Talbot *et al.* (2014) carried out a study to determine the molecular identities of fungal species causing sino-nasal aspergillosis (SNA). in dogs. Genomic DNA was extracted from 91 fungal isolates from 90dogs diagnosed with SNA in Australia, the USA and Belgium, and the ITS1-5.8S-ITS2 ribosomal DNA and partial β-tubulin regions were sequenced. Eighty-eight of 91 (96.7%) isolates were identified as A. fumigatus and 3/91 (3.3%) belonged to Aspergillus section Nigri spp. (Aspergillus tubingensis: 2/91; Aspergillus uvarum: 1/91). These findings confirm that A. fumigatus is the most common aetiological agent of canine SNA. This is the first report to document a pathogenic role for A. tubingensis and A. uvarum in dogs.

1.11. Treatment of aspergillosis in dogs

Sharp and Sullivan (1989) treated 15 dogs with **nasal aspergillosis** with ketoconazole (5 mg/kg of body weight, q 12 h, PO) for 2 to 18 weeks. Four dogs whose conditions deteriorated during treatment received ketoconazole for less than the prescribed 6 weeks. Six months or more later, only 47% of the dogs were determined to be disease-free, on the basis of no fungal growth on culture. It was concluded that ketoconazole at this dosage is a useful treatment for canine nasal aspergillosis, but is no more effective than thiabendazole.

Pavletic and Clark (1991) treated 5 dogs with **nasal aspergillosis** by surgical exposure and delayed closure of the nasal cavity and involved frontal sinus. Diseased tissue was excised, and 10% povidone-iodine solution was applied three times daily with cotton-tipped applicators. Skin wounds were closed at weeks 6 through 8. In one dog, the frontal sinus was partially obliterated with a temporalis muscle flap before skin closure. At months 6 through 34, all dogs were clinically free of aspergillosis. Open treatment has potential clinical application as a primary approach to nasal aspergillosis or for cases that are unresponsive to previous medical management.

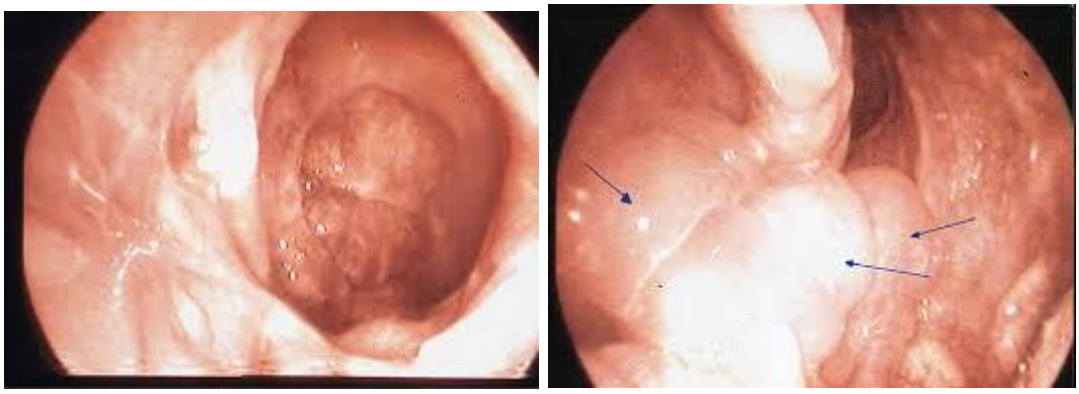
Dallman *et al.* (1992) treated a German shepherd dog initially for signs of urinary tract infection; subsequently, signs of spinal pain and neurologic deficits developed. Fungal hyphae were found in the urine sediment, and spinal radiography revealed changes in the vertebrae and intervertebral disks at the levels of T3 to T8, T12 to T13, L3-4, and L5-6, consistent with **diskospondylitis.** Fungal cultures of urine and specimens from spinal lesions yielded ***Aspergillus terreus***. Itraconazole (5 mg/kg of body weight, PO, q 24 h) was used to treat this infection, and locomotion improved. Sudden death occurred 4 weeks after treatment was initiated; this was attributed to exsanguination associated with a weakened renal artery. This dog was raised in Florida and resided in central Virginia.

Sharp *et al.* (1993) treated 24 dogs with **nasal aspergillosis** were treated with (10 mg/kg bid for 7-14 days) administered topically through tubes surgically implanted into the nasal chambers. Aspergillosis was eliminated in 19 dogs over a median follow-up period of 18 months. Another dog died, but at necropsy there was no evidence of causative fungus. Two of the four dogs that were not cured had infection of periorbital soft tissues. An additional seven dogs received 6 weeks **ketoconazole** (5 mg/kg bid PO) and **enilconazole** therapy topically. Six of these dogs were disease-free over a median follow-up period of 35 months. The seventh dog responded to repeated treatment with enilconazole. Twenty-six of the 29 dogs (90%) without extranasal aspergillosis were cured.

Kelly *et al*. (1995) treated 4 dogs with **disseminated aspergillosis** caused by ***Aspergillus terreus*** were treated with oral **itraconazole** for 190 to 1095 days. Infection was eliminated in 1 dog. Two dogs were treated for 1000 and 1095 days but were eventually euthanased 572 and 485 days after treatment was stopped. At necropsy both dogs had widespread aspergillosis. The fourth dog was euthanased for other reasons after 190 days of treatment when it was showing a good clinical response although there was radiographic evidence that the disease was progressing.

Zonderland *et al.* (2002) conducted a study to determine effectiveness of infusion of 1 and 2% enilconazole for treatment of nasal and **sinusal aspergillosis**, respectively,

in 26 client-owned dogs with aspergillosis. All dogs had typical clinical signs of aspergillosis and rhinoscopically visible intrasinusal or intranasal fungal plaques associated with turbinate destruction. During rhinoscopy, affected nasal cavities and frontal sinuses were debrided meticulously. Nineteen dogs (group A) were treated with 1% enilconazole by use of a modified noninvasive infusion procedure. Seven dogs (group B) were treated with 2% enilconazole via catheters that were placed via endoscopic guidance into the frontal sinuses. All dogs underwent follow-up rhinoscopy for determination of further treatment until cure was established. Age, disease duration, clinical score, and rhinoscopic score were similar for both groups before treatment. In group A, 17 of 19 dogs were cured; 9, 6, and 2 dogs were cured after 1, 2, or 3 treatments, respectively. The remaining 2 dogs were euthanatized before the end of the treatment protocol. In group B, all dogs were cured; 6 dogs and 1 dog were cured after 1 or 2 treatments, respectively. Only minor adverse effects such as nasal discharge, epistaxis, and sneezing developed. After extensive rhinoscopic debridement, 1 and 2% enilconazole infused into the nasal cavities and the frontal sinuses, respectively, were effective for treatment of aspergillosis in dogs. Intrasinusal administration via endoscopically placed catheters appeared to require fewer infusions for success. Follow-up rhinoscopy is strongly advised.



Intranasal infusion of enilconazole for treatment of sinonasal aspergillosis in dogs
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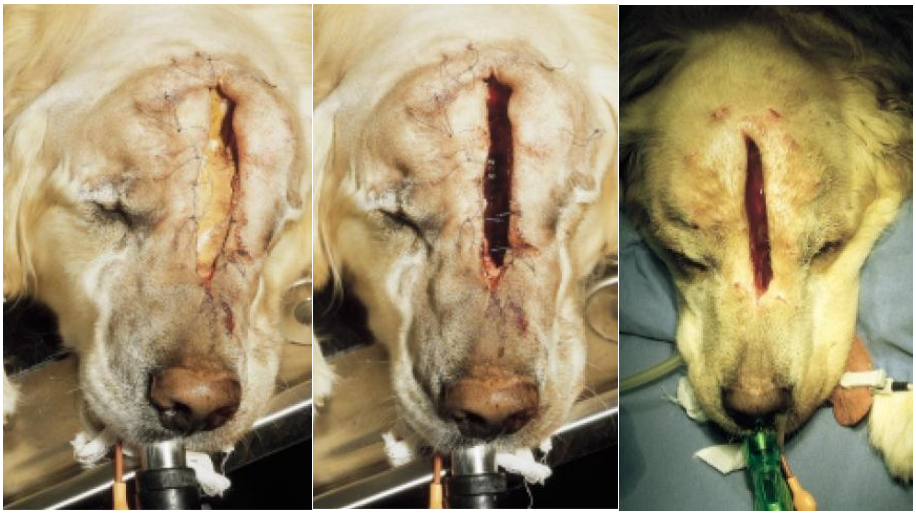
Moore (2003) treated 3 dogs with **mycotic rhinitis** were treated with a proprietary wound dressing product intended to produce a sustained release of povidone-iodine. All of the dogs had been refractory to other treatments. One dog had extensive soft tissue involvement, including extension into the orbital tissues, and another had evidence of involvement of the supporting bones of the nose. In all cases, the affected nasal cavity and/or frontal sinus was exposed via a dorsal approach and partial turbinectomy was performed. The wound dressing was applied and retained with a 'tie-over' dressing. The dressing was replaced every 48 to 72 hours until all exposed tissue was covered by healthy granulation tissue, at which time the rhinotomy was closed by soft tissue reconstruction. There was no evidence of recurrence of the fungal infection at follow-up times of up to 20 months postsurgery.



At day 3 the open sinorhinotomy is shown with the skin edges sutured to the bone margins and suture loops placed to retain the dressing, Povidone-iodine dressing has been packed into the sinorhinotomy to cover all the exposed surfaces, At 21 days postoperatively the surfaces are all covered with healthy granulation tissue. (Shown immediately before preparation for closure)

Schochet and Lappin (2005) diagnosed A two-year-old, female spayed Australian cattle dog with **nasal aspergillosis**. The dog was treated topically with clotrimazole. Clinical signs recurred two months later and the clotrimazole **treatment** was repeated and 5 mg/kg itraconazole twice daily was added to it. The recommended dose of itraconazole for nasal aspergillosis is 5 mg/kg twice daily administered orally. The dog's symptoms completely resolved, but it developed an adverse febrile reaction to the Itraconazole. The Itraconazole was discontinued and the dog remained asymptomatic for four years. The dog then developed mucopurulent discharge from the right nostril and was diagnosed as having recurrent nasal aspergillosis. Itraconazole at 5 mg/kg twice daily was prescribed, which again induced a fever. When the itraconazole was decreased to 5 mg/kg once daily there were no fever episodes, but the nasal discharge was not completely resolved. The dog was then treated with topical clotrimazole Infusion, and maintained on 5 mg/kg itraconazole daily.

Sissener *et al.* (2006) evaluated the effect of short duration 1 per cent **clotrimazole** flush when combined with 1 per cent clotrimazole cream instilled into the frontal sinuses for the treatment of **nasal aspergillosis** in 14 dogs.with clinical, radiological, serological and rhinoscopic findings consistent with nasal aspergillosis. Dogs were treated by frontal sinus trephination and a short, five-minute flushing of 1 per cent topical clotrimazole solution followed by a 1 per cent clotrimazole cream instilled as a depot agent. Twelve of the 14 dogs (86 per cent) responded well to treatment and either had no clinical signs after treatment or had signs consistent with mild rhinitis during a minimum follow-up period of six months. Only one dog required multiple treatments. Treatment was well tolerated by all patients, with minimal complications.

Billen *et al.* (2010) evaluated the effect of 1% bifonazole cream in the treatment of canine **sino-nasal aspergillosis** (SNA). The cream was instilled through perendoscopically placed catheters into the frontal sinuses and was used either as single therapy after debridement (DC) or as adjunctive therapy after 2% enilconazole infusion (DEC). Twelve dogs were treated initially with DEC: 7 and 3 of these dogs were free of disease after 1 and 2 procedures, respectively, while 2 dogs were cured after DC was used as a second procedure. Five dogs were treated

with DC only: in 3 dogs with moderate disease, cure was obtained after a single procedure while, in 2 debilitated patients, cure could not be confirmed. Topical administration of 1% bifonazole cream appears as an effective therapy in SNA, either as an adjunctive therapy to enilconazole infusion or as sole therapy in moderately affected patients.

Sharman *et al.* (2012) treated 9 client-owned dogs diagnosed with mycotic **rhinosinusitis** between March 2008 and December 2009 with either **clotrimazol**e (1% in polyethylene glycol) or **enilconazole** (10% solution), after imaging and rhinoscopic assessment. Both frontal sinuses were trephined, debrided and flushed with saline. Infusion was administered via frontal sinuses with dogs in sternal recumbency and computed tomography (CT) performed 5 minutes after completion. Distribution was scored 1 to 4 at the canine tooth, premolar 4, cribriform plate and frontal sinus on both sides, for a maximum score of 32. Distribution of antifungal agents to all regions of the nasal cavity and frontal sinuses was achievable, but varied considerably. Retention was poor in 10 of 18 regions assessed.

Talbot *et al.* (2015) assessed the susceptibilities of isolates collected between 1988 and 2014 from 46 **dogs** and 4 **cats** to **itraconazole, posaconazole, voriconazole, fluconazole and ketoconazole** using Sensititre Yeast One microdilution trays; and to enilconazole and clotrimazole, following the CLSI M38-A2 standard. For the majority of isolates MICs were high for ketoconazole, low for enilconazole and clotrimazole, and less than established epidemiological cut-off values for itraconazole, posaconazole and voriconazole. One canine isolate from 1992 had multiazole resistance and on Cyp51A gene sequencing a mutation associated with azole resistance (F46Y) was detected. There is no evidence of emerging azole resistance among A. fumigatus isolates from dogs and cats and topical azole therapy should be effective against most isolates.

Corrigan *et al.* (2016) performed a study to determine the safety and efficacy of **posaconazole** for the **treatment** of naturally occurring **disseminated** Aspergillus infections in dogs. Posaconazole was administered to 10 dogs at dosage of 5 mg/kg PO q12h. The treatment response for dogs with disseminated disease while receiving posaconazole was defined as clinical remission (n = 4) and clinical improvement (n = 6). There was a high rate of relapse during treatment or after cessation of treatment in both groups, and most dogs died or were euthanized due to progressive disease. Excluding 1 dog concurrently treated with terbinafine that remains alive 5 years after diagnosis, the mean survival time for dogs was 241 days (range 44-516 days). Three other dogs lived >1 year after starting treatment. No clinically relevant adverse events or increases in serum liver enzyme activity occurred during treatment with posaconazole.

1.12. Reports on aspergillosis in cats

Hamilton *et al.* (2000) reported a case of nasal, frontal sinus, and orbital aspergillosis in a cat . Diagnostics for exophthalmos and therapy for retrobulbar abscesses were discussed.

Whitney *et al*. (2005) reported four cats with fungal rhinitis. Serological testing was not useful in two cats tested. The cats in this study were treated with oral itraconazole therapy. When itraconazole therapy was discontinued prematurely, clinical signs recurred. Hepatotoxicosis is a possible sequel to itraconazole therapy.

Kano *et al.* (2008) reported for the first time ***A. udagawae*,** a previously recognised but rare opportunistic pathogen causing fatal orbital aspergillosis in a cat. Identification of this isolate was secured by comparative sequence based analyses of the ITS and the beta tubulin region. Antifungal susceptibility testing results revealed that this isolate had high in vitro MIC to amphotericin B (AMB) that correlated with in vivo failure of therapy with AMB.

Barachetti *et al.* (2009) reported a 12-year-old, 4 kg, castrated male Persian cat with a 2-month history of sneezing and bilateral mucopurulent nasal discharge. Rhinoscopically acquired nasal biopsies at this time revealed bilateral lymphoplasmacytic **rhinitis**. A tapering dose of oral prednisone caused the complete remission of the clinical signs, but 2 months after discontinuation of the therapy, the rhinitis recurred and the OD became exophthalmic. Computed tomography showed a soft tissue mass in both sides of the nasal cavity, both frontal sinuses, the right orbit, and to a lesser extent the left orbit. A fine needle aspirate of the right orbit revealed **pyogranulomatous inflammation** and Aspergillus spp. hyphae. Repeat nasal biopsy demonstrated multi-focal necrosis and a mixed inflammatory cell process which now included macrophages and scattered septate fungal hyphae. A few days later the cat became bilaterally blind and a contrast enhancing lesion involving the optic chiasm was found on magnetic resonance imaging. Despite a poor prognosis, therapy consisted of exenteration of the right orbit and trephination of both frontal sinuses before the planned initiation of medical antifungal therapy. Unfortunately, the cat died of cardiac arrest intraoperatively. ***Aspergillus fumigatus*** was cultured from both orbits at necropsy. Orbital aspergillosis has been rarely reported in cats and its relationship with lymphoplasmacytic rhinitis is unclear. In this patient lymphoplasmacytic rhinitis or previous antibiotic/corticosteroid therapy may have allowed secondary fungal invasion of the nasal mucosa and subsequently both orbits and the brain. Alternatively, Aspergillus infection may have preceded the lymphoplasmacytic rhinitis.

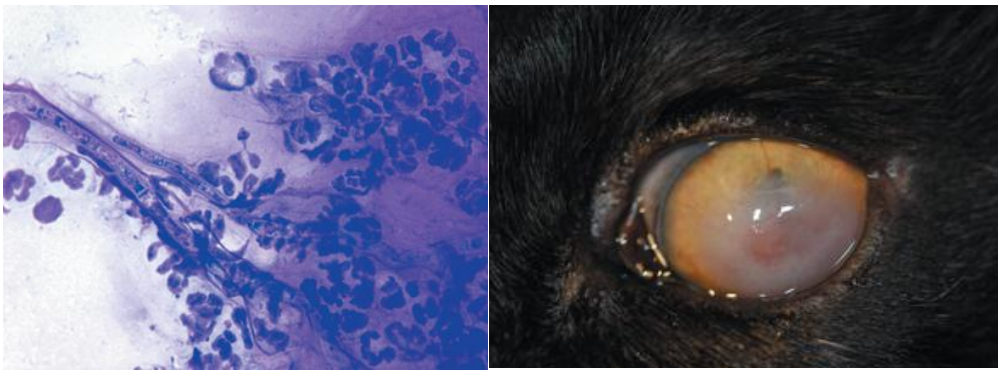
Furrow and Gromen (2009) examined 2 cats (13 and 11 years old) to determine the cause of nasal discharge of varying duration (4 days and 5 months, respectively). Computed tomography revealed marked turbinate destruction and soft tissue densities in the nasal passages. Histologic examination of nasal specimens revealed chronic active inflammation and branching fungal hyphae consistent with Aspergillus spp. Fungal culture of nasal specimens resulted in growth of **Aspergillus spp**. Testing yielded negative results for antibodies against Aspergillus spp.Both cats were anesthetized and treated with a 1-hour intranasal infusion of clotrimazole. Recovery from the procedure was uncomplicated, and both cats had complete resolution of clinical signs.

Karnik *et al.* (2009) reported the computed tomographic (CT) findings of fungal rhinitis/sinusitis in cats. The CT images of 10 cats ranging in age from 7 to 13 years were examined. The mean age was 10.8 years and all were neutered males. **Nasal aspergillosis** was diagnosed in five cats, cryptococcosis in three cats, hyalohyphomycosis in one cat, and trichosporonosis in one cat. Bilateral disease was present in eight cats, seven had abnormal soft tissue attenuation in two-thirds of the nasal cavity, and six had turbinate lysis. Seven cats had also lysis of the hard palate, nasal septum, or frontal bone. One cat had lysis of the cribriform plate. Five of the nine cats whose lymph nodes were imaged had lymph node enlargement. There was contrast medium enhancement in the nasal cavity in all cats, with either a primarily peripheral rim or heterogeneous pattern. There appears to be an overlap of clinical

signs, age, and CT features of cats with nasal neoplasia and those with fungal rhinitis/ sinusitis.

Labelle *et al*. (2009) reported an 8-year-old male castrated Domestic Short-haired cat with a 1-week history of blepharospasm and mucoid ocular discharge. Examination revealed **ulcerative keratitis** with stromal loss, stromal infiltrate, corneal edema, perilimbal vascularization and miosis. Cytology of the cornea revealed multiple dichotomously branching, septate fungal hyphae and severe, predominantly neutrophilic inflammation. PCR of the cytology samples confirmed the presence of ***Aspergillus flavus*** while fungal and bacterial cultures were negative. Treatment with topical 1% voriconazole solution was successful in resolving the keratomycosis.

OS at initial presentation. Note ulceration of the cornea with loss of approximately 50% of the corneal stroma and a necrotic focus in the axial cornea, diffuse corneal edema, white/yellow stromal infiltrate and mid to deep stromal vascular invasion of the cornea. OS cornea after 11 days of topical antifungal therapy. Note the progression of the vascularization. **Labelle *et al*. (2009)**



Corneal cytology demonstrating suppurative inflammation and fungal hyphae. OS cornea after 29 days of treatment. Note the clear peripheral cornea and remodeling of the axial cornea. At this time the cat was visual and comfortable. **Labelle *et al*. (2009)**

Giordano et al. (2010) described a cat diagnosed with sinonasal orbital ***Aspergillus fumigatus*** infection using advanced imaging, histopathology and culture.

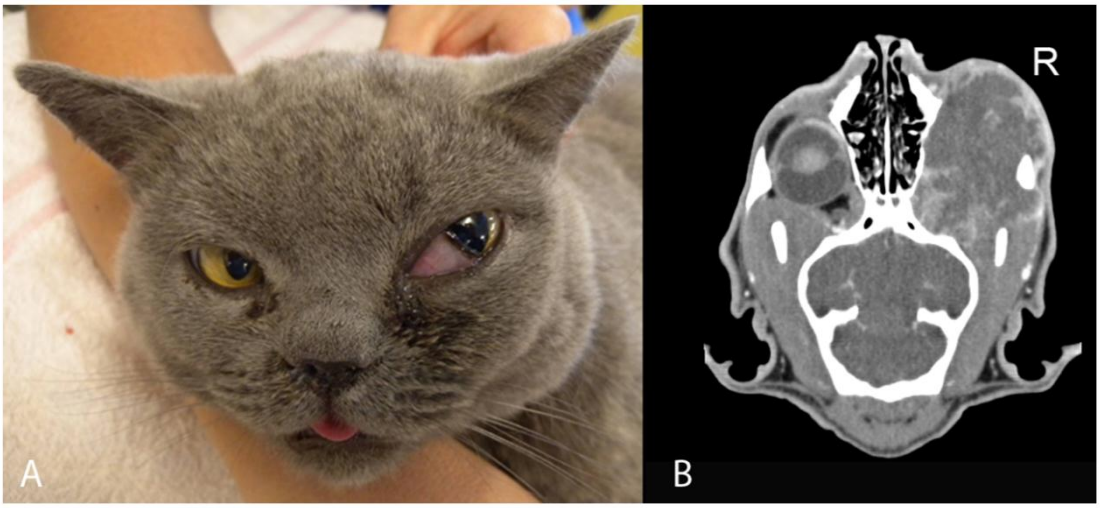
Smith and Hoffman (2010) diagnosed feline **orbital aspergillosis** in three cats with **exophthalmos**, significant dorso-temporal globe deviation and pronounced resistance to retropulsion. Advanced imaging was performed in all three cases to evaluate the extent of disease as well as to obtain guided orbital biopsies in two cases. Surgical intervention in the form of a lateral orbitotomy was pursued in the first case with the other two cases treated with enucleation or medical management alone. The available published reports concerning sino-orbital aspergillosis are reviewed. Oral therapy with a novel **triazole, voriconazole**, was instituted in two cases. Although voriconazole was apparently effective against the fungal organisms, it is also resulted in adverse reactions.

Hazell *et al.* (2011) reported a 7-year-old, spayed female Domestic Longhair cat with a 6-week history of coughing. Thoracic radiography revealed a pleural effusion. Thoracic ultrasound revealed a pleural effusion and a focal lung mass. The cat underwent exploratory thoracotomy and a total left pneumonectomy was performed. Histopathology and cultures revealed fungal **pneumonia and pyothorax** caused by ***Aspergillus fumigatus***. Abdominal ultrasound, repeat thoracic radiography, urinalysis with culture, and retroviral screening failed to detect evidence of systemic disease. The cat's poorly regulated diabetes mellitus is suspected to be the predisposing factor allowing a fungal pulmonary infection to become established. At 18 months after surgery the cat was still disease-free. To our knowledge this is the first reported case of successful treatment of pulmonary aspergillosis in the cat.

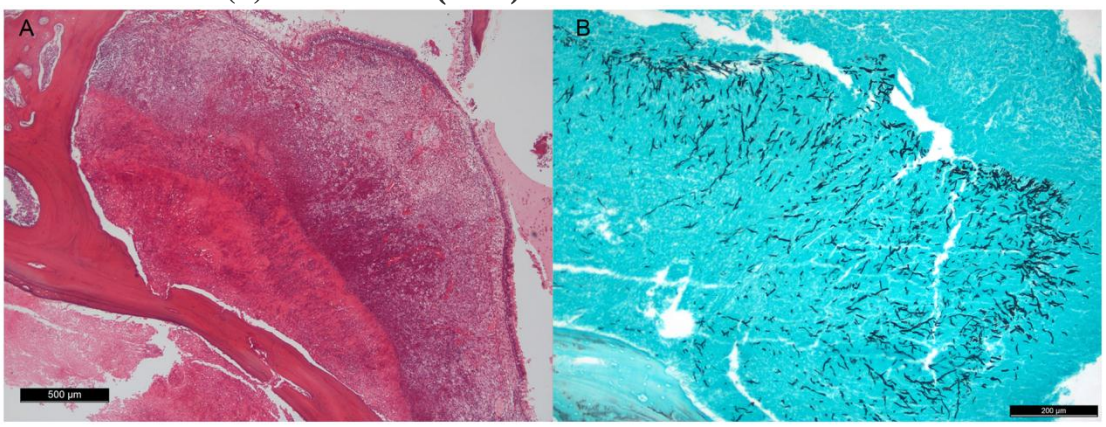
Barrs *et al.* (2012) documented the aetiology, clinicopathological findings and treatment outcomes in 23 cats (1.5-13 years of age) with sinonasal (SNA, n=6) or sino-orbital (SOA, n=17) aspergillosis. Cases recruited retrospectively and prospectively were included if fungal hyphae were identified on cytological or histological examination and the fungal pathogen was identified by PCR and DNA sequencing (ITS1 or ITS1-5.8S-ITS2 regions, rDNA gene cluster). Fungal culture was positive in 22/23 cases. In cases of SNA, the fungal pathogen was Aspergillus fumigatus (n=4), Neosartorya fischeri or A. lentulus (n=1) or a non-speciated Neosartorya spp. (n=1). In all cases of SOA (n=17), the fungal pathogen was identified as Neosartorya spp. Nine cats had brachycephalic conformation. Cats with SNA were more likely to be infected with A. fumigatus and had a better prognosis than cats with SOA.

Eye and orbital cavity of a cat with sino-orbital aspergillosis. Top: a. Exophthalmos, b. mass in the left pterygopalatine fossa and c. ulceration of the hard palate. Bottom left: Cleistothecia (small spherical structures) at the colony junction in paired cultures of *Neosartorya* spp. isolates from two cases (inset). *Neosartorya* spp. ascospores with roughened side walls and two axial crests. Scanning electron micrograph (Zeiss EVO LS15). Scale bar = 2 µm. Bottom right: Periodic acid-Schiff-stained section of frontal sinus epithelium from a cat with invasive sino-orbital aspergillosis. Fungal hyphae are present deep within the sinus epithelium, demonstrating the invasive nature of this mycosis. Scale bar = 60 µm. **Barrs *et al.* (2012)**

Barrs *et al.* (2013) described *A. felis* (neosartorya-morph) isolated from three host species with invasive aspergillosis including a human patient with chronic invasive pulmonary aspergillosis, domestic cats with invasive fungal rhinosinusitis and **a dog with disseminated invasive aspergillosis.** Disease in all host species was often refractory to aggressive antifungal therapeutic regimens. Four other human isolates previously reported as *A. viridinutans* were identified as ***A. felis*** on comparative sequence analysis of the partial β-tubulin and/or calmodulin genes.

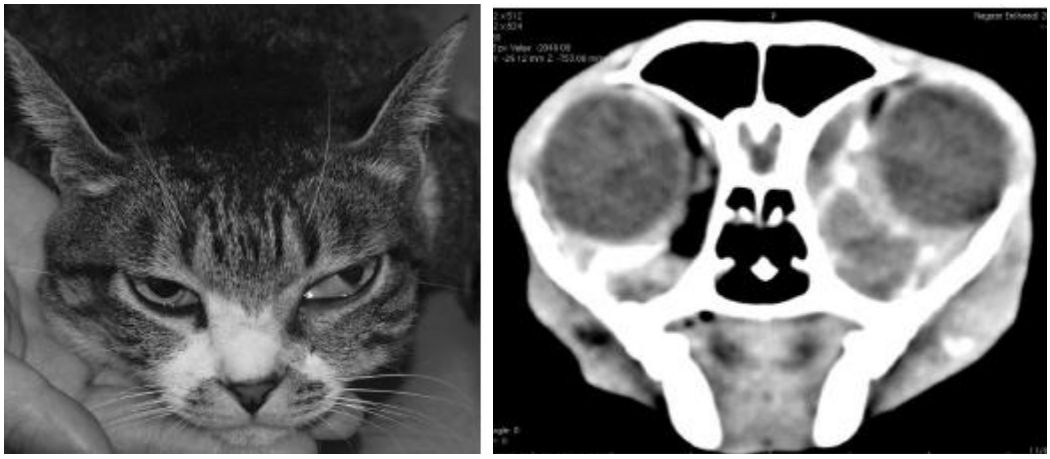


Cat with sino-orbital aspergillosis (invasive fungal rhinosinusitis) caused by A. felis with exophthalmia and prolapse of the nictitating membrane (third eyelid) associated with a retrobulbar fungal granuloma (A).Coronal CT scan soft-tissue post-contrast view showing retrobulbar fungal granuloma occupying the inferior aspect of the orbit with involvement of the adjacent paranasal subcutaneous tissues (B). **Barrs *et al.* (2013)**

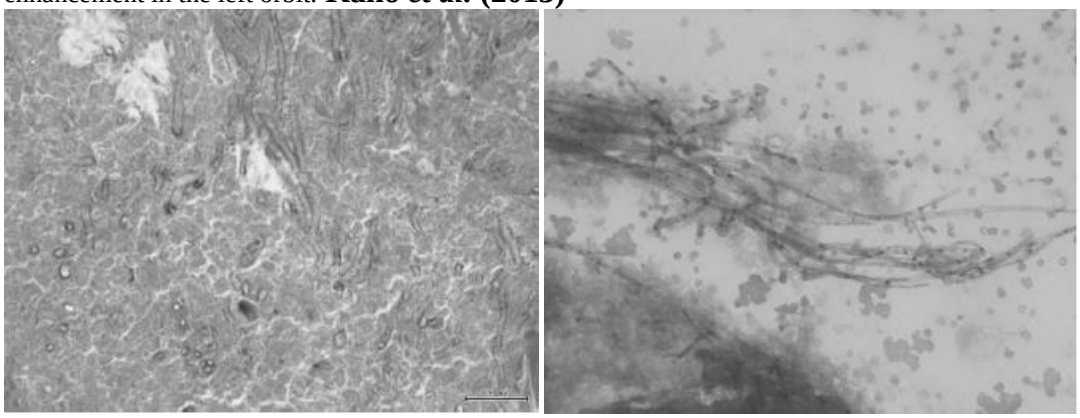


Tissue invasion by fungal hyphae in a cat with SOA.Hematoxin & Eosin- (A) and Grocott- (B) stained section of nasal mucosa and turbinates demonstrating granulomatous rhinitis (A) and submucosal invasion by septate branching fungal hyphae (B). **Barrs *et al.* (2013)**

Kano *et al.* (2013) reported two cases of feline **orbital aspergillosis**, one caused by ***A. udagawae*** and the other by ***A. viridinutans***. Case was treated with a high dosage of itraconazole, and in case A. viridinutans infection was associated with sarcoma. Identification of the etiologic agents of these cases was confirmed by comparative analyses of the sequences of β-tubulin-encoding genes. With the spread of non-fumigatus aspergillosis, increasing emphasis should be placed on molecular identification of the infecting Aspergillus species and the use of in vitro drug susceptibility tests to ensure the selection of appropriate antibiotics.

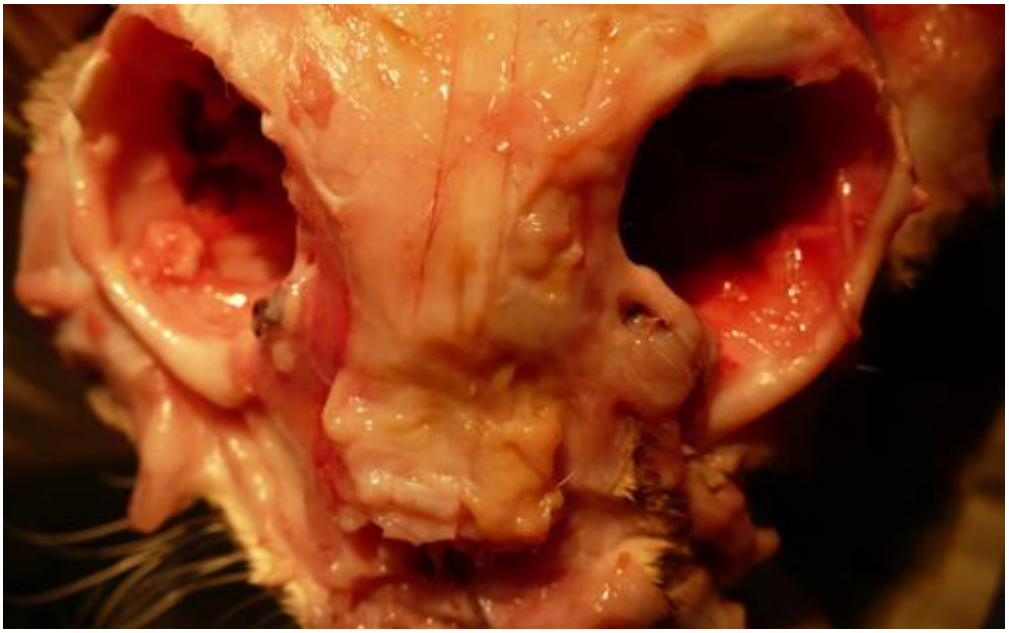


The progressive protrusion of the left third eyelid and eyeball of case 1, Computed tomography (CT) image of Case 1 with contrast demonstrating a soft tissue mass lesion with nonhomogeneous enhancement in the left orbit. **Kano *et al.* (2013)**



Histopathologic examination of the mass from the left orbit of Case 1 revealed granulomatous inflammation with many branching hyphal filaments (PAS stain)., Impression smear of the biopsy specimen of the mass from the left eye orbit of Case 2 revealed many branching hyphal filaments (PAS stain). **Kano *et al.* (2013)**

Veterinary hospital in Oxfordshire (2013) reported a 13-year old female Burmilla cat with a history of left-side unilateral nasal discharge. Rhinoscopy revealed mucopurulent discharge on the left side but was otherwise unremarkable. Aspiration of the swelling over the left frontal sinus produced pus and this abscess was lanced and flushed. The frontal sinus was trephined and the sinus and nasal cavity flushed with saline. Cytology of the material flushed from the frontal sinus and nasal cavity revealed fungal hyphae consistent with Aspergillus species and culture of this material yielded growth of a fungus which was morphologically similar to ***Aspergillus candidus***. The cat was then started on oral itraconazole (Itrafungol, Janssen) 10 mg/kg p.o. SID. The abscess over the rostral frontal sinus did not heal and a second abscess appeared over the nasal bone just dorsal to the nose. The left nasal cavity and sinus were full of pus and debris and there was severe bone erosion from the nasal cavity into the rostromedial orbit through which pus was protruding. There was also severe bone erosion rostrally through the nasal bone and less severe bone erosion dorsally over the rostral part of the frontal sinus, these sites of bone erosion being at the location of the two subcutaneous abscesses.

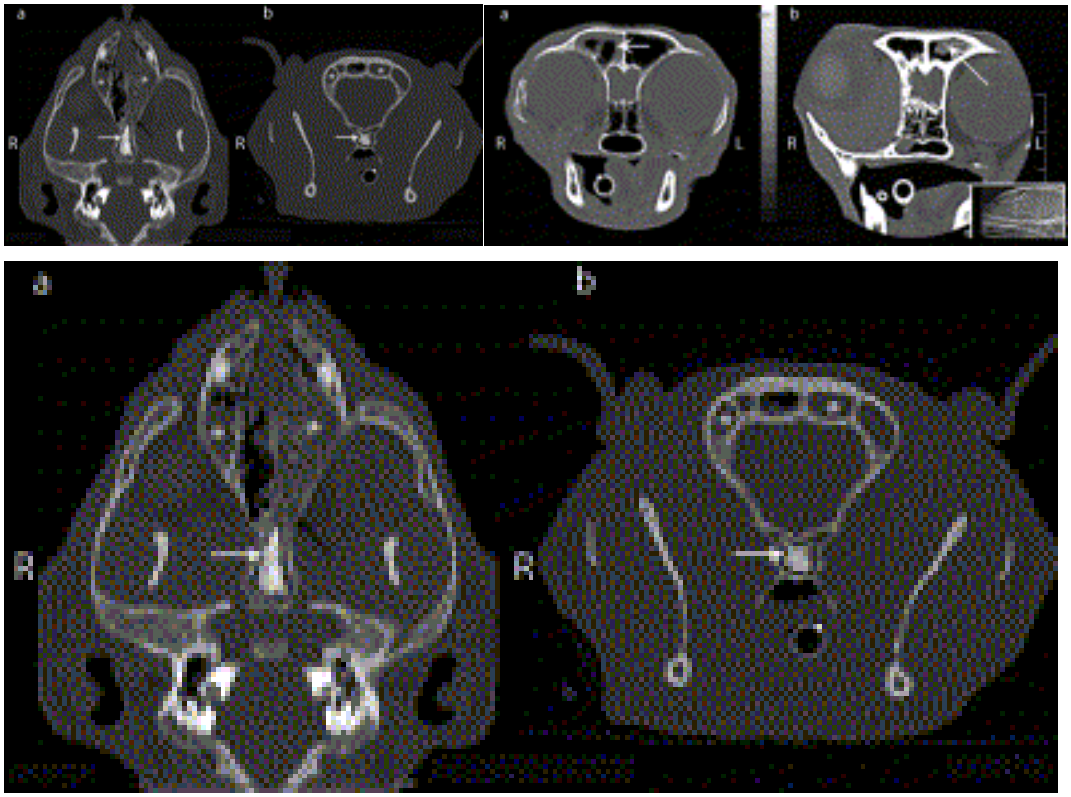


www.aspergillus.org.ukNasal, sinus and orbital aspergillosis in a cat. Nose- severe bone erosion rostrally through the nasal bone

Whitney *et al.* (2013) evaluated serum GM measurement as a non-invasive diagnostic test for URT aspergillosis in cats. A one-stage, immunoenzymatic sandwich ELISA was used to detect serum GM in 4 groups of cats; Group 1 (URT aspergillosis) - confirmed URT aspergillosis (n=13, sinonasal aspergillosis (SNA) n=6 and sino-orbital aspergillosis (SOA) n=7), Group 2 (URT other) - other URT diseases (n=15), Group 3 (β-lactam) - cats treated with β-lactam antibiotics for non-respiratory tract disease (n=14), Group 4a - healthy young cats (≤ 1 y of age, n=28), Group 4b - healthy adult cats (>1 y of age, n=16). One cat with SNA and two cats with SOA caused by an Aspergillus fumigatus-mimetic species, tested positive for serum GM. For a cut-off optical density index of 1.5, the overall sensitivity and specificity of the assay was 23% and 78% respectively. False positive results occurred in 29% of cats in Group 3 and 32% of cats in Group 4a. Specificity increased to 90% when Groups 3 and 4a were excluded from the analysis. Overall, serum GM measurement has a poor sensitivity but is a moderately specific, non-invasive screening test to rule out infection in patients with suspected feline upper respiratory tract aspergillosis.

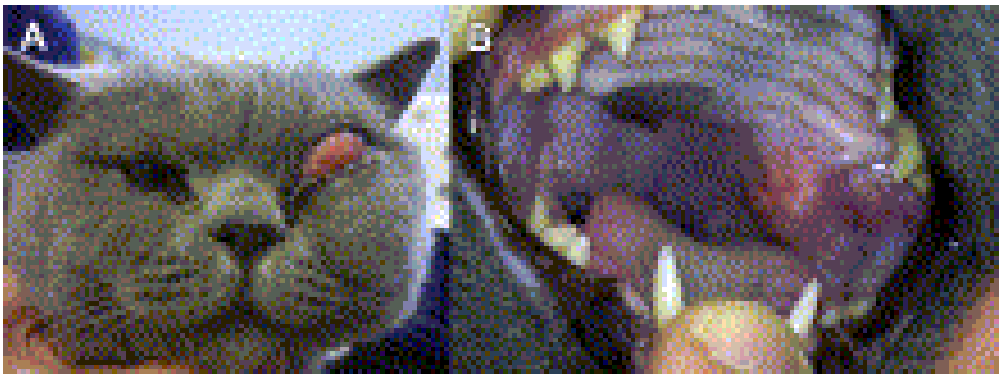
Barrs *et al.* (2014) reported feline upper respiratory tract aspergillosis (URTA) as two distinct anatomical forms, namely, **sino-nasal aspergillosis** (SNA) and **sino-orbital aspergillosis** (SOA). Computed tomography was used to investigate the route of fungal infection and extension in 16 cases (SNA n = 7, SOA n = 9) where the infecting isolate had been identified by molecular testing. All cases had nasal cavity involvement except for one cat with SNA that had unilateral frontal sinus changes. There was a strong association between the infecting species and anatomic form (P = 0.005). ***A. fumigatus*** infections remained within the sino-nasal cavity, while cryptic species infections were associated with orbital and paranasal soft-tissue involvement and with orbital lysis. Cryptic species were further associated with a mass in the nasal cavity, paranasal sinuses or nasopharynx. Orbital masses showed heterogeneous contrast enhancement, with central coalescing hypoattenuating foci and peripheral rim enhancement. Severe, cavitated turbinate lysis, typical of canine SNA, was present only in cats with SNA. These findings support the hypothesis that the nasal cavity is the portal of entry for fungal spores in feline URTA and that the route of extension to involve the orbit is via direct naso-orbital communication from

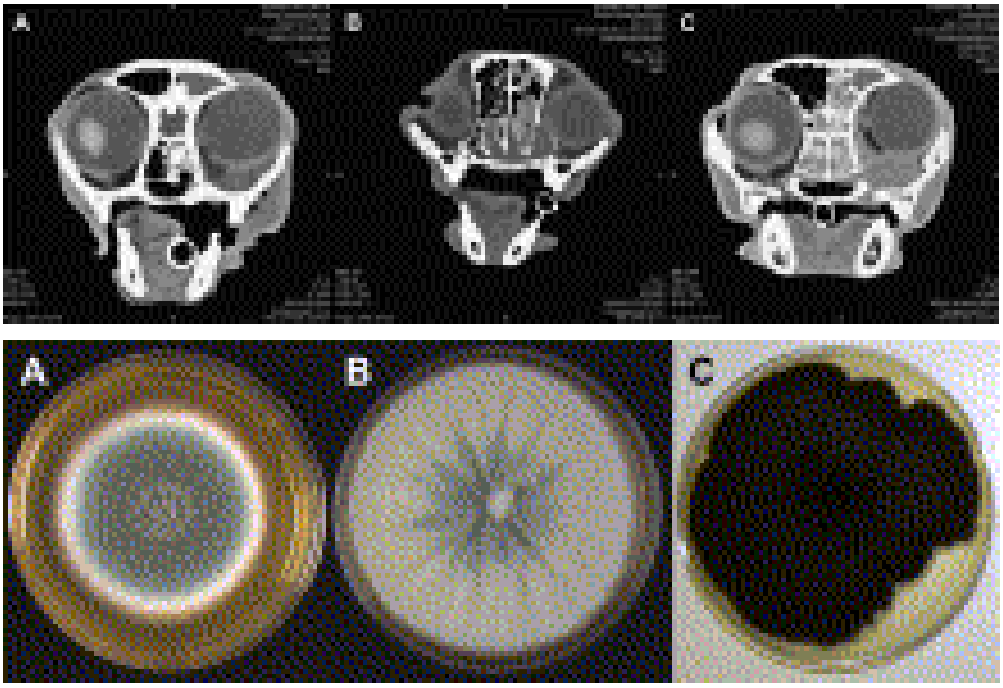
bone lysis. Additionally, a pathogenic role for ***A. wyomingensis*** and a sinolith in a cat with ***A. udagawae*** infection were reported for the first time.



Barrs *et al.* (2014)

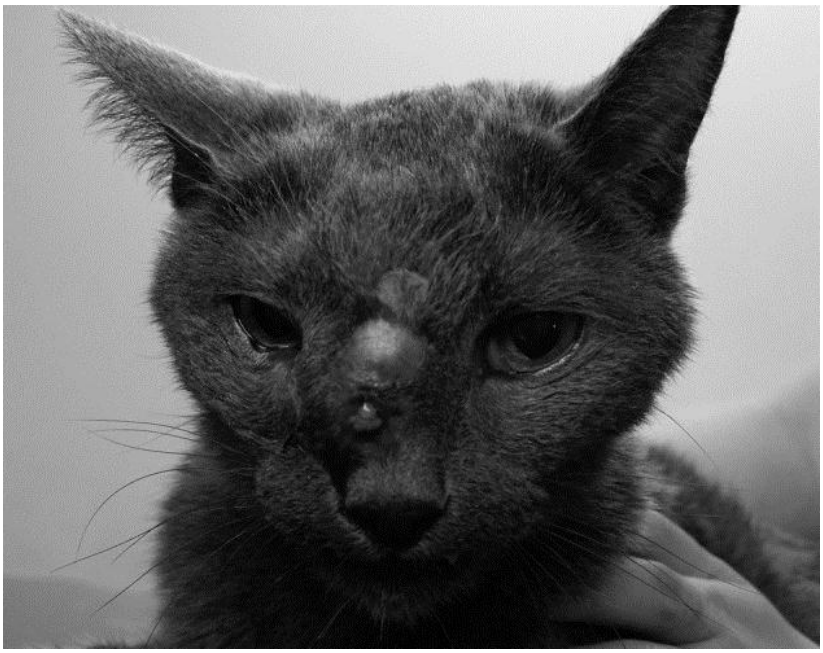
Barrs and Talbot (2014) mentioned that feline aspergillosis includes sinonasal aspergillosis (SNA), sino-orbital aspergillosis (SOA), other focal invasive forms, and disseminated disease. SOA is an invasive mycosis that is being increasingly recognized, and is most commonly caused by a recently discovered pathogen Aspergillus felis. SNA can be invasive or noninvasive and is most commonly caused by ***Aspergillus fumigatus*** and ***Aspergillus niger***. Molecular methods are required to correctly identify the fungi that cause SNA and SOA. SNA has a favorable prognosis with treatment, whereas the prognosis for SOA remains poor.



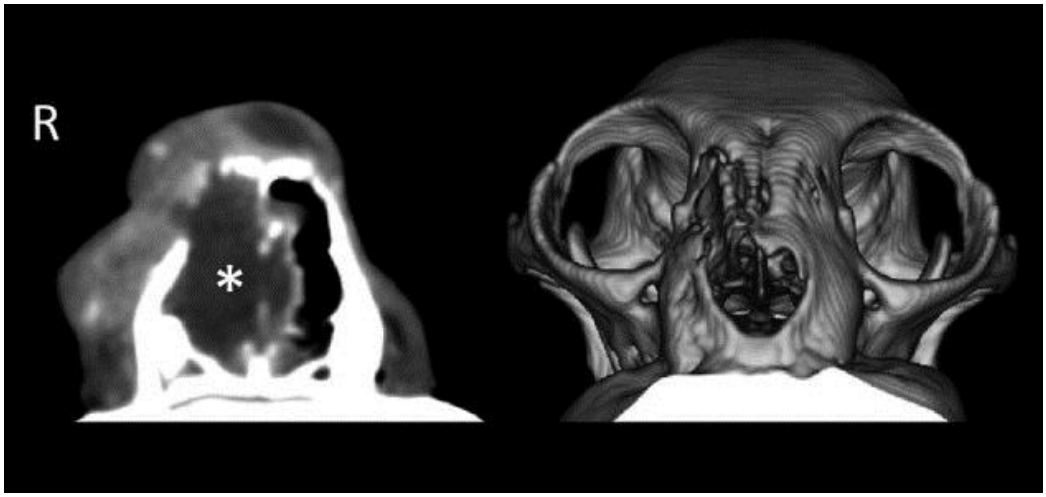


Barrs and Talbot (2014)

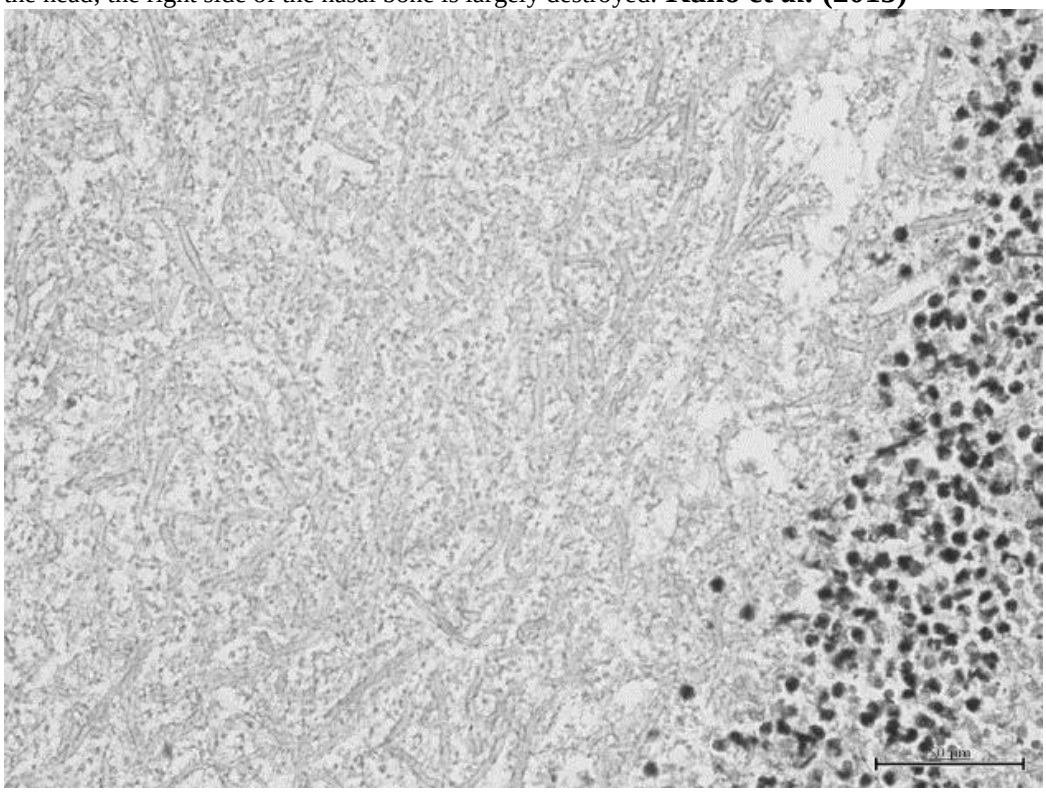
Kano et *al.* (2015) considered feline upper respiratory tract infection due to **Aspergillus spp**. is considered an emerging disease, with the number of reported cases continuing to rise. They repored the first case of feline **sinonasal aspergillosis** caused by ***Aspergillus fischeri*** in Japan. The patient presented after 2 months of progressive facial deformity around the nose and nasal discharge. The isolate from this case was susceptible to itraconazole (ITZ), voriconazole and micafungin, but was resistant to amphotericine B. However, the infected cat died approximately 1 month after referral, despite treatment for 12 days ITZ administered orally at 10 mg/kg.



Pus discharge and an ulcer were observed on the mass on the left side of the bridge of the nose. **Kano et *al.* (2015)**



The left panel shows a computed tomographic (CT) image after administration of a contrast agent at the level of the canine teeth. The animal's right nasal cavity (R) is occupied by a large mass (*) that lacks contrast enhancement. The right panel shows a reconstructed image from multiple CT images of the head; the right side of the nasal bone is largely destroyed. **Kano et *al.* (2015)**



Histopathologic examination of the mass from the right nasal cavity of the case revealed chronic purulent inflammation with many branching hyphal filaments (HE stain). **Kano et *al.* (2015)**

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2. Penicilliosis in cats and dogs

2.1. Introduction

Infections with *Penicillium* spp are rare in domestic animals. In dogs, infections of the nasal cavity, lungs, lymph nodes, and bones have been reported. Nasal disease is most common and behaves similar to nasal aspergillosis. In cats, the fungus has been isolated from the nasal cavity, orbital cellulitis and sinusitis, and lungs. It has also been reported to cause systemic disease in captive toucanets (*P griseofulvum*) and bamboo rats (*P marneffei*) in southeast Asia. *Penicillium* spp are widely distributed in nature and are found in soils, grains, and various foods and feeds **(Joseph Taboada, 2014, http://www.merckvetmanual.com).**

2.2. Clinical Findings and Lesions

Dogs with nasal penicilliosis have chronic sneezing and an acute to chronic nasal discharge that varies from intermittent hemorrhagic to intermittent or continuous mucoid or mucopurulent. Radiographic findings include areas of turbinate destruction with increased radiolucency. Grossly, the nasal mucosa has foci of necrosis and ulceration; microscopically, fungal hyphae may form a thick mat over an intact mucosa adjacent to these foci. Systemic disease often affects long bones, resulting in lameness.

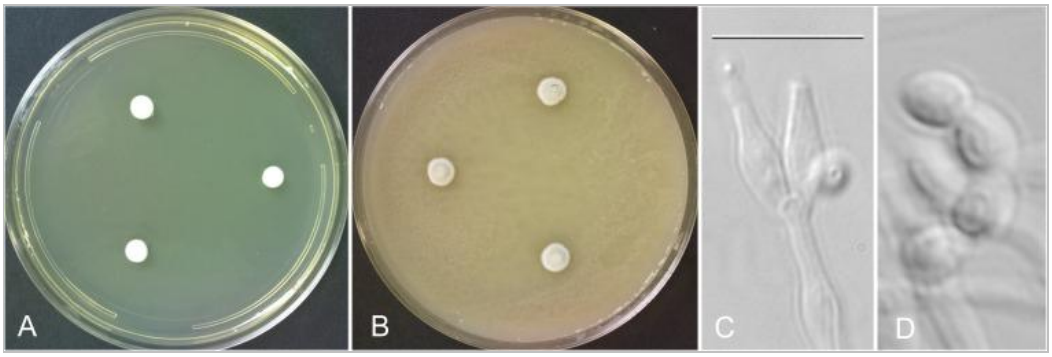
2.3. Reported causes of penicilliosis in cats and dogs

P. species **(Harvey, 1994), Whitney *et al.*, 2005, Soonthornsit *et al.*, 2013)**
P. purpurogenum **(Zanatta *et al.*, 2006)**
P. marneffei ***(Chaiwun et al., 2011)***
P. canis **(Langlois *et al.*, 2014)**

2.4. *Aetiology*

2.4.1. *Penicillium canis* S. W. Peterson, Journal of Clinical Microbiology 52 (7): 2450 (2014)

Colonies on CYA attain diameters of 6 to 7 mm after 7 days of incubation at 25°C, are white, and form a 2-mm-high cushion of loose vegetative hyphae. Sporulation is sparse and basal, with no exudate, soluble pigments, sclerotia, or ascomata. The reverse is yellowish near chamois in color. Colonies on MEA attain diameters of 7 to 8 mm after 7 days of incubation at 25°C, are white, and form a 2- to 3-mm raised cushion of largely vegetative hyphae. Sporulation is sparse, with no exudate or soluble pigments and no sclerotia or ascomata, and the reverse is a pale drab. Colonies on MGA incubated for 7 days at 25°C attain diameters of 5 to 6 mm, form a 2- to 3-mm raised cushion, and sporulated heavily in a greenish gray color with no exudate, soluble pigments, sclerotia, or ascomata. The colony reverse is not visible on this medium. There is no growth on CYA at 5°C; at 37°C, small, white, 2- to 3-mm-diameter colonies are formed after 7 days. Colonies grown at 25°C on CYA—5% NaCl or CYA–20% sucrose are 2 or 5 mm in diameter, respectively. Conidiophores are simple, arising from basal and aerial hyphae, smooth walled, hyaline, 5 to 25 by 1.5 to 2.5 μm, nonvesiculate, bearing an apical whorl of two to five ampuliform phialides 5 to 7 (uncommonly, up to 10) by 2 to 3 μm with a 1- to 2-μm collula, bearing ovoid, smooth-walled conidia 4 to 5 by 2 to 3 μm.

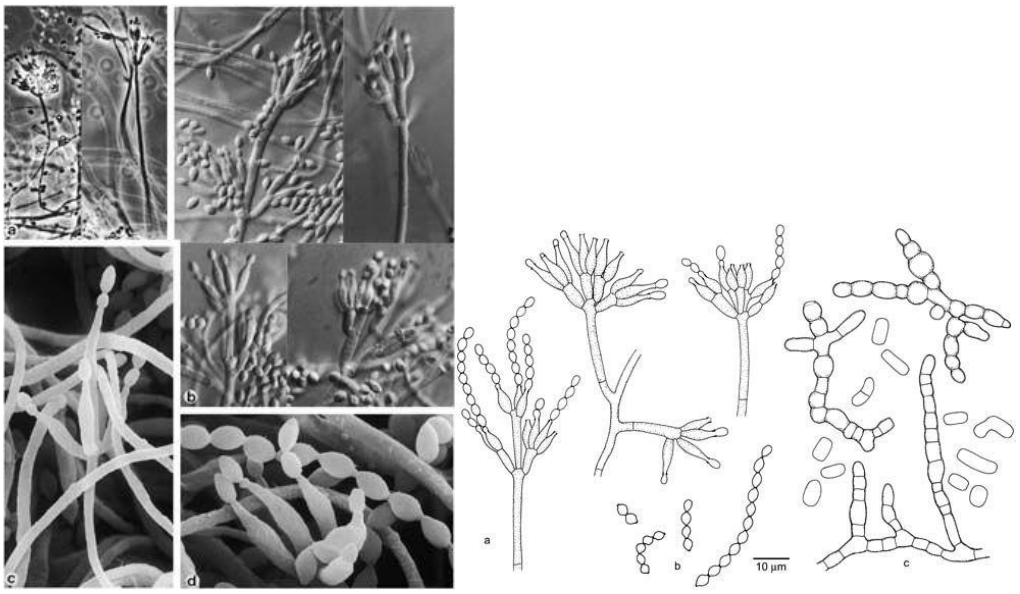


P. canis NRRL 62798. (A) Seven-day-old colonies grown on CYA at 25°C. (B) Seven-day-old colonies grown on MGA at 25°C. (C) Typical conidiophore with two apical phialides. (D) Characteristic elongate-ovoid, smooth-walled conidia. Bar = 10 µm for panels C and D

2.4.2. *Penicillium marneffei* Segretain, Capponi & Sureau, Bulletin de la Société Mycologique de France 75: 416 (1959)

Colonies (CzA, 30°C) flat, sparse, compact, greenish to purplish, on MEA exuding an orange or red pigment into the medium; primary cultures often canary yellow due to sterile aerial mycelium. At 37°C colonies restricted, whitish, yeast-like. Microscopy. Hyphae in part spirally twisted. Conidiophores creeping or fasciculate, 70-150 x 2.5-3.0 µm; penicilli generally biverticillate but also irregularly monoverticillate or more complex. Metulae 7-11 µm long, in whorls of 3-5. Phialides in whorls of 4-7, ampulliform to acerose, 6-10 x 2.5-3.0 µm. Conidia smooth-walled, ellipsoidal, 2.5-4.0 x 2-3 µm, often with prominent scars, borne in short, disordered chains.

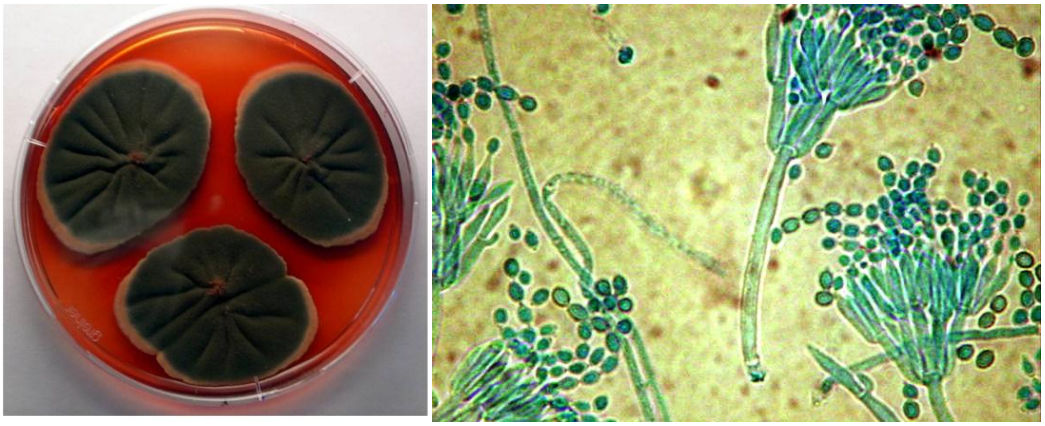
Penicillium marneffei en.wikipedia.org , citeseerx.ist.psu.edu



Mycobank

2.4.3. ***Penicillium purpurogenum*** Stoll sensu Raper & Thom, A manual of the Penicillia: 563-633 (1945)

Colonies on Czapek's solution agar (Col. Pl. X) growing rather restrictedly, attaining a diameter of 1.5 to 2.5 cm, in 12 to 14 days at room temperature (fig. 162A and E), sometimes definitely wrinkled, zonate or azonate, consisting of a yellow to orange-red mycelial felt bearing abundant conidial structures, or of massed conidial heads arising from aerial hyphae or directly from the substratum and superficially appearing velvety, or in some strains tending to become floccose with growing margin white or yellowish from an admixture of encrusted sterile hyphae; usually heavily sporing in central and sub-central areas, in deep yellow-green shades near lily green through deep slate green to dull greenish black (Ridgway, Pl. XLVII); exudate usually limited but in some strains fairly abundant, in orange-red shades; odor indistinct or slightly moldy; reverse in deep red to dark reddish purple shades, often approximating ox blood red (R., Pl. I), with surrounding agar similarly colored in somewhat lighter shades; conidiophores arising from the substratum and measuring up to 100 to 150 µm in length by 2.5 to 3.0 or 3.5 µm in diameter, or as branches from aerial hyphae and much shorter, about 40 to 50 µm, smooth-walled; penicilli typically biverticillate and symmetrical (fig. 162C and D), compact, usually consisting of a single verticil of 5 to 7 or 8 metulae, each terminating in a compact cluster of 4 to 6 parallel sterigmata bearing short conidial chains; metulae 10 to 14 µm by 2.5 to 3.0 µm; sterigmata mostly 10 to 12 µm by 2.0 to 2.5 µm, lanceolate in form, characteristically tapered; conidia elliptical to sub-globose in some strains, sometimes more or less apiculate, mostly 3.0 to 3.5 µm by 2.5 to 3.0 µm with walls typically heavy and irregularly roughened, sometimes showing distinct transverse bands, but in some strains almost smooth.



Penicillium purpurogenum microbialcellfactories.biomedcentral.com, www.drthrasher.org

2.5. Diagnosis

Diagnosis is based on fungal culture, character of the lesions, presence of fungal hyphae, and a positive agar-gel double-diffusion test. Cultural isolation of a *Penicillium* sp must be accompanied by demonstration of tissue invasion by the fungus for confirmation. In tissues, *P marneffei* closely resemble the yeast phase of *Histoplasma capsulatum*.

2.6. Treatment

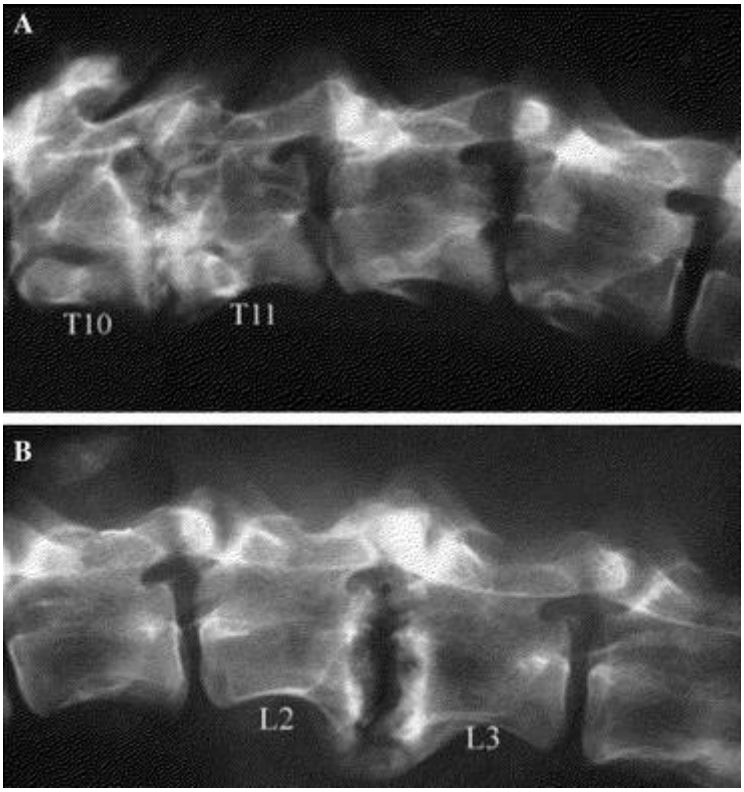
Very little has been reported concerning treatment of penicilliosis. Surgical turbinectomy with curettage has been combined with flushing of the nasal cavity with 1% tincture of iodine or povidone-iodine (10:1) and oral thiabendazole. Fluconazole, 2.5–5 mg/kg/day for 2 mo, has been used to successfully treat some dogs with nasal penicilliosis.

2.7. Reports:

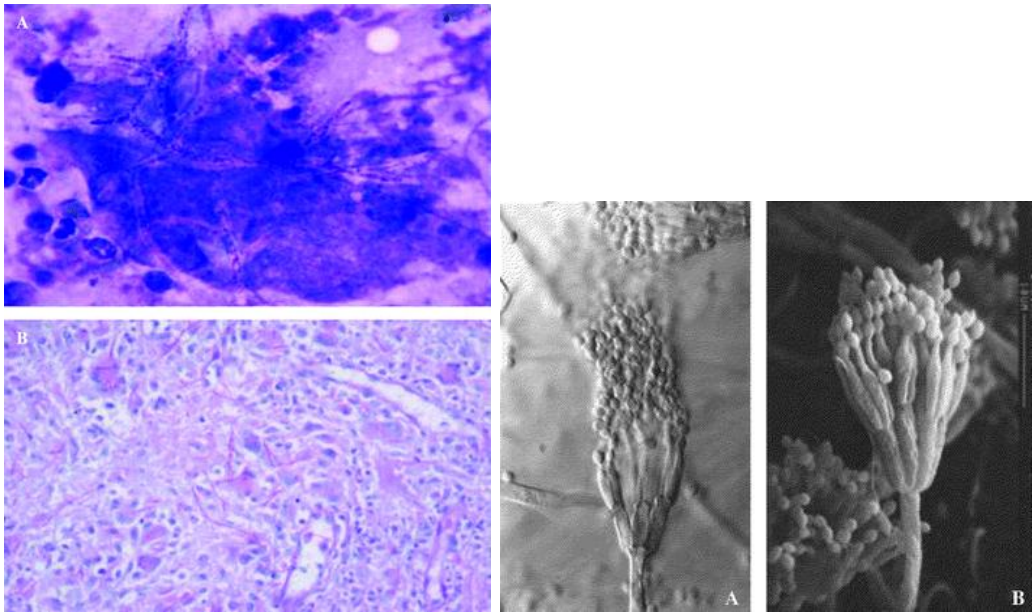
Harvey (1994) treated 47 dogs with nasal aspergillosis or penicilliosis with thiabendazole (20 mg/kg orally for 6 weeks). Nasal turbinectomy was performed on 26 of the dogs. Six months or more later, 43% of the dogs were clinically normal or considerably improved; results were better in dogs not treated surgically. It was concluded that thiabendazole at a dosage of 20 mg/kg is not an effective treatment for nasal aspergillosis or penicilliosis in dogs.

Whitney *et al.* (2005) mentioned that fungal rhinitis is uncommon in the cat and cases of nasal aspergillosis and penicilliosis have been rarely reported. Signs of fungal rhinitis include epistaxis, sneezing, mucopurulent nasal discharge and exophthalmous. Brachycephalic feline breeds seem to be at increased risk for development of nasal aspergillosis and penicilliosis. Computed tomography (CT) imaging and rhinoscopy are useful in assessing the extent of the disease and in obtaining diagnostic samples. Fungal culture may lead to false negative or positive results and must be used in conjunction with other diagnostic tests. Serological testing was not useful in two cats tested. The cats in this study were treated with oral itraconazole therapy. When itraconazole therapy was discontinued prematurely, clinical signs recurred. Hepatotoxicosis is a possible sequel to itraconazole therapy.

Zanatta *et al.* (2006) reported a 4-year-old female German shepherd dog (GSD) with forelimb instability and back pain. Clinical examination showed hyperthermia, generalized lymphadenomegaly and kyphosis. Radiological findings of the spine revealed areas of discospondilitis involving thoracic and lumbar vertebrae. Microscopic observations of fine needle aspiration biopsies (FNAB) of lymph-nodes showed regular, septate, branching fungal hyphae. Itraconazole therapy was started but the subject died six days later. Disseminated necrotic areas were detected in enlarged lymph-nodes, liver and spleen. Vertebral granulomas within lytic areas in T10-T11 and L2-L3, were observed. Cultures inoculated with samples obtained from lymph-node FNAB and bioptic material from necropsied organs revealed the presence of pure cultures of Penicillium, subsequently identified as ***P. purpurogenum***. Apart from female GSD's suspected predisposition to disseminated mycoses described in literature, no other predisposing factors were ascertained in this case.



(A) Radiographic examination of the spine. Area of discospondylitis involving T10–T11. The intervertebral disc space between T10–T11 is poorly defined. Diffuse periosteal reactions. (B) Radiographic examination of the spine. Area of discospondilitis involving L2 and L3. Litic area in the intervertebral disc, with irregular and sclerotic margins. Ventral periosteal reaction with poor defined margins. **Zanatta *et al.* (2006)**



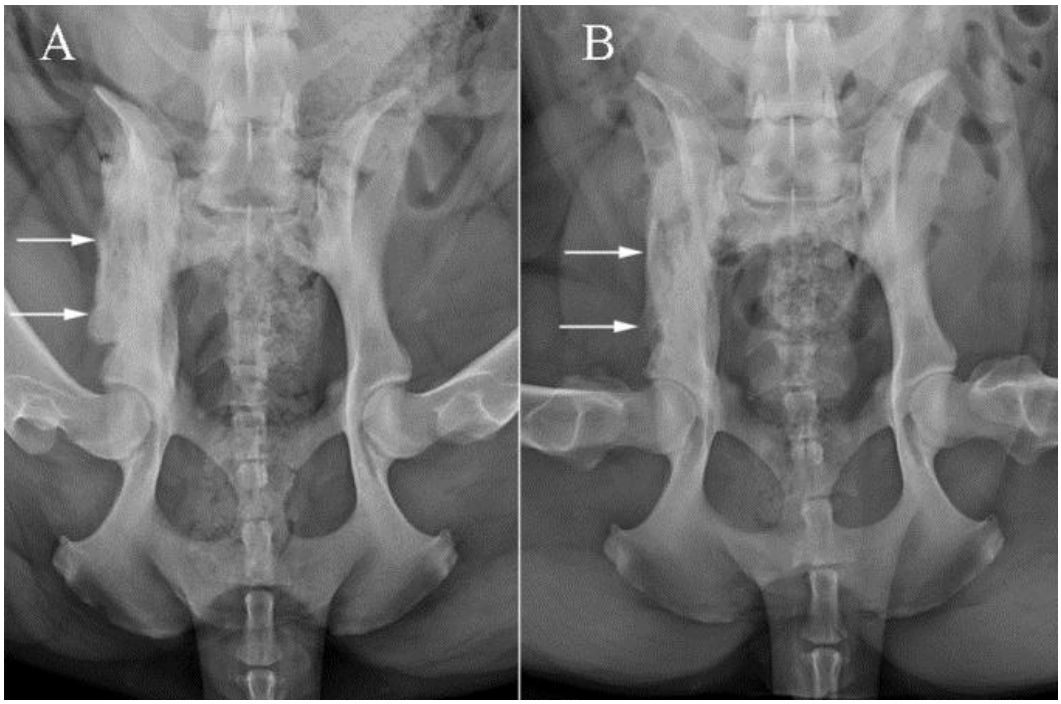
(A) Cytological examination of a prescapular lymph node. Granulomatous process with the presence of regular branching septate hyphae. (May-Grünwald Giemsa, ×100). (B) Histological examination of a prescapular lymph node. Granulomatous chronic reaction characterized by numerous giant multinucleated cells and encapsulating fibrosis. Diffuse branching septate hyphae at the periphery of the necrotic foci (PAS×400). (A) Conidiophore of *Penicillum purpurogenum*. Differential interference contrast (×1200). (B) Conidiophore of *P. purpurogenum*. Scanning electron microscopy. **Zanatta *et al.* (2006)**

Chaiwun et al. (2011) found that approximately 13% of nasal swabs from dogs in Chiang Mai, Thailand were positive for ***P. marneffei*** when tested by two different PCR methods, but culture results were negative. Sequencing the products from both PCR reactions showed 100% identity with *P. marneffei*, whereas no other known fungi shared both sequences. These results suggest that dogs might be an animal reservoir for *P. marneffei* in northern Thailand. This observation should be confirmed by additional studies.

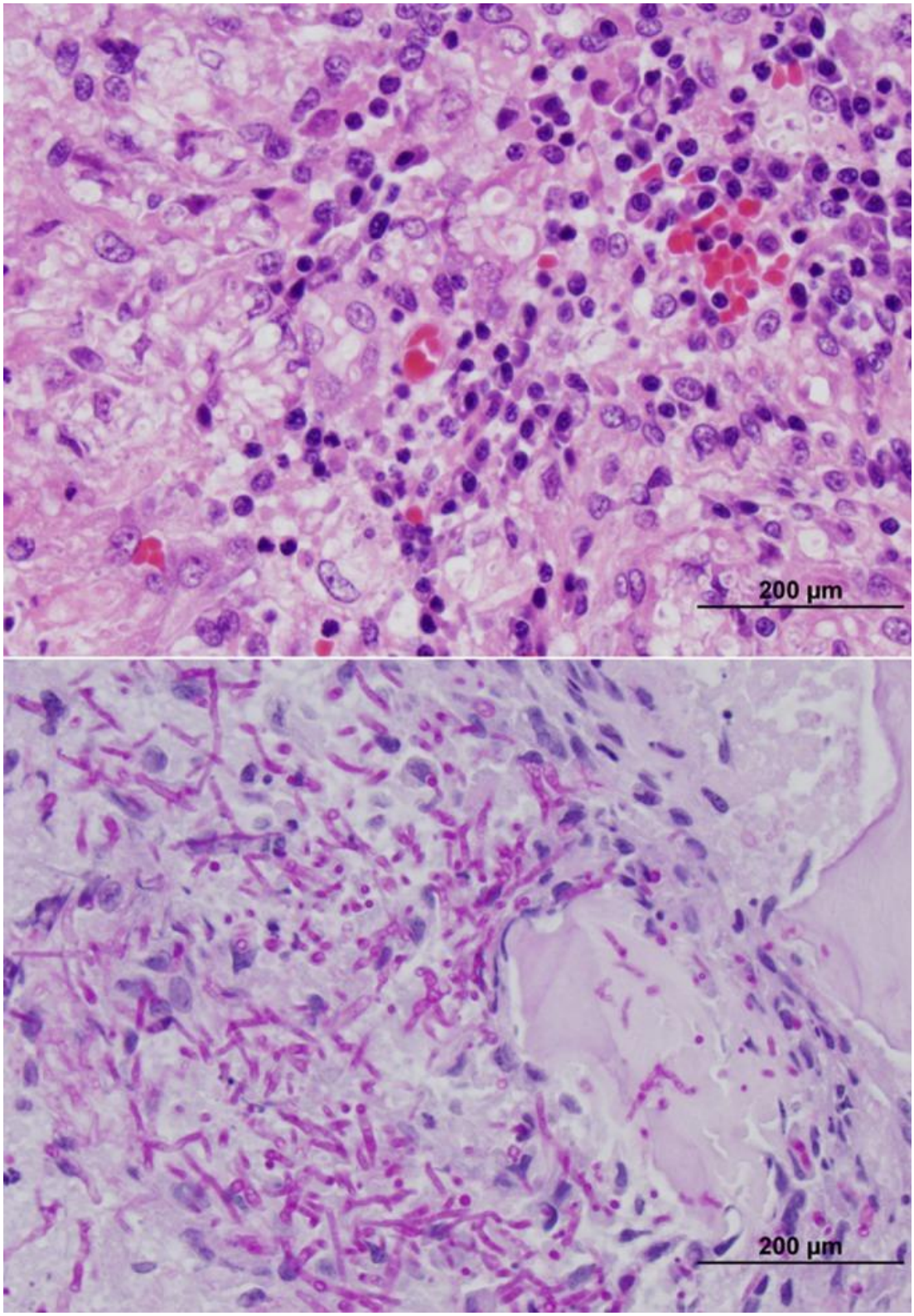
Soonthornsit *et al.* (2013) reported a 5-year-old, female neutered Persian cat with clinical signs of dysuria, haematuria and partial urethral obstruction that had manifested over several months. The animal also had hyperkalaemia and severe azotaemia at the time of presentation. Urinalysis showed haematuria, pyuria and the presence of several transitional cells. In addition, ultrasonography demonstrated an extraluminal mass between the neck of urinary bladder and the colon. Fine-needle aspiration of the mass revealed a fungal form with branching and septate hyphae. Consequently, itraconazole treatment was prescribed and clinical signs of improvement were seen after 7 days. However, 1 month later, the cat died of acute anaemia. Necropsy revealed the presence of extraluminal multifocal fungal granuloma at the neck of the urinary bladder, and contracted kidneys. Histopathological analysis of the fungal granuloma was found to be composed of branching, septate hyphal fungi together with inflammatory cells. Subsequent fungal culture and identification revealed this to be a species of Penicillium.

Langlois *et al.* (2014) isolated an unknown species of Penicillium from a bone lesion in a young dog with osteomyelitis of the right ilium. Extensive diagnostic evaluation did not reveal evidence of dissemination. Resolution of lameness and clinical stability

of disease were achieved with intravenous phospholipid-complexed amphotericin B initially, followed by long-term combination therapy with terbinafine and ketoconazole. A detailed morphological and molecular characterization of the mold was undertaken. Sequence analysis of the internal transcribed spacer revealed the isolate to be closely related to Penicillium menonorum and Penicillium pimiteouiense. Additional sequence analysis of β-tubulin, calmodulin, minichromosome maintenance factor, DNA-dependent RNA polymerase, and pre-rRNA processing protein revealed the isolate to be a novel species; the name ***Penicillium canis sp. nov***. is proposed. Morphologically, smooth, ovoid conidia, a greenish gray colony color, slow growth on all media, and a failure to form ascomata distinguish this species from closely related Penicillium species.



Ventrodorsal projections of the pelvis at presentation (A) and 3 months later (B). There is extensive mixed periosteal proliferation and permeative lysis along the right ilial body and wing in the initial radiograph, which are smoother and less severe in the subsequent image. **Langlois *et al.* (2014)**



Photomicrographs of sections from the bone biopsy specimen. (A) Hematoxylin-and-eosin staining; magnification, ×40. Bone segments were surrounded by marked granulomatous inflammation and small regions of necrosis. Intrahistiocytic and extracellular nonpigmented fungal organisms were documented throughout the section. Small numbers of lymphocytes, plasma cells, and neutrophils were also present throughout the section. (B) Periodic acid-Schiff staining; magnification, ×40. Note the large number of organisms within the cytoplasm of macrophages and free throughout the section. Intracellular and extracellular fungal organisms were nonpigmented and septate (3 to 4 µm in diameter by 10 to 20 µm in length) and exhibited occasional dichotomous branching. There were also round, 5-

to 7-µm-diameter fungal structures that likely represent cross sections of conidia. **Langlois *et al.* (2014)**

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3. Paecilomycosis in cats and dogs

3.1. Introduction

Paecilomycosis is a rare disease primarily of dogs, horses, reptiles, and humans. Clinical manifestations in veterinary patients vary but include disseminated disease and diskospondylitis, particularly in dogs: pneumonia in dogs, horses, and reptiles; keratitis in horses; and miscellaneous local infections. It is important to have an appropriate index of suspicion because the diagnosis can be difficult, particularly in localized disease where it is difficult to determine whether a positive culture represents an etiology or a contamination with an environmental saprophyte. Spinal radiographs, transtracheal washes, histopathology, and fungal culture have proven to be valuable diagnostic tools. The prognosis for paecilomycosis is poor, although some treatment success has been reported, and success rates could improve if additional information were available regarding fungal species occurring in veterinary patients and drugs to which these fungi are susceptible **(Foley *et al.*, 2002)**

- ***Paecilomyces* spp. infections** have been rarely reported in dogs,4–9 cats, and other animals. ***Paecilomyces variotii*** and ***Paecilomyces lilacinus*** are responsible for most human and animal hyalohyphomycosis infections. Wound contamination is the most common mode of entry of *P. variotii* organisms in humans and disseminated disease may develop via spread from the primary skin inoculation site to various tissues.

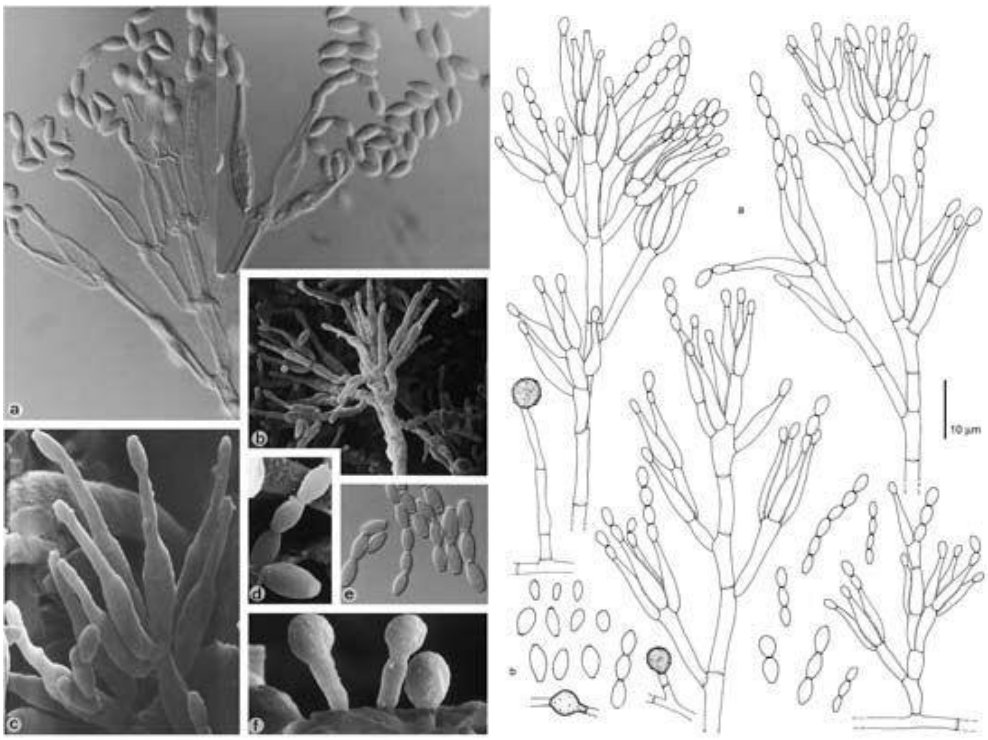
- The majority of reported cases of canine paecilomycosis were disseminated, presenting with diskospondylitis, prostatitis, cystitis, rhinitis and/or dermatitis/cellulitis.

3.2. Aetiology

3.2.1. *Paecilomyces variotii* Bainier, Bulletin de la Société Mycologique de France 23: 27 (1907)

≡Penicillium variotii (Bainier) Sacc., Sylloge Fungorum 22: 1273 (1913) [MB#212281]
=Penicillium aureocinnamomeum Biourge, La Cellule 33: 213 (1923)

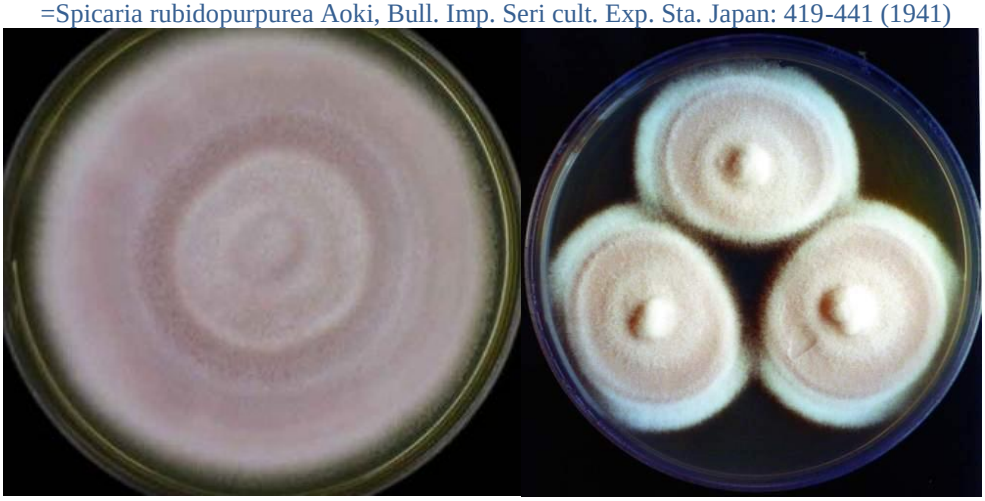
Colonies growing very rapidly, 5-8 cm in diameter after 10 days, appearing powdery or granular with abundant sporulation, occasionally loosely funiculose or fasciculate, or with an overgrowth of white to buff-colored aerial mycelium; soon developing dull olive shades, from ecru olive to light yellow olive (Ridgway Pl. XXX), dark olive-buff (XL), or greyish olive (XLVI). Reverse colorless, or in yellow, dull greenish, drab or orange shades; in age nearly black in some strains. Exudate produced as small, colorless droplets. Odor indistinct. Mycelium smooth- walled, hyaline, 1.8-15.0 µm wide. Conidiophores arising as upright branches from hyphal ropes or aerial mycelium, 25-170 µm long, 3.0-7.5 µm wide, smooth-walled, hyaline, cylindrical or tapering toward the apex, usually with several stages of irregular branching; branches 6-22 µm long, frequently bearing secondary branches. Conidiophores and their branches terminating with a group of 1-6 adpressed or divergent phialides. Phialides variable in size and shape, 9-32 x 2.5-6.0 µm, cylindrical to ellipsoidal in the lower part, usually narrowing abruptly into a long, cylindrical neck 1-2 µm wide. Conidia subhyaline to olivaceous, smooth-walled, ellipsoidal to cylindrical or clavate, often truncate at the base, highly variable in size, 3.4-11.8 x 1.8-6.1 µm, produced in long, strongly divergent chains



Paecilomyces variotii, Mycobank

3.2.2. *Paecilomyces lilacinus* (Thom) Samson, Studies in Mycology 6: 58 (1974)

≡Penicillium lilacinum Thom, Bull. Bur. Anim. Ind. U.S. Dep. Agric.: 73 (1910)
≡Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, FEMS Microbiology Letters 321: 144 (2011)
=Penicillium amethystinum Wehmer
=Spicaria rubidopurpurea Aoki, Bull. Imp. Seri cult. Exp. Sta. Japan: 419-441 (1941)



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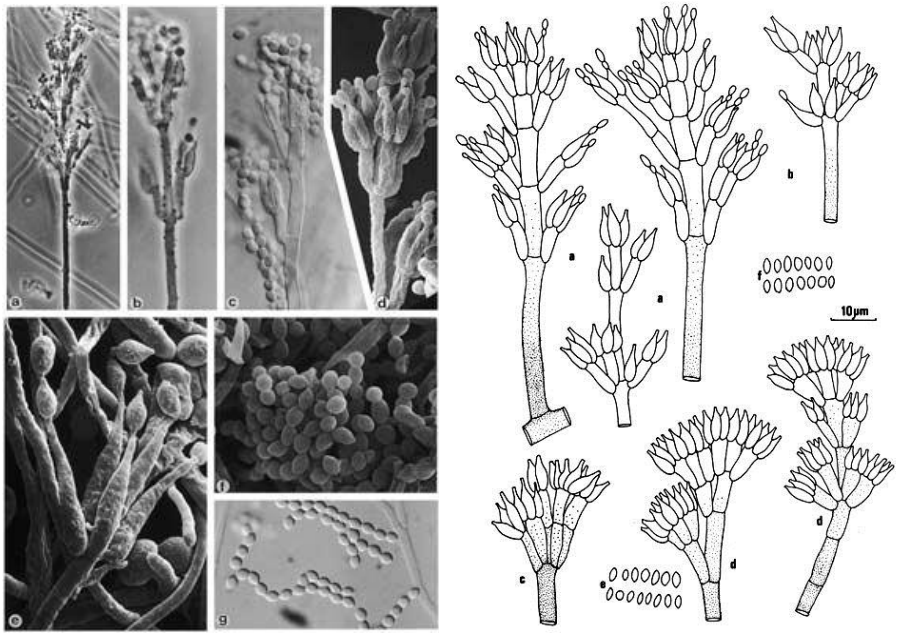


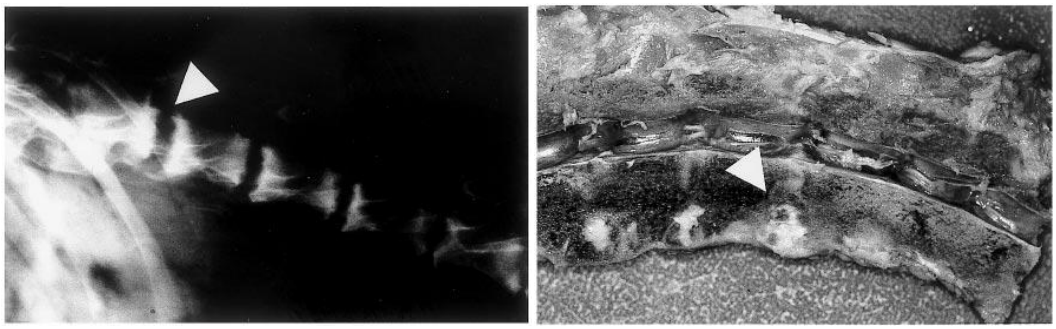
Paecilomyces lilacinus Mycobank

3.3. Reports:

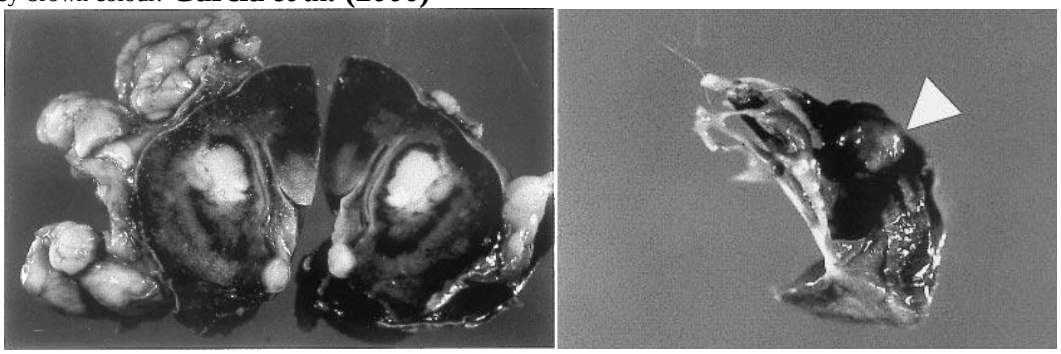
Littman and Goldschmidt (1987) reported disseminated paecilomycosis in an adult dog without underlying immunosuppressive disease. During the 3-month illness (before euthanasia), the dog had ulcerative granulomatous inguinal lymphadenitis, fever, anorexia, dyspnea, generalized lymphadenopathy, retinochoroiditis, and seizures. Fungal organisms isolated from inguinal and prescapular lymph nodes before the dog was euthanatized were identified histologically.***Paecilomyces variotii*** was isolated from the prescapular lymph node specimen. Paecilomyces variotii may be more pathogenic (once it has gained bodily entry) than previously thought.

Nakagawa *et al.* (1996) reported a 5-year-old dog with a remarkable edematous swelling of the left hock, lameness and local cellulitis. ***Paecillomyces* sp.** was isolated from ulcerative lesion of the hock joint and mediastinum. At autopsy severe effusive pleuritis was shown and numerous necrotizing and granulomatous lesions with fungal elements were seen in the liver, pancreas, kidney and mediastinal lymph nodes.

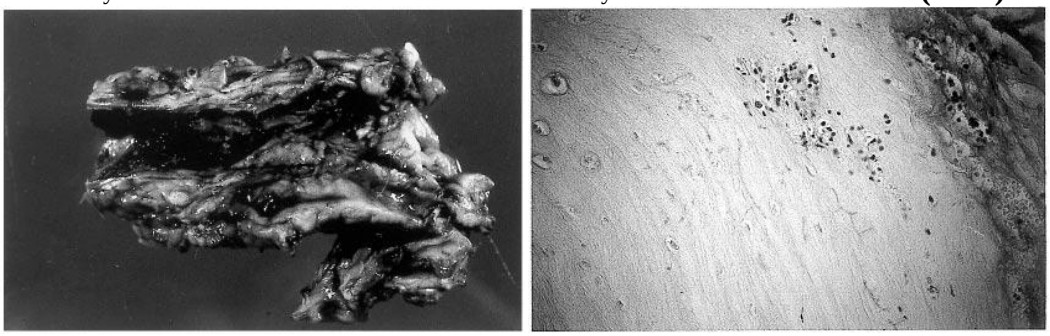
García *et al.* (2000) described a case of canine mycoses initially diagnosed by clinical signs and enzyme-linked immunosorbent assay anti-fungal test, and later confirmed by the isolation of ***Paecilomyces sp***. during the post-mortem examination. The fungus was isolated from lesions in the kidneys, mitral valve, abdominal aorta and vertebral discs.



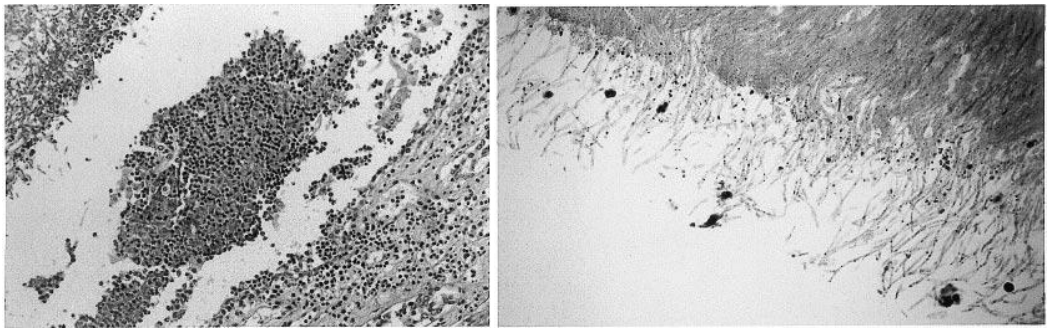
X-ray latero-lateral observation in the thoracic region; discospondylitis, Intervertebral discs with small cavities and grey-brown colour. **García *et al.* (2000)**



Kidney with nodular lesion. Mitral valve with friable and yellow nodule **García *et al.* (2000)**



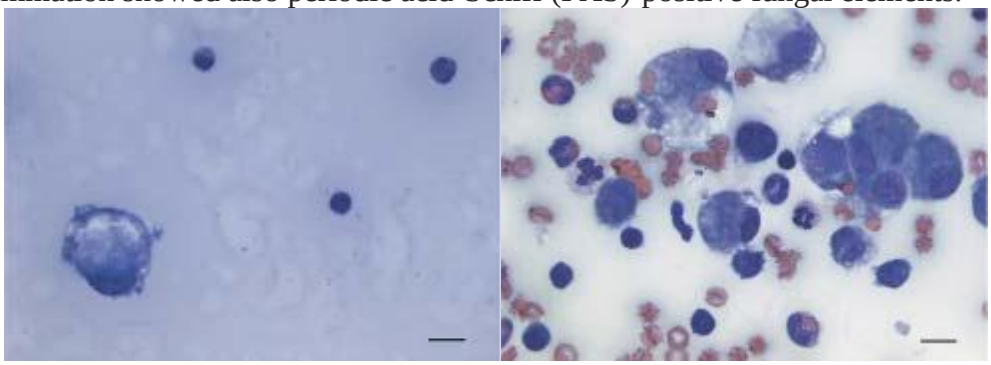
Abdominal aorta with irregularities in the endothelium and mixed thrombi adhered to its Surface, Intervertebral discs: multifocal areas of necrosis with slight mononuclear inflammatory infiltrates and branching fungal hyphae (haematoxylin and eosin stain, _400). **García *et al.* (2000)**



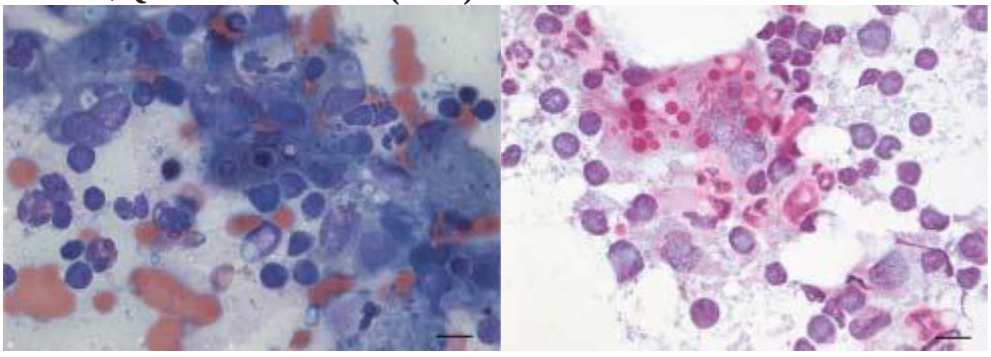
Kidney; irregular foci of necrosis with polymorphonuclear inflammatory infiltrates, macrophages and abundant branching fungal hyphae (haematoxylin and eosin stain, _200). Nodule in mitral valve; eosinophilic and acellular formation, almost completely constituted by foci of branching fungal hyphae (haematoxylin and eosin stain, _400) **García *et al.* (2000)**.

Booth *et al.* (2001) described disseminated mycosis caused by ***Paecilomyces varioti*** in a female German shepherd dog presented with chronic forelimb lameness. Radiographs of the swollen carpal joint revealed geographic lysis of the radial epiphysis. Diagnosis was based on cytological demonstration of fungal hyphae and chlamydiospores, as well as fungal culture of fluid obtained by arthrocentesis. Temporary remission was characterised by markedly improved clinical signs and laboratory parameters, following treatment with ketoconazole. The dog was euthanased 9 months after the initial diagnosis, following the diagnosis of multifocal discospondylitis.

Quance-Fitch *et al.* (2002) reported a 7-year-old, 28-kg spayed female mixed-breed dog with discospondylitis, spondylosis, hilar lymphadenopathy and moderate thoracolumbar spondylosis. Abdominal ultrasound revealed hepatosplenomegaly with echogenicity changes and diffuse abdominal lymphadenopathy. Fine-needle aspirate of spleen confirmed the presence of pyogranulomatous inflammation and numerous fungal elements, Fine-needle aspirate of lymph node showing pyogranulomatous inflammation showed also periodic acid-Schiff (PAS)-positive fungal elements.



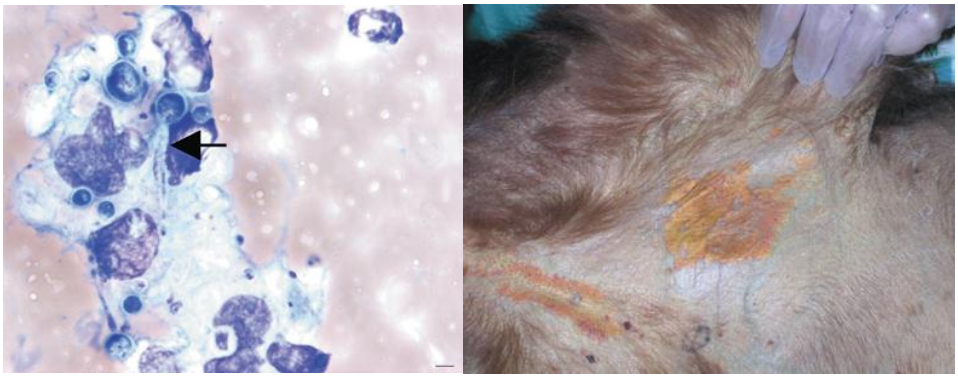
Direct smear of pleural fluid from a dog. Wright-Giemsa, Sediment smear of pleural fluid from a dog. Wright- Giemsa, **Quance-Fitch *et al.* (2002)**



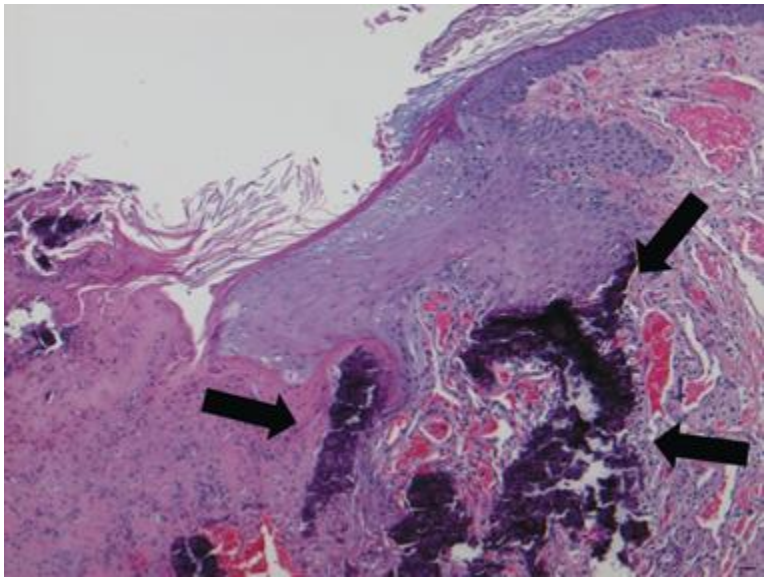
Fine-needle aspirate of spleen from a dog with disseminated *Pacilomyces* infection. Pyogranulomatous inflammation and numerous fungal elements are seen. Wright-Giemsa, Fine-needle aspirate of lymph node showing pyogranulomatous inflammation and periodic acid-Schiff (PAS)-positive fungal elements. PAS, **Quance-Fitch *et al.* (2002)**

Rosser (2003) presented a cat presented with a 2-year history of a recurrent, soft-tissue swelling of the left metacarpal region. The mass was excised and submitted for aerobic and anaerobic bacterial culture, fungal culture, and histopathological examination. Cultures revealed the organism ***Paecilomyces lilacinus***, and histopathological examination showed a nodular mycotic granuloma. Itraconazole (10 mg/kg body weight, per os [PO], q 24 hours) was administered and continued for a total of 60 days, with a swelling of the upper lip occurring 3 months after the initial presentation. Subsequent surgical excisions and debridements along with treatment with itraconazole (20 mg/kg body weight, PO, q 24 hours) for a total of 4 months were curative.

Holahan *et al.* (2008) reported a 4-year-old spayed female mixed breed dog with vomiting, lethargy and anorexia of 2 weeks duration. Abdominal radiographs and ultrasonography showed hepatosplenomegaly. Cytological evaluation of ultrasound-guided fine needle aspirates of the liver and spleen revealed fungal organisms and pyogranulomatous inflammation; fungal culture documented ***Paecilomyces variotii*** infection. The dog received antifungal therapy and supportive care. Multiple firm plaque-like skin lesions, predominantly involving the inguinal region, developed 18 days after initial presentation and were diagnosed histopathologically as calcinosis cutis. While generalized calcinosis cutis has been reported in three dogs with blastomycosis and one dog with leptospirosis, the association with disseminated Paecilomyces spp. infection is novel.



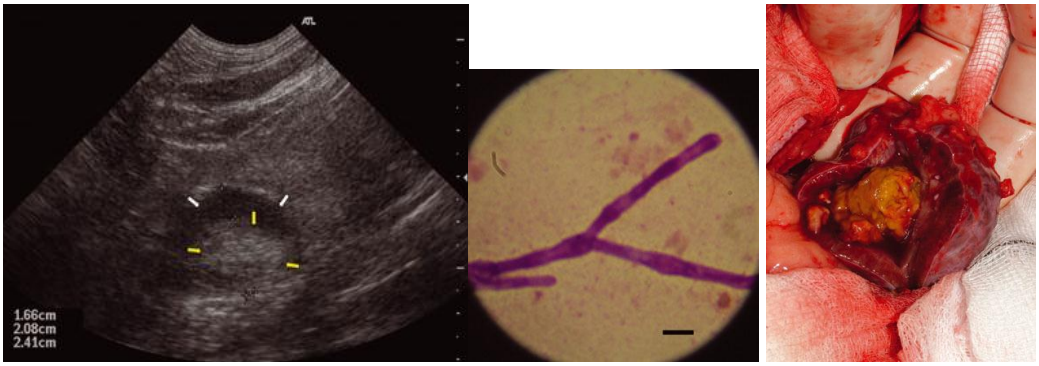
Modified Wright stain of splenic aspirate showing rare fungal organisms with a clear cell wall and occasional hyphal forms (arrow). Inguinal calcinosis cutis lesions (caudal ventrum and right dorsal-medial thigh), **Holahan *et al.* (2008)**



Skin section displaying regions of stromal mineralization (black arrows), and surface extrusion of mineralized debris. The overlying epidermis transitions from regions of irregular hyperplasia to ulceration. Haematoxylin and eosin. **Holahan *et al.* (2008)**

Pawloski *et al.* (2010) reported a 6-year-old, spayed female domestic shorthair cat with an intermittent cough and wheezing of 3 to 4 months' duration. Thoracic radiography revealed atelectasis of the right middle and caudal lung lobes with hyperinflation of the accessory lobe, consistent with bronchial obstruction. Bronchoscopy confirmed a narrowing of the right mainstem bronchial lumen; however, positive-pressure ventilation resulted in a severe pneumothorax. A lateral thoracotomy and right caudal lung lobectomy resulted in complete resolution of the pneumothorax and respiratory signs. Histopathology and culture of the lung revealed ***Paecilomyces lilacinus***. The cat was placed on itraconazole therapy for 6 months. Since dismissal from the hospital, the cat has not exhibited clinical evidence of wheezing, coughing, or dyspnea and is neurologically normal.

Tappin *et al*. (2012) reported a six-year-old female entire German shepherd dog with polyuria, polydipsia and lethargy. Investigations revealed a mild azotaemia and abdominal ultrasound revealed marked bilateral dilation of the renal pelves with echogenic material and proximal left hydroureter. Urine cytological examination and aspirates from the right renal pelvis revealed mats of fungal hyphae consistent with fungal bezoar formation. Fungal cultures revealed a profuse growth of ***Paecilomyces variotii***. Initial treatment with oral itraconazole was unsuccessful, leading to bilateral nephrotomies to remove the fungal material. Postoperatively the Paecilomyces infection persisted despite continued itraconazole therapy. Treatment was commenced with amphotericin B, leading to resolution of the dog's clinical signs.



Ultrasound image of the right renal pelvis (white arrows) revealing a well-defined echogenic mass (yellow arrows), Cytological examination of urine sample revealing branching fungal hyphae. Wrights Giemsa stain, A fungal granuloma within the left renal pelvis at nephrotomy, **Tappin *et al.* (2012)**

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4. Scedosporiosis in cats and dogs

4.1. *Introduction*

Scedosporium apiospermum, an anamorphs of *Pseudallescheria boydii*, is a eutrophic filamentous fungus commonly isolated from soil, vegetation, polluted water, and animal faeces in temperate climates. It is now recognized as an emerging agent of severe fungal infections in immunosuppressed human patients. In humans, *S. apiospermum* has been classically implicated in subcutaneous infections and in

asymptomatic pulmonary colonization, whereas systemic infections are more frequently observed in patients with an impaired immune system.

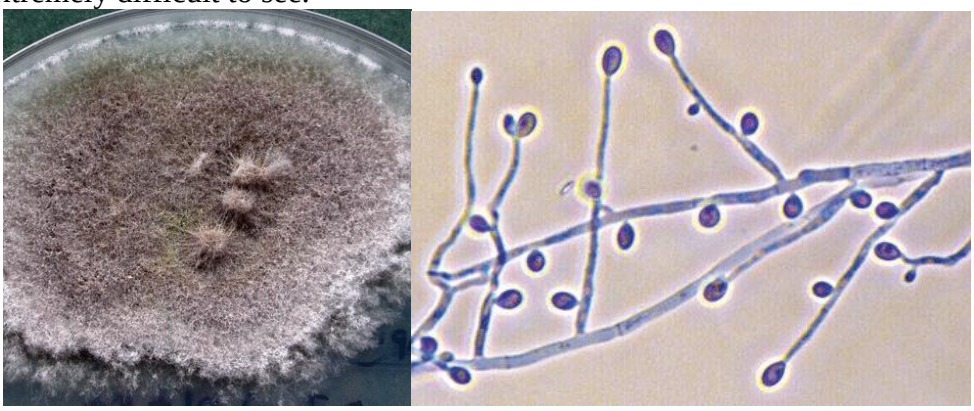
Infections caused by fungi belonging to the *Scedosporium*/*Pseudallescheria* complex (SPCF) in dogs are restricted to a small number of cases including localised infections involving the skin, upper respiratory tract and eyes and disseminated disease.

4.2. Aetiology

4.2.1. *Scedosporium apiospermum* (Sacc.) Sacc. ex Castell. & Chalm., Manual of Tropical Medicine: 1122 (1919)

≡Monosporium apiospermum Sacc., Annales Mycologici 9 (3): 254 (1911)
≡Aleurisma apiospermum (Sacc.) Maire, Bull. Soc. Pathol. Exot.: 290 (1921)
=Actinomyces albus Tarozzi, Archivio Sci. Med.: 535-632 (1909)
=Monosporium sclerotiale Pepere, Soc. Cult. Sci. Med. Nat. Cagl.: 543 (1914)
=Dendrostilbella boydii Shear, Mycologia 14 (5): 242 (1922)
=Glenospora clapieri Catanei, Bull. Soc. Pathol. Exot.: 502 (1927)
=Indiella americana Delamare & Gatti, Compt. Rend. Hebd. Séances Acad. Sci., Sér. D: 1264 (1929)
=Acremonium suis Bakai, Bolezni Svinei, Kiev: 198 (1967)
=Polycytella hominis C.K. Campb., Journal of Medical and Veterinary Mycology 25 (5): 302 (1987)

Colonies are fast growing, greyish-white, suede-like to downy with a greyish-black reverse. Numerous single-celled, pale-brown, broadly clavate to ovoid conidia, 4-9 x 6-10 mm, rounded above with truncate bases are observed. Conidia are borne singly or in small groups on elongate, simple or branched conidiophores or laterally on hyphae. Conidial development can be described as annellidic, although the annellations (ring-like scars left at the apex of an annellide after conidial secession) are extremely difficult to see.

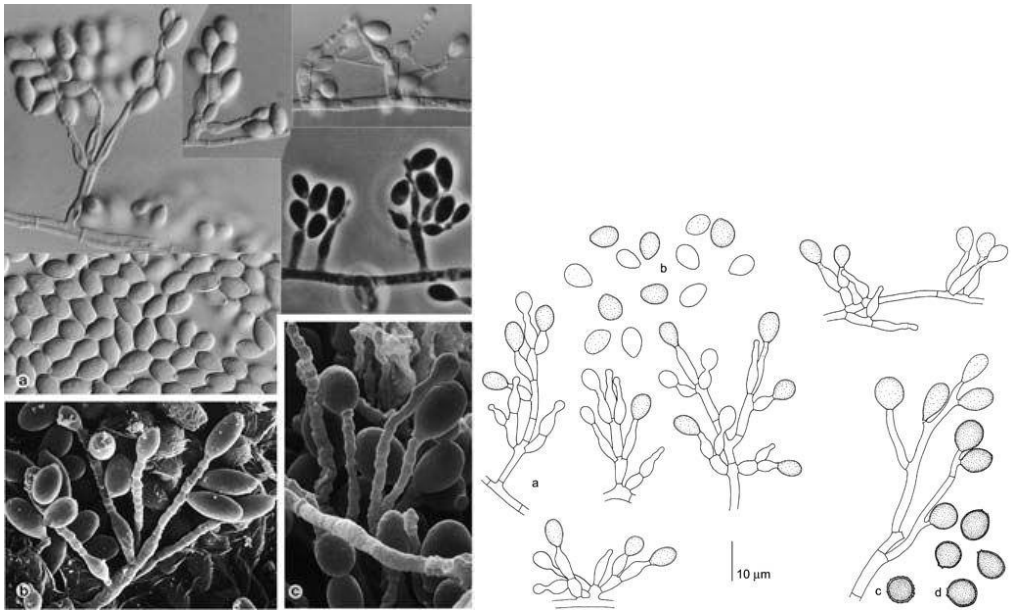


Scedosporium apiospermum http://www.mycology.adelaide.edu.au/

4.2.2. *Scedosporium prolificans* (Hennebert & B.G. Desai) E. Guého & de Hoog, Journal de Mycologie Medicale 1: 8 (1991)

≡Lomentospora prolificans Hennebert & B.G. Desai, Mycotaxon 1 (1): 47 (1974)
=Scedosporium inflatum Malloch & Salkin, Mycotaxon 21: 249 (1984)

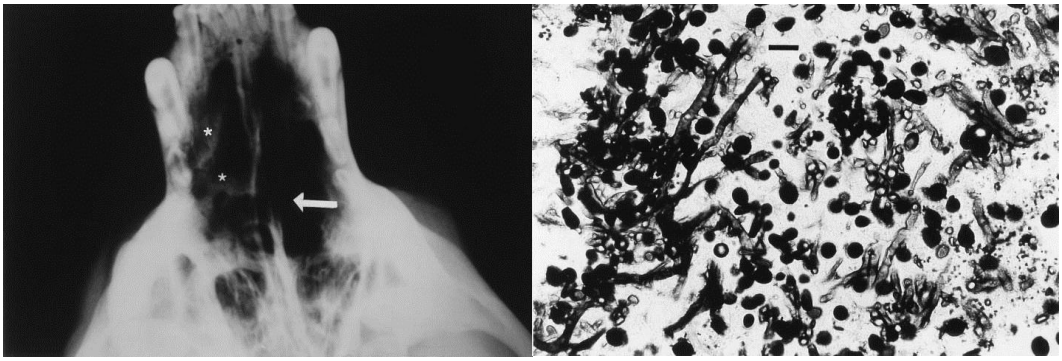
Colonies (OA, 30°C) expanding, flat, moist with depressed, cobweb-like aerial mycelium, olivaceous grey to blackish. Conidiogenous cells locally aggregated in small brushes, flask-shaped, often bearing a long, inconspicuously annellated zone. Darker and more inflated conidia may arise alongside hyphae. Conidia smooth-walled, aggregating in dense, slimy heads, soon becoming rather thick-walled and olivaceous brown, 3-7 x 2-5 µm.



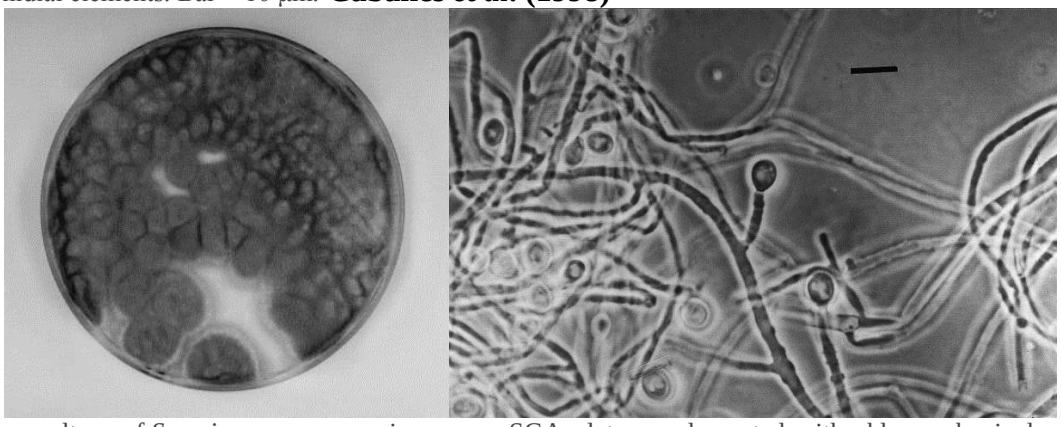
Mycobank

4.3. Reports:

Cabañes *et al*. (1998) presented a 10-month-old male American Staffordshire terrier with a 6-month history of a mucopurulent bilateral **nasal** discharge. The dog had not responded to antibiotics. A follow-up X ray revealed a mixed pattern of osteolysis and increased radiodensity confined to the nasal cavity. Histologic sections of the biopsy specimens revealed the presence of granules containing numerous septate hyphae that were hyaline to pale brown and smooth, one-celled, subspherical-to-elongate conidia that were hyaline to brownish green, and bacteria. Cultures yielded numerous colonies belonging to ***Scedosporium apiospermum***. Susceptibility tests were performed on the isolated strain. The isolate was sensitive to ketoconazole, intermediate to clotrimazole, and resistant to amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole. The dog was treated with oral ketoconazole. During the treatment a general improvement in the lesions was observed. To our knowledge, S. apiospermum has not been implicated previously as an etiologic agent of nasal disease in dogs. This report provides its first description as such.

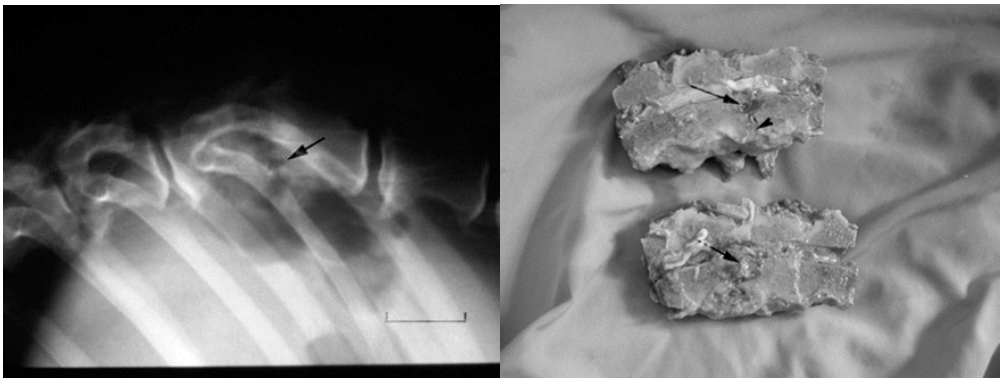


Intraoral radiograph showing lysis of the vomer bone (arrow) and increased radiodensity confined to the nasal cavity, which is more pronounced on the right side (asterisks). The turbinate pattern is less apparent than in normal dogs on both sides (black areas in the nasal cavity). Detail of a Grocott-methenamine-silver-stained section of a tissue biopsy sample showing a granule containing hyphal and conidial elements. Bar = 10 μm. **Cabañes *et al*. (1998)**

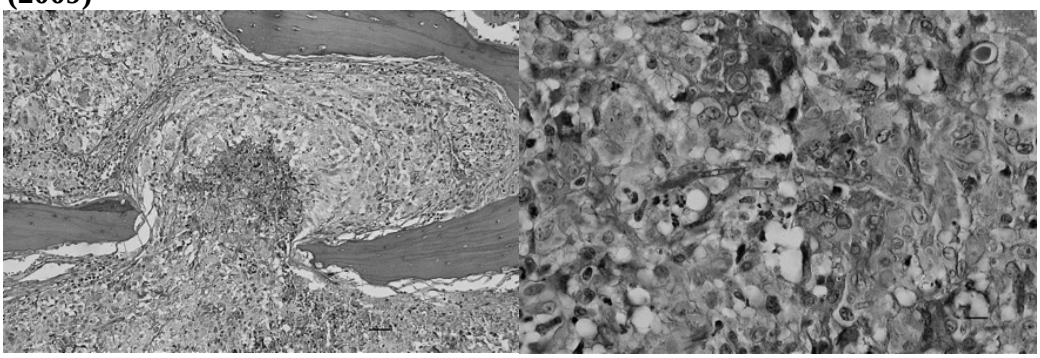


Pure culture of *S. apiospermum* growing on an SGA plate supplemented with chloramphenicol and inoculated with material from a biopsy after 8 days of incubation at 37°C. Conidiogenesis of *S. apiospermum*. Note the conidia situated terminally on annellides without swollen bases. Phase-contrast microscopy was used. Bar = 10 μm. **Cabañes *et al*. (1998)**

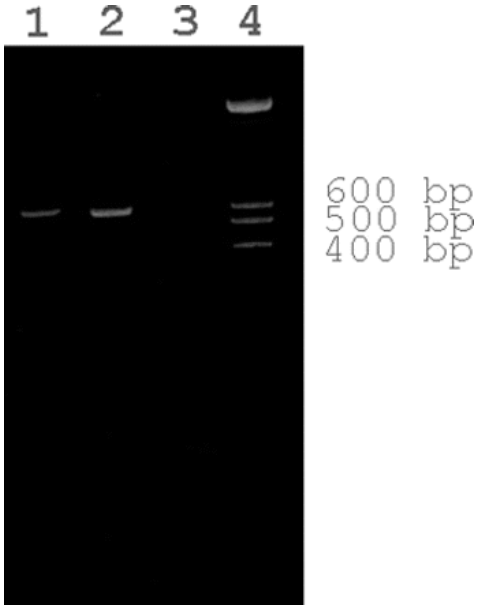
Hugnet *et al.* (2009) presented a 6-year-old, 30-kg, female German Shepherd Dog with a severe rear limb motor disorder and a medical history of acute onset of fever. Routine hematology indicated neutrophilia. Spinal survey radiographs were consistent with **osteomyelitis and discospondylitis**. Because of the poor clinical prognosis and the painful nature of the lesions, the dog was euthanized at the owners' request. At necropsy, T13-L1 vertebrae had large areas of necrosis within the vertebral bodies. Histopathological findings were consistent with chronic, severe, fungal osteomyelitis and discospondylitis. Polymerase chain reaction identified ***Scedosporium apiospermum***, a eutrophic filamentous fungus now recognized as an emerging agent of severe infections in immunosuppressed human patients.



Lateral spinal survey radiograph. Notice the areas of osteolysis and osteoproliferation involving the T13 and L1 vertebrae (arrow). The radiographic changes are consistent with discospondylitis and vertebral osteomyelitis. Bar = 3 cm. The T13 and L1 vertebrae, dissected at necropsy, contain large foci of necrosis within the vertebral bodies (arrows). The contour of both vertebrae are uneven, and there is destruction of the intervertebral disk (arrowhead). The spinal cord appears normal. **Hugnet *et al.* (2009)**

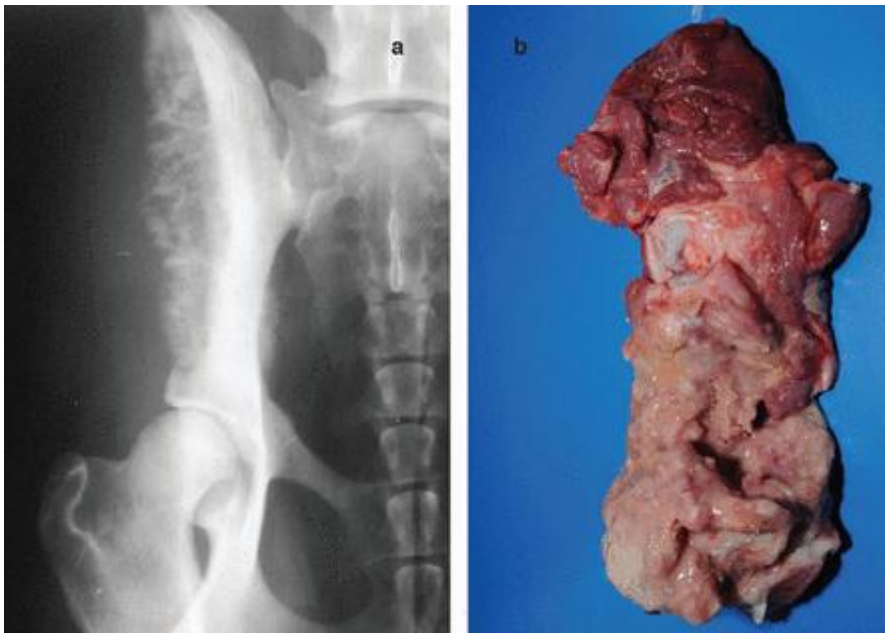


Pyogranulomatous inflammation of the bone marrow with a focus of central necrosis. Note the severe osteolysis of the bone trabeculae. Hematoxylin and eosin. Bar = 40 μm, Pyogranulomatous infiltrate with thin, septate, branching hyphae and large encapsulated spores. Periodic acid–Schiff stain. Bar = 10 μm. **Hugnet *et al.* (2009)**

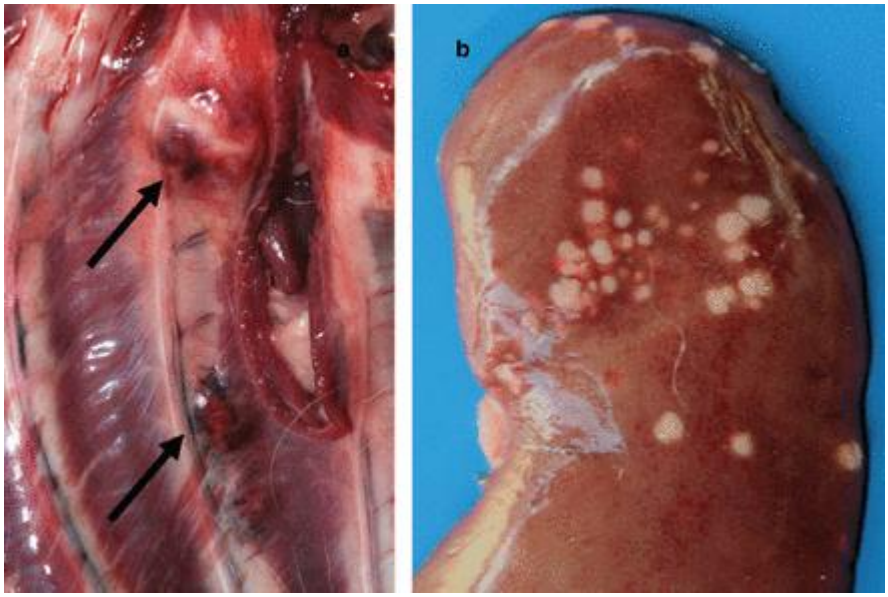


Ethidium bromide-stained, agarose, polymerase chain reaction (PCR) gel. Lane 1: positive amplification of *Scedosporium apiospermum* DNA from paraffin-embedded sample; lane 2: PCR positive control (DNA from *Aspergillus fumigatus* strain CBS 144–89); lane 3: PCR negative control (distilled water substituted for DNA template); lane 4: 1-kb molecular marker. **Hugnet *et al.* (2009)**

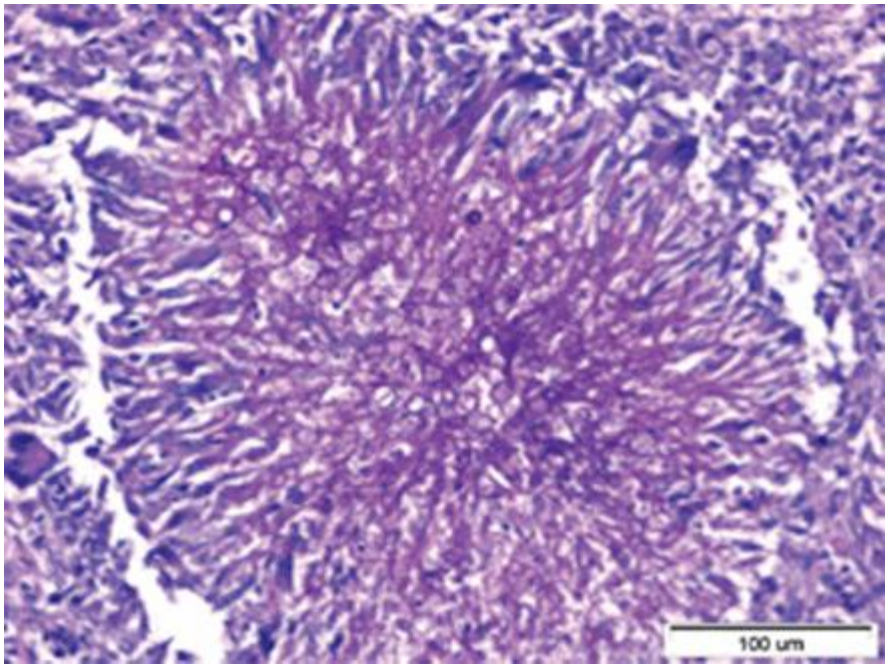
Elad *et al.* (2010) presented a case of **disseminated pseudallescheriasis** in a German Shepherd bitch. Bones (ilium, a rib and phalanges), joints (elbow and acetabulum) and the surrounding tissues were the principal organs affected. In addition, ***Pseudallescheria boydii*** was isolated, in lower numbers, from the eye, kidney, lymph nodes draining the affected regions and urine. The dog was euthanized. P. boydii was identified by morphologic characteristics and molecular techniques (beta tubulin sequence). In addition, an ITS nucleotide sequence analysis showed that this strain differed from another isolate identified as Scedosporium apiospermum that had caused a disseminated infection in another German Shepherd. The importance of the molecular characterization of fungi belonging to the Pseudallescheria/Scedosporium complex, isolated from animals was stressed in light of the ongoing attempts to recharacterize these fungi.



Iliac region. (a) Radiography — note extensive bone destruction. (b) Necrotic purulent material around the Ilium. **Elad *et al.* (2010)**



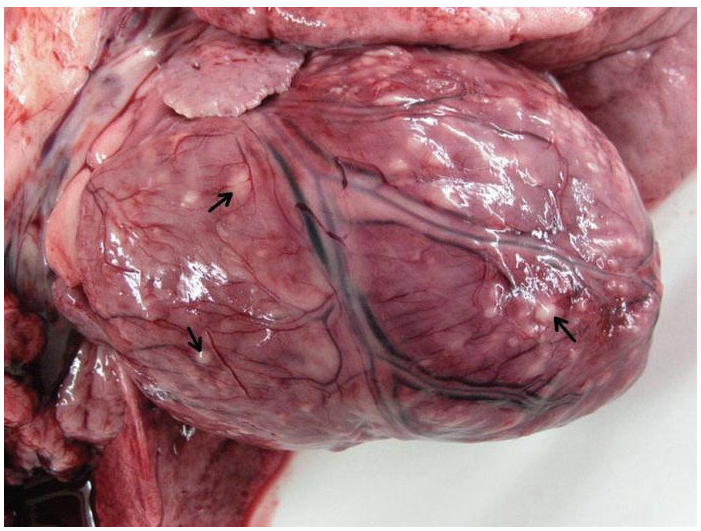
Foci of fungal infection. (a) Rib (arrows indicate lesions). (b) Kidney. **Elad *et al.* (2010)**



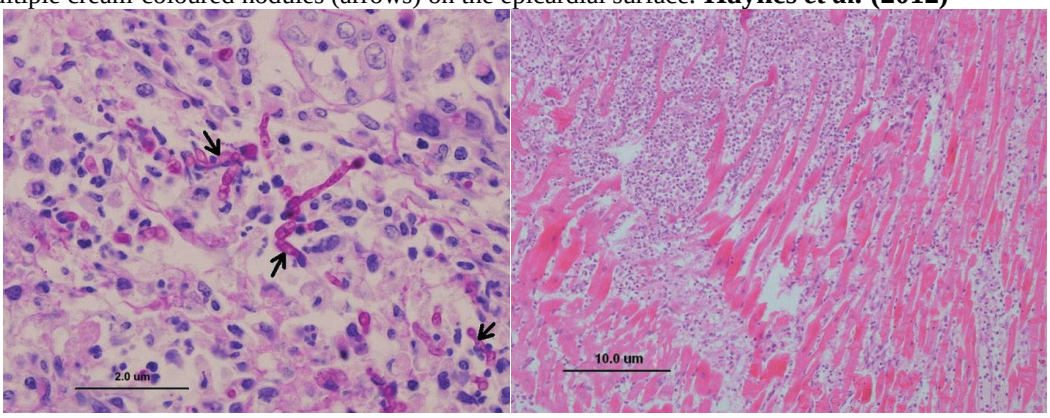
Fungal aggregate in bone tissue (PAS stain). **Elad *et al.* (2010)**

Leperlier *et al.* (2010) reported a 3-year-old neutered male Bengal cat with severe fungal **rhinosinusitis**. A diagnosis was obtained after computer tomography imaging, histopathological examination and fungal culture. The mold ***Scedosporium apiospermum*** was identified as the aetiological agent. Aggressive surgical debridement combined with topical and systemic antifungal therapy was performed. Unfortunately, the treatment resulted only in a partial remission of signs.

Haynes *et al.* (2012) described **disseminated *Scedosporium prolificans*** infection in a 1-year-old female spayed German Shepherd dog. Clinical signs were predominantly associated with fungal pyelonephritis and the organism was cultured from the urine. The dog was treated with itraconazole and later, terbinafine was added. Subsequent antifungal susceptibility testing of the isolate showed it to be resistant to all available antifungal drugs. The dog was euthanased because of acute abdominal haemorrhage and associated clinical deterioration. Postmortem examination revealed extensive pyogranulomas containing fungal organisms in the renal parenchyma, myocardium, bone marrow, skeletal muscle, liver, lung, spleen, multiple lymph nodes and pancreas.

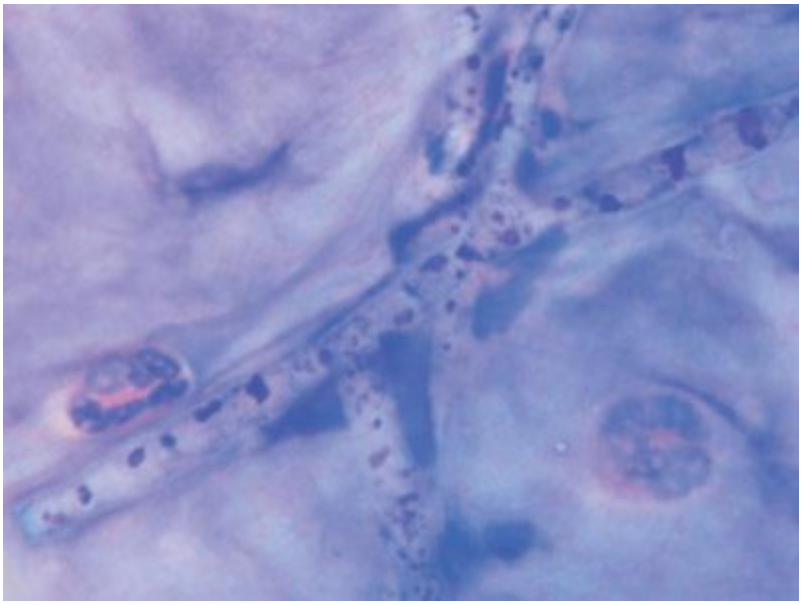


Heart from the 1-year-old female spayed German Shepherd dog at postmortem examination showing multiple cream-coloured nodules (arrows) on the epicardial surface. **Haynes *et al.* (2012)**



Photomicrograph of the renal cortex from the 1-year-old female spayed German Shepherd dog showing a pyogranuloma containing septate fungal hyphae (arrows) (¥100, PAS). Photomicrograph of myocardium from the 1-year-old female spayed German Shepherd dog showing myocardial fibres disrupted by large numbers of neutrophils and macrophages (¥20, H&E). **Haynes *et al.* (2012)**

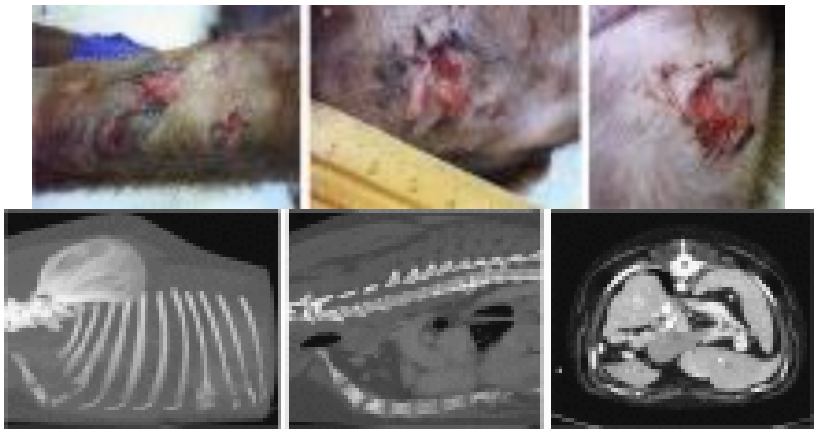
Newton *et al* (2012) reported a 6-year-old male castrated Norfolk Terrier dog with a 21-day history of an increasingly painful eye. Examination revealed marked blepharospasm and purulent ocular discharge associated with an ulcerative keratitis. There was panstromal corneal opacity with raised gray to white lesions. Corneal cytology demonstrated branching septate fungal hyphae identified by polymerase chain reaction as ***Scedosporium apiospermum***. Treatment with topical 1% voriconazole solution was successful in resolving the keratomycosis.



Corneal cytology. Fungal hyphae and a few neutrophils are visible. Modified Wright stain. ·100 objective.

Taylor et al. (2014) reported a case of disseminated scedosporiosis due to ***Scedosporium prolificans*** in a Labrador retriever dog that was receiving immunosuppressive drug therapy for treatment of immune-mediated haemolytic anaemia. Despite cessation of immunosuppressive medications and an initial response to aggressive treatment with voriconazole and terbinafine the dog developed

progressive disease with neurological signs necessitating euthanasia six months from diagnosis.



Taylor *et al.* (2014)

Nevile *et al.* (2015) diagnosed and treated 5 cases of canine keratomycosis. Clinical presentations varied between dogs. Predisposing factors were identified in 4 of 5 cases. Diagnostic modalities utilized were corneal cytology and fungal culture. Corneal cytology confirmed the presence of fungal organisms in all five cases. A 7-month-old male Australian Shepherd presented for corneal ulceration OD. The referring veterinarian had removed a small vegetable matter foreign body from the right cornea 4 days previously and prescribed fusidic acid 1% ointment four times daily and 0.3% gentamycin sulfate four times daily. On examination, OD had marked blepharospasm, moderate perilimbal hyperemia, and an axial superficial stromal corneal ulceration with several axial corneal opacities surrounding it. Corneal cytology was performed as described above and numerous fungal hyphae were seen. A corneal swab was submitted for fungal culture. Treatment was commenced with topical 1% itraconazole ointment (Stenlake Compounding Chemist) six times daily and oral carprofen 2 mg/kg daily. Topical 0.3% gentamycin sulfate was continued four times daily. Scedosporium apiospermum was cultured 6 days after sample collection. Treatment was continued for 34 days at which time the corneal ulcer had healed and the axial corneal opacities had resolved.

References:

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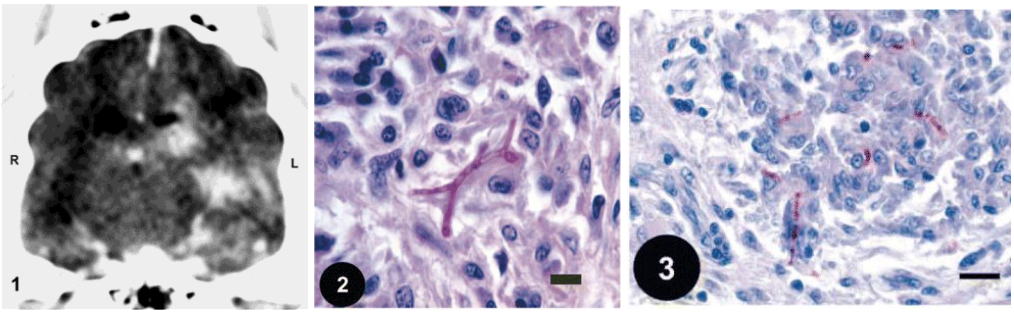
6. **Nevile JC**, **Hurn SD**, **Turner AG**. Keratomycosis in five dogs.**Vet Ophthalmol.** 2015 Sep 24.

7. Newton EJ. Scedosporium apiospermum keratomycosis in a dog. Vet Ophthalmol. 2012 Nov;15(6):417-20.
8. Taylor A, Talbot J, Bennett P, Martin P, Makara M, Barrs VR. Disseminated Scedosporium prolificans infection in a Labrador retriever with immune mediated haemolytic anaemia. Med Mycol Case Rep. 2014 Nov 4;6:66-9.

5. Fusariosis in cats and dogs

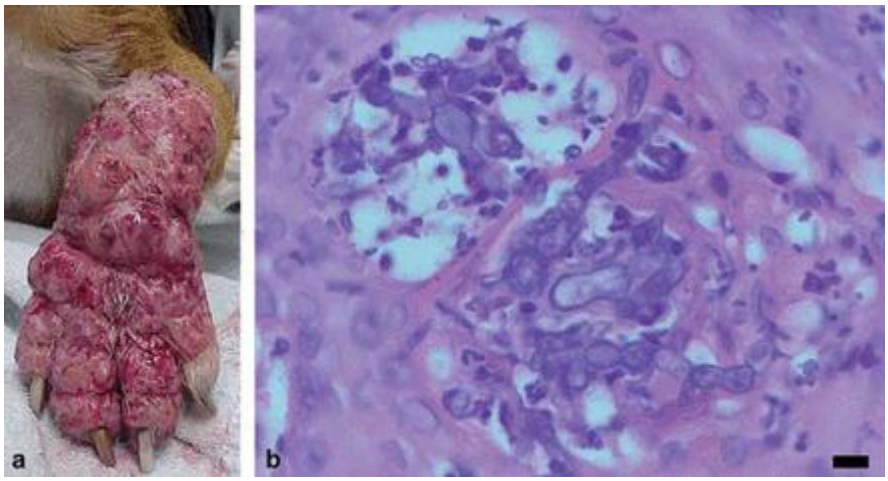
Evans *et al.* (2004) described a 2-year-old, spayed, female German Shepherd Dog with **meningoencephalitis** secondary to infection with Fusarium spp. Meningoencephalitis in dogs secondary to a species of Fusarium has not been previously reported. The diagnosis was made based on the histopathologic examination of brain tissues postmortem and special immunohistochemical stains specific for ***Fusarium solani***. The clinical signs in this dog were indicative of multifocal brain disease and included seizures and a paradoxical vestibular syndrome. The clinical findings, diagnostic and histopathologic test results, and the comparative characterizations of other disseminated fungal diseases, especially aspergillosis, are described.



1. Transverse contrast-enhanced CT image of the brain at the level of the dorsum sellae showing multiple, diffusely coalescing areas of contrast enhancement centered in the left hippocampus, with ventrolateral and medioventral extension into the adjacent internal capsule and rostral midbrain, respectively. There is extensive white matter edema in the left cerebral hemisphere collapsing the left lateral ventricle and deviating the falx cerebri to the right of midline. R, right; L, left.2. fungal granuloma from the cerebral cortex showing multiple, short, septate hyphae with dichotomous branching, 3.Positive immunohistochemistry reaction using an indirect PAP technique with heterologously absorbed anti-*Fusarium* antibodies.17 Harris' hematoxylin counterstain. Bar = 25 µm. **Evans *et al.* (2004)**

Kano *et al.* (2011) described the isolation of Fusarium sporotrichioides from a canine cutaneous ulceration. A 2-year-old male Beagle dog weighing 8.6 kg, with a history of immune-mediated hemolytic anemia (IMHA), had been treated with prednisone for 9 months. Physical examination revealed cutaneous ulceration on the left foreleg. Histopathological examination of skin samples from the ulcerative area revealed many branching hyphae surrounding neutrophils. Since itraconazole (ITZ) is recommended for miscellaneous fungal infections, the dog was treated with ITZ. However, the ulcerative lesions did not improve and after 3 weeks of treatment the dog died due to renal failure. No autopsy was performed. Since the isolate recovered from the biopsy specimen was identified as Fusarium species by morphological characteristics, the animal was diagnosed as having had an infection caused by this mould. The dog's prior prednisone treatment may have played a role in establishing the fungal infection. Comparative sequence analyses of the ITS regions of the clinical

isolate with those in GenBank showed that it was 100% identical to F. sporotrichioides and less than 96% similar to ITS of other Fusarium species. Based on these findings, F. sporotrichioides was established as the etiologic agent of the canine infection, a situation that has not been previously reported in dogs, as well as humans.



(a) Cutaneous ulceration on the left foreleg. (b) Microscopic examination of a biopsy specimen from the cutaneous lesion disclosed numerous branching hyphae around neutrophils in the dermis. Black bar indicates 15 µm long.

Kano *et al.* (2011) isolated a strain of ***Fusarium solani*** from a dog showing many cutaneous and submucosal nodules and pyogranulomatous kidney lesions. Clinical isolates from this systemic infection were identified using microscopic examination and confirmed by molecular analysis.

Namitome *et al*. (2011) reported an 8-year-old male Golden Retriever with lameness and claw abnormality in the second digit of the left forelimb. Radiography revealed osteomyelitis in the distal phalanx bone of the affected limb. Microscopic examination of the claw revealed numerous hyphae in the claw matrix. Fungal DNA fragments coding the ribosomal internal transcribed spacer region (ITS) were detected from the claw matrix as well as fungal colonies of the clinical isolates by PCR. Nucleotide sequencing revealed that the amplicons shared > 99% homology with Fusarium sp. Therapy including oral itraconazole resulted in regrowth of a new claw, in which no hyphae were detected. To the authors' knowledge, this is the first case report of canine onychomycosis in which Fusarium sp. was isolated from the affected claw.

References

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2. Kano R, Okayama T, Hamamoto M, Nagata T, Ohno K, Tsujimoto H, Nakayama H, Doi K, Fujiwara K, Hasegawa A. Isolation of Fusarium solani from a dog: identification by molecular analysis. Med Mycol. 2002 Aug;40(4):435-7.
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6. Pheohyphomycoses in cats and dogs

6.1. Introduction

Phaeohyphomycoses in dogs and cats are rare opportunistic fungal infections caused by numerous genera of fungal moulds that characteristically produce melanin-pigmented 'dematiaceous' (dark-coloured) hyphal elements in tissues and in culture. These fungi are widespread in the environment and are found in soil, wood and decomposing plant debris and are usually inoculated into the host by direct trauma from a wood splinter, via bite wounds, or by contamination of an existing open wound. Most infections with dematiaceous fungi cause subcutaneous nodules and/or tracts that contain purulent exudates. In some cases the infection disseminates or involves the central nervous system (CNS).
The number of reports of infections is increasing in humans and animals, often associated with immunosuppressive treatment or an immunosuppressive condition.

Cytological examination of exudates usually reveal pyogranulomatous inflammation that may contain fungal hyphae. Fungal culture is usually recommended to diagnose this disease.
Treatment involves surgical excision in cases of localised skin disease followed by systemic antifungal therapy, with itraconazole as the agent of first choice. Relapses after treatment are common. Itraconazole and other systemic antifungal agents have been used to treat systemic or neurological cases, but the response is unpredictable. The prognosis is guarded to poor in cats with multiple lesions and systemic or neurological involvement. Combination therapy with terbinafine and an azole anti-fungal drug appears to be effective in dogs.

6.2. Phaeohyphomycoses reported in dogs and cats

Dogs

1. Cerebral phaeohyphomycosis: **(Dillehay *et al*., 1987, Migaki *et al.*, 1987**, **Bentley *et al.*, 2011**, **Giri *et al*, 2011)**
2. Systemic phaeohyphomycosis: **(Schroeder *et al.*, 1994, Añor *et al.*, 2001**, **Singh *et al.*, 2006)**
3. Cutaneous phaeohyphomycosis: **(Herráez *et al.*, 2001**, **Swift *et al*., 2006)**
4. Pulmonary phaeohyphomycosis (**Sutton *et al*., 2008)**
5. Osteomyelitis (**Lomax *et al.*, 1986)**

Cats

1. Cutaneous/ subcutaneous phaeohyphomycosis: **(Muller *et al*., 1975, Haschek and Kasali, 1977, Bostock *et al.*, 1982, Sousa *et al.*, 1984, Dhein *et al*. , 1988, VanSteenhouse *et al.*, 1988, Kettlewell *et al.*, 1989, Roosje *et al*., 1993, Outerbridge *et al.*, 1995, Fuchs *et al*., 1997, McKay *et al*.,2001, Abramo *et al*.,2002)**
2. Nasal: **(Bostock *et al.*, 1982, Dhein *et al*., 1988, McKay *et al*., 2001)**
3. Cerebral phaeohyphomycosis **: (Shinwari *et al.*, 1985, Dillehay *et al*.,1987, Bouljihad *et al.*, 2002, Mariani *et al*., 2002)**
4. Systemic phaeohyphomycosis: **(Elies *et al.*, 2003)**
5. Pulmonary phaeohyphomycosis: (**Evans *et al.*, 2011)**
6. Ocular **(Miller et al.,** 1983)

7. Renal **(.Reed et al.,** 1974**)**

6.3. Dematiaceous fungi recorded as causes of phaeohyphomycoses in dogs

1. ***Alternaria infectoria,*** **Dedola *et al.* (2010)**
2. **Bipolaris *sp.*, Giri *et al.* (2011)**
3. **Cladophialophora sp., Bentley *et al.* (2011)**
4. ***Cladophialophora bantianum*, Dillehay *et al*. (1987), Añor *et al.* (2001**
5. **Curvularia sp, Herráez *et al.* (2001)**
6. ***Curvularia lunata*, Swift *et al*. (2006)**
7. ***Ochroconis gallopavum,* Singh *et al.* (2006)**
8. ***Phialemonium obovatum* , Lomax *et al.* (1986)**
9. ***Xylohypha bantiana.* Schroeder *et al.* (1994)**

6.4. Dematiaceous fungi recorded as causes of phaeohyphomycoses in cats

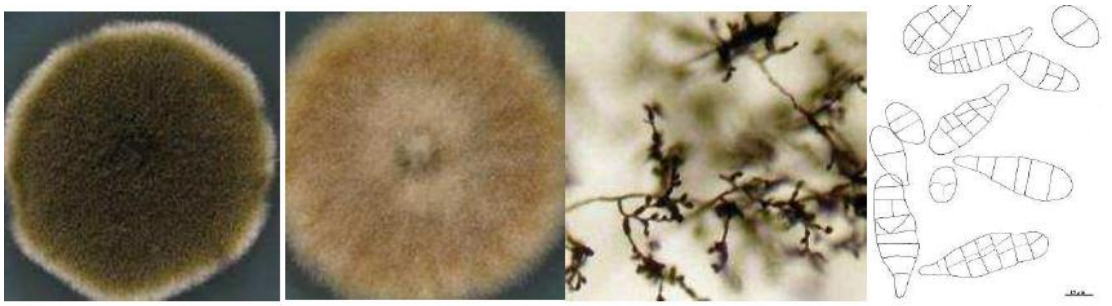
1. ***Alternaria alternate,* Dhein *et al*. (1988, Outerbridge *et al.* (1995), McKay *et al*. (2001)**
2. ***Alternaria infectoria*, Roosje *et al*. (1993)**
3. **Cladosporium sp, Sousa *et al.* (1984), Mariani *et al*. (2002)**
4. ***Cladosporium bantianum,* Shinwari *et al.* (1985), Dillehay *et al*. (1987), Abramo *et al.* (2002), Bouljihad *et al.* (2002), Elies *et al.* (2003), Evans *et al.* (2011)**
5. ***Drechslera spicifera,* Muller *et al*. (1975), Maeda *et al.* (2008)**
6. ***Exophiala spinifera*, Kettlewell *et al.* (1989)**
7. ***Exophiala jeanselmei*, Bostock *et al.* (1982), Pukay and Dion (1984)**
8. ***Fonsecaea pedrosoi,* Fondati *et al.* (2001)**
9. ***Moniliella suaveolens*, McKenzie *et al*. (1984)**
10. ***Phialophora gougerotii* , Haschek and Kasali (1977)**
11. ***Phialophora verrucosa*, Pukay and Dion (1984), Beccati *et al.* (2005)**
12. ***Scolecobasidium humicola,* VanSteenhouse *et al.* (1988)**
13. ***Staphylotrichum coccosporum*, Fuchs *et al.* (1997).**
14. **Stemphylium sp , Sousa *et al.* (1984)**
15. **Ulocladium species, Knights *et al.* (2008)**

6.5. Description of demattiaceous fungi recorded as causes of phaeohyphomycoses in dogs and cats

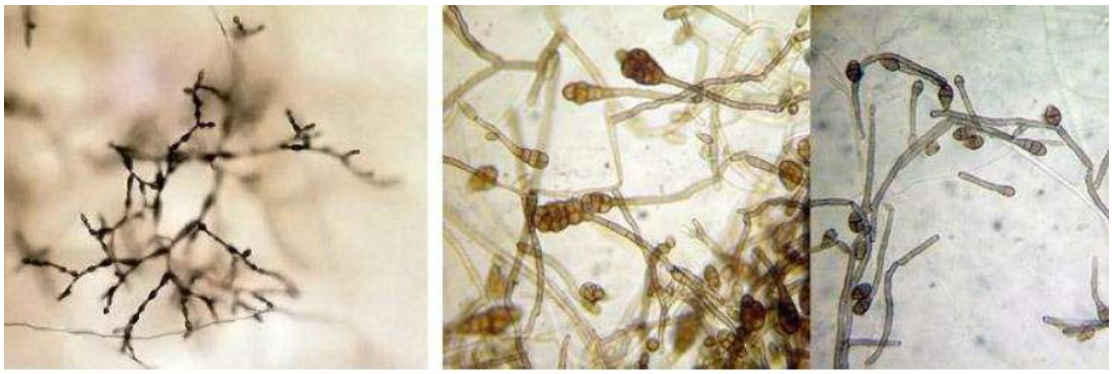
6.5.1. *Alternaria alternata* (Fr.) Keissl. (1912)

Synonyms: *Alternaria tenuis* Nees 1917
Macrosporium fasciculatum Cooke & Ellis (1817),
Torula alternata Fr. (1832),*Alternaria fasciculata* Jones & Grout (1897),
Alternaria rugosa McAlpine (1896)

Alternaria species grow rapidly producing flat, downy to woolly colonies, covered by grayish, short, aerial hyphae. The surface is greyish white at the beginning which later darkens and becomes greenish black or olive brown with a light border. Microscopically, the fungus develops septate, brown hyphae. Conidiophores are also septate and brown in colour, occasionally producing a zigzag appearance. They bear simple or branched large conidia, which have both transverse and longitudinal septations (muriform conidia). They are dark in colour, elongated and found in chains. The conidia may be observed singly or in acropetal chains and may produce germ tubes. They are ovoid to obclavate, darkly pigmented, muriform, smooth or roughened. The end of the conidium nearest the conidiophore is round while it tapers towards the apex.



Alternaria alternata

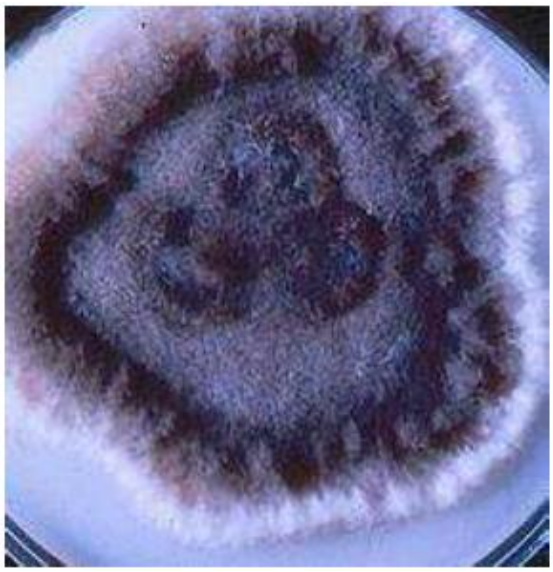


A. infectoria

6.5.2. *Bipolaris spicifera* (Bainier) Subram., (1971)

Synonyms: *Brachycladium spiciferum* Bainier, (1908)
Brachysporium spiciferum (Bainier) Corbetta, (1963)
Curvularia spicifera (Bainier) Boedijn, (1909)
Dendryphion spiciferum (Bainier) Sacc. & Traverso,(1910)
Drechslera spicifera (Bainier) Arx, (1970)
Helminthosporium spiciferum (Bainier) Nicot, (1953)

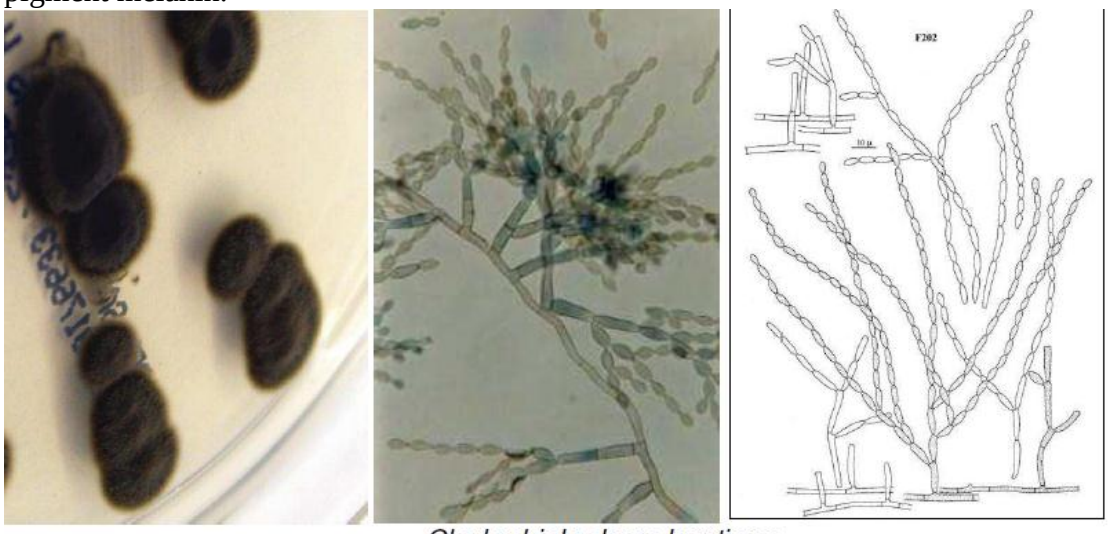
Colonies on potato dextrose agar at 25°C are initially white, soon becoming gray to black with a black reverse. Rapid growth. Texture is woolly tocottony. Hyphae are septate and dark. Conidiophores may be up to 150 µm in length, are sympodial, geniculate, simple or branched, bearing conidia through pores or openings (poroconidia). Conidia have 2 to 5 transverse distosepta or pseudosepta (septa that do not extend to the cell wall with cells inclosed within sacs) and 3 to 6 cells. They measure approximately 14-40 x 6-11 µm. A flattened hilum or point of attachment is seen on the basal cell. Conidia germinate from both poles (bipolar).




6.5.3. *Cladophialophora bantiana* de Hoog, Kwon-Chung & McGinnis, (1995)

Synonyms: Torula bantiana Sacc., in Saccardo, (1912)
Cladosporium bantianum (Sacc.) Borelli, (1960)
Xylohypha bantiana (Sacc.) McGinnis, Borelli, Padhye & Ajello, (1986)
Cladosporium trichoides Emmons Binford, Thompson & Gorham, (1952)
Cladosporium bantianum (Sacc.) Borelli, (1960)
Cladosporium trichoides var. chlamydosporum Kwon-Chung, (1978)

In culture, the colony is black with a velvety texture or dark grey in colour, depending on the type of agar medium it is grown on. It grows slowly under temperatures ranging from 14-42 °C with optimal growth around 30 °C. It can be distinguished from other species of the genus *Cladophialophora* by the presence of urease activity. Microscopically, the fungus produces predominantly hyphal growth both *in vivo* and *in vitro*, that consists of dark coloured largely unbranched, wavy chains of conidia, individually 5–10 µm in length. The dark colour is due to the presence of the dark pigment melanin.

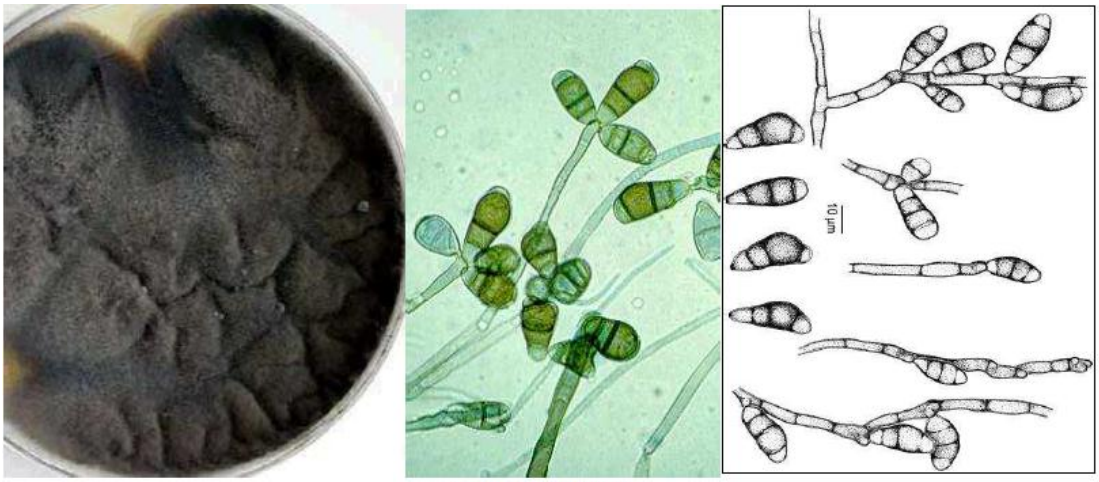


Cladophialophora bantiana

6.5.4. *Curvularia lunata* (Wakker) Boedijn, 127 (1933)

Synonyms: *Acrothecium lunatum* Wakker, (1898)]
Helminthosporium curvulum Sacc., (1916)
Helmisporium curvulum Sacc. (1916)

Curvularia produces rapidly growing, woolly colonies on potato dextrose agar at 25°C. From the front, the color of the colony is white to pinkish gray initially and turns to olive brown or black as the colony matures. From the reverse, it is dark brown to black. *Curvularia* produces septate, brown hyphae, brown conidiophores, which are simple or branched and are bent at the points where the conidia originate. The conidia are straight or pyriform, brown, multiseptate with transverse septa, and have dark basal protuberant hila. The central cell is typically darker and enlarged compared to the end cellsin and usually gives the conidium a curved appearance.

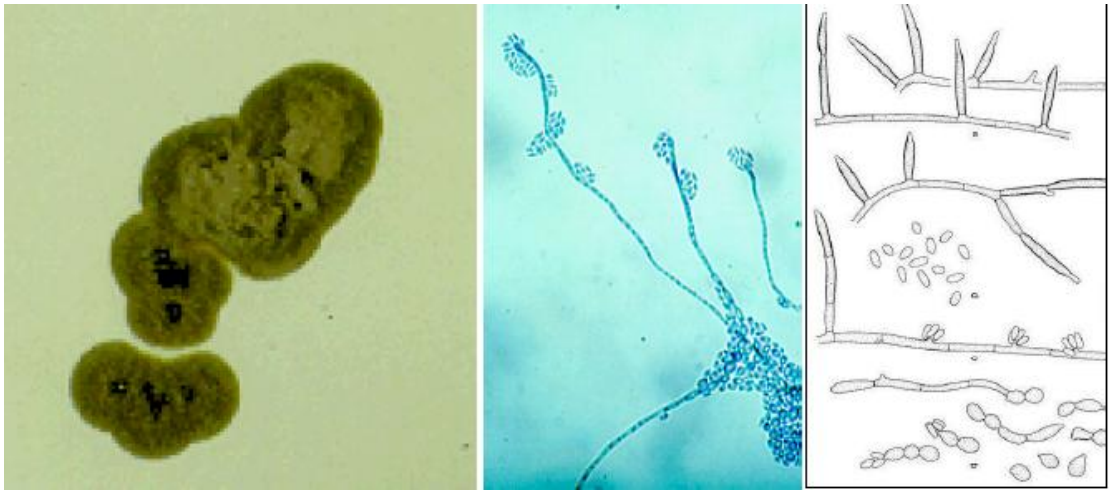


Curvularia lunata.

6.5.5. *Exophiala jeanselmei* (Langeron) McGinnis & A.A. Padhye (1977)

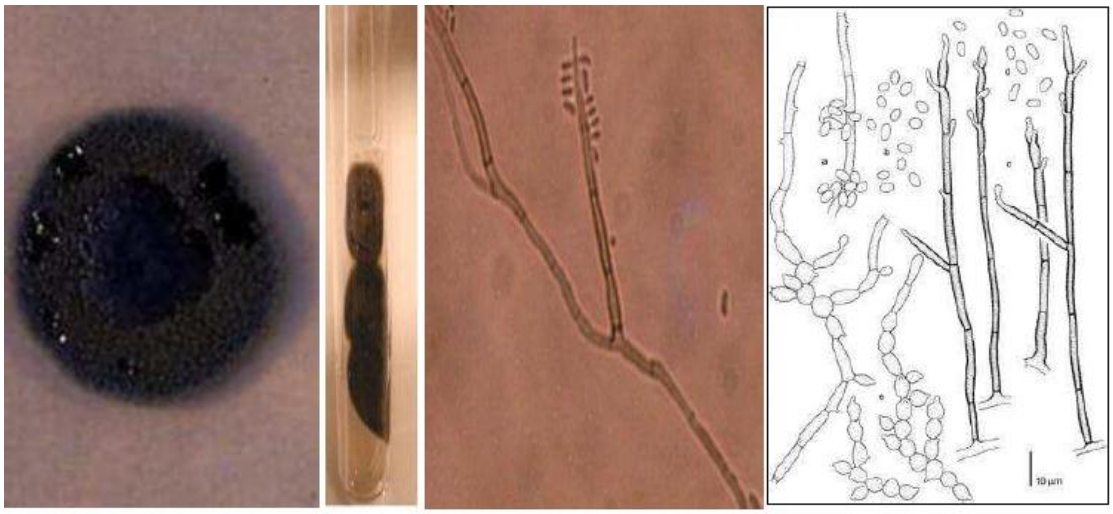
Synonyms: *Torula jeanselmei* Langeron, (1928)
Pullularia jeanselmei (Langeron) Dodge, (1935)
Phialophora jeanselmei (Langeron) Emmons (1945)
Exophiala jeanselmei var. jeanselmei (1977)

Colonies are initially smooth, greenish-grey to black, mucoid and yeast-like, becoming raised and developing tufts of aerial mycelium with age, often becoming dome-shaped and suede-like in texture. Reverse is olivaceousblack. Numerous ellipsoidal, yeast-like, budding cells are usually present, especially in young cultures. Scattered amongst these yeast-like cells are larger, inflated, subglobose to broadly ellipsoidal cells (germinating cells) which give rise to short torulose hyphae that gradually change into unswollen hyphae. Conidia are formed on lateral pegs either arising apically or laterally at right or acute angles from essentially undifferentiated hyphae or from strongly inflated detached conidia. Conidiogenous pegs are 1-3 µm long, slightly tapering and imperceptibly annellate. Conidia are hyaline, smooth, thin-walled, broadly ellipsoidal, 3.2-4.4 x 1.2-2.2 µm, and with inconspicuous basal scars. Cultures grow at 37C but not at 40C.



6.5.6. *Exophiala spinifera* (H.S. Nielsen & Conant) McGinnis (1977)

Synonyms: *Phialophora spinifera* (1968)
Rhinocladiella spinifera (H.S. Nielsen & Conant) de Hoog, (1977)



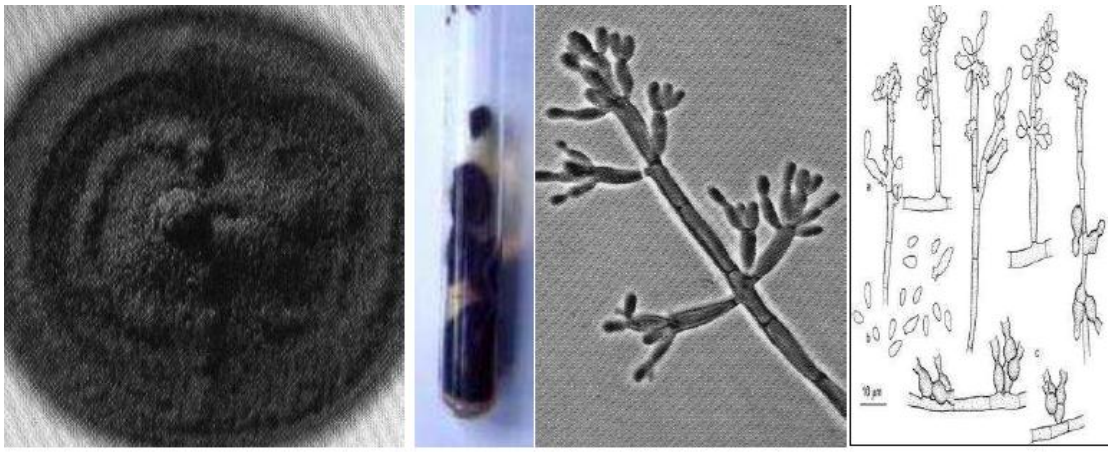
Exophiala spinifera

6.5.7. *Fonsecaea pedrosoi* (Brumpt) Negroni (1936)

Synonyms: *Hormodendrum pedrosoi* Brumpt, (1922)
Acrotheca pedrosoi (Brumpt) Fonseca & Leão (1923)
Trichosporum pedrosoi (Brumpt) Brumpt (1927)
Trichosporum pedrosianum (Brumpt) M. Ota (1927)
Gomphinaria pedrosoi (Brumpt) C.W. Dodge (1935)
Hormodendroides pedrosoi (Brumpt) M. Moore & F.P. Almeida (1936)
Phialophora pedrosoi (Brumpt) Redaelli & Cif: 592 (1941)
Carrionia pedrosoi (Brumpt) Bric.-Irag (1942)
Rhinocladiella pedrosoi (Brumpt) Schol-Schwarz (1968)
Hormodendrum algeriense Montpell (1927)
Hormodendrum rossicum Jacz. & Merlin (1929)
Hormodendrum compactum Carrion (1935)
Phialoconidiophora guggenheimia M. Moore & F.P. Almeida (1936)
Fonsecaea compactum (Carrion) Carrion (1940)

Fonsecaea pedrosoi var. communis Carrion (1940)
Rhinocladiella compacta Carrion ex de Hoog, (1977)

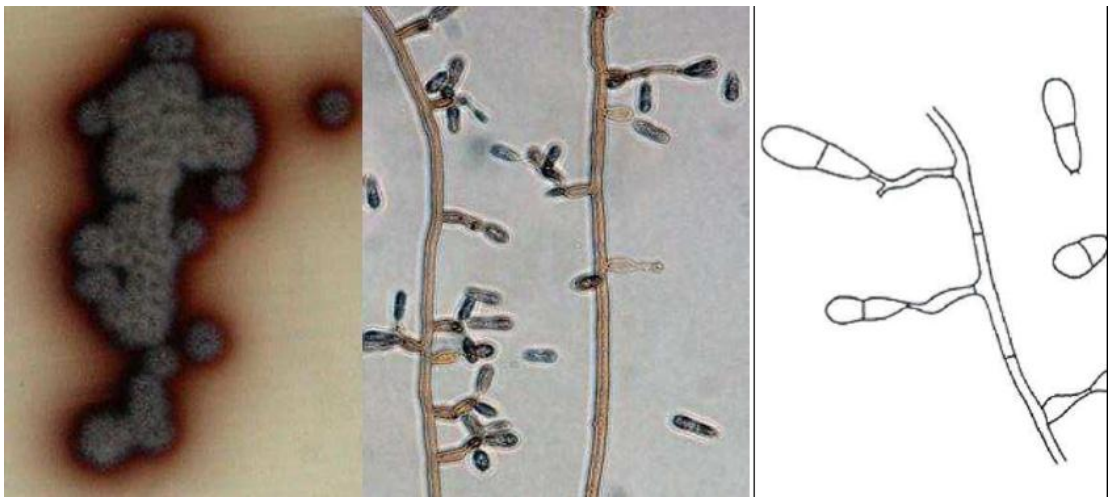
Colonies are slow growing, flat to heaped and folded, suede-like to downy, olivaceous to black with black reverse. Conidiogenous cells pale olivaceous, arranged in loosely branched systems, with prominent denticles. Conidia pale olivaceous, clavate to ellipsoidal, in short chains, subhyaline, smooth and thinwalled, 3.5-5 x 1.5-2 µm. *F. monophora* on average has slightly longer conidial chains and slightly shorter denticles than *F. pedrosoi.* All strains grow at 37C but not at 40C.



6.5.8. *Ochroconis gallopava* (W.B. Cooke) de Hoog (1983)

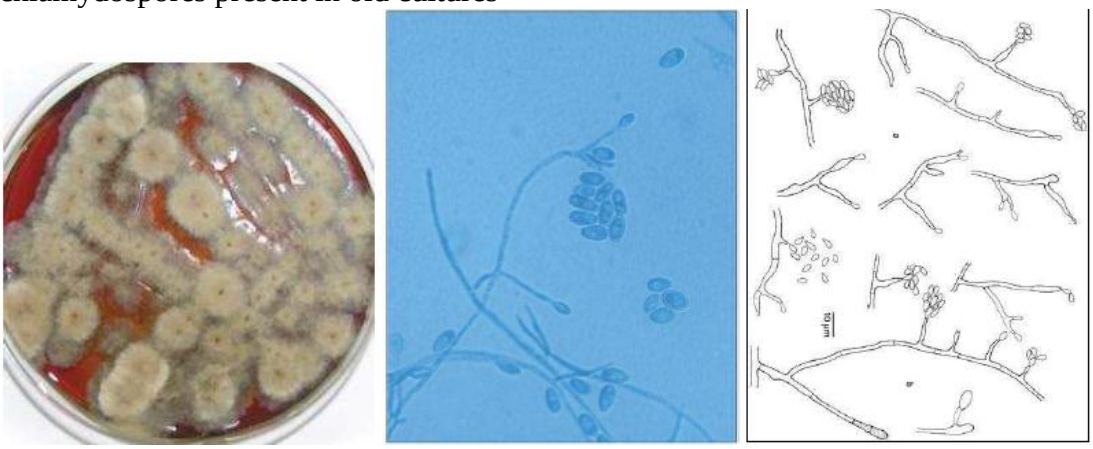
Synonyms: *Diplorhinotrichum gallopavum* W.B. Cooke (1964)
Dactylaria gallopava (W.B. Cooke) G.C. Bhatt & W.B. Kendr. (1968)
Dactylaria constricta var. gallopava (W.B. Cooke) Salkin & Dixon, (1987)

Colonies are smooth to suede-like, dry, flat, tobacco-brown to brownish-black with a dark brown diffusible pigment. Hyphae are brown with relatively thick walls. Conidiophores are mostly cylindrical to acicular, sometimes poorly differentiated, bearing a few conidia at the tip. Conidia are two-celled, subhyaline to pale brown, smooth-walled to verrucose, cylindrical to clavate, constricted at the septum, 11-18 x 2.5-4.5 µm in size, with the apical cell wider than the basal cell. A remnant of a denticle may also be seen at the conidial base. Optimum growth at 35C, tolerant to 40C.



6.5.9. *Phialemonium obovatum* W. Gams & McGinnis (1983)

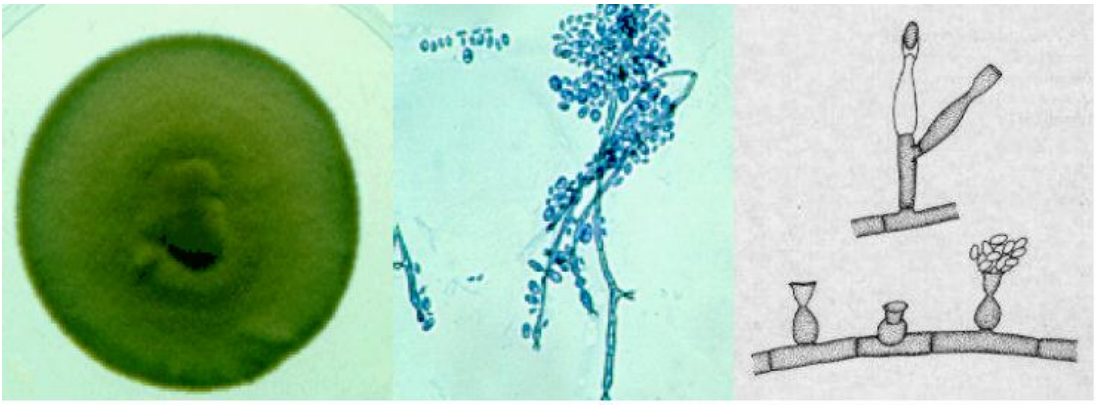
Colonies (PDA) spreading, flat, smooth, pale ochraceous or greenish. Microscopy. Conidia produced from lateral, non-septate outgrowths of creeping hyphae or from terminal, elongate phialides up to 15 µm in length. Conidia hyaline, smooth- and thin-walled, obovoidal, 3.5-6.0 x 1.5 µm, aggregating in slimy heads. Pale brown chlamydospores present in old cultures



6.5.10.Phialophora verrucosa Medlar (1915)

Synonyms: Phialophora calyciformis G. Sm. (1962)
Cadophora richardsiae Nannf (1934)
Cadophora brunnescens R.W. Davidson (1935)
Phialophora richardsiae (Nannf.) Conant (1937)
Cadophora richardsiae Nannf (1934)
Cadophora brunnescens R.W. Davidson (1935)
Phialophora calyciformis G. Sm (1962)
Pleurostomophora richardsiae (Nannf.) Mostert, Gams & Crous, (2004)

Colonies of *Phialophora* grow moderately slowly and attain a diameter of 2 3 cm following an incubation of 7 days at 25°C. The texture is wooly to velvety and may be heaped and granular in some strains. From the front, the color is initially white and later becomes dark grey-green, brown or black. From the reverse, it is iron grey to black. Microscopically, members of the genus *Phialophora* produce clusters of single-celled conidia in basipetal succession from characteristic flask-shaped or cylindrical phialides which have distinctive collarettes. Conidia are hyaline to olivaceous brown, smoothwalled, ovoid to cylindrical or allantoid, and usually aggregate in slimy heads at the apices of the phialides, which may be solitary, or in a brush-like arrangement.



P. verrucosa.

6.5.11.Stemphylium macrosporoideum (Berk.) Sacc., (1881)

Synonyms: Epochnium macrosporoideum Berk., (1838)
Acrospeira macrosporoidea (Berk.) Wiltshire, (1838)
Hyphelia castaneae Wallr., (1833)
Hyphelia castanea Wallr., (1833)

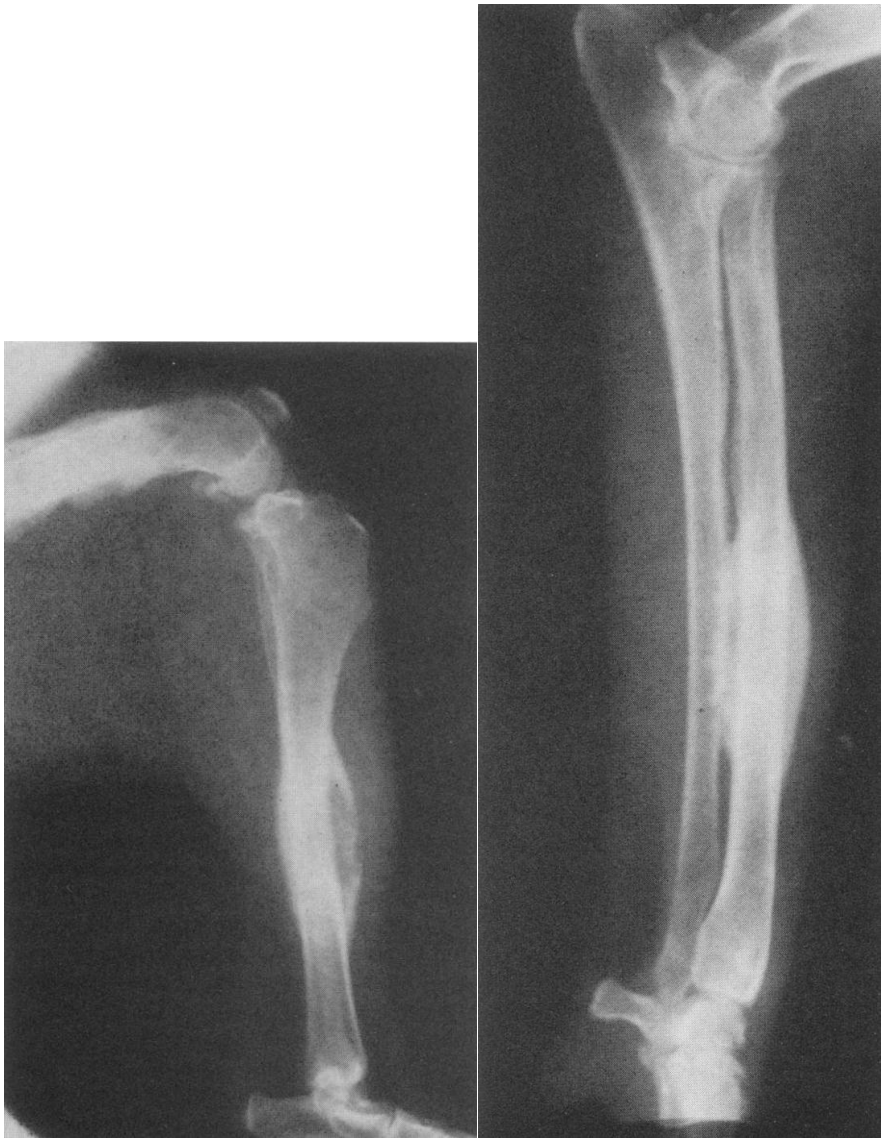
Colonies of *Stemphylium* grow rapidly, they are velvety to cottony in texture, gray, brown, or brownish-black in colour. Reverse is black. Microscopically, the fungus develops septate hyphae, which are pale brown to brown in colour. Conidiophores are black, may be simple or branched, bear a number of vesicular swellings or nodes. Conidiogenous cells are terminally located and percurrent (the proliferation which grows through the tip of the conidiogenous cell). Conidia (12-20 x 15-30 μm) are solitary, light brown to black in color, and rough- or smooth-walled. They are oblong or subspherical, rounded at the tips and have transverse and vertical septations (=muriform conidia) with a typical constriction at the central septum. They have thickened scars at their base

6.6. Reports on Phaeohyphomycoses in dogs and cats

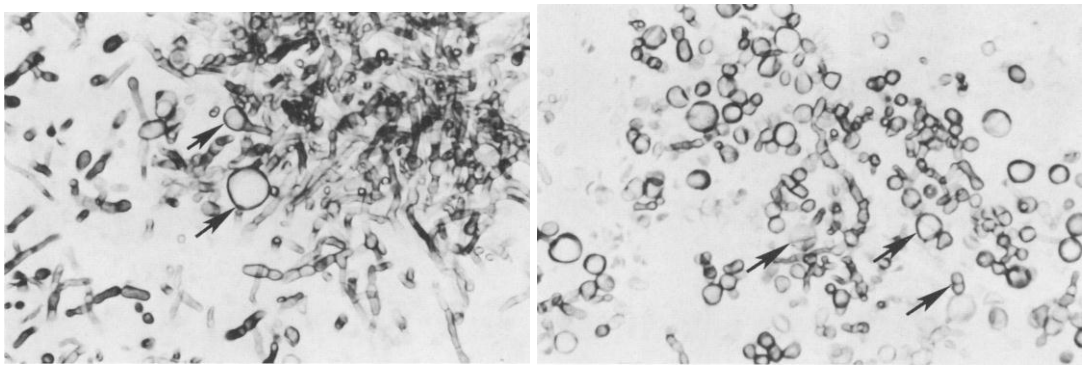
6.6.1. Reports on Phaeohyphomycoses in dogs

Lomax *et al.* (1986) identified ***Phialemonium obovatum*** as the cause of osteomyelitis in a German shepherd dog. Histologic examination of the biopsied material from the

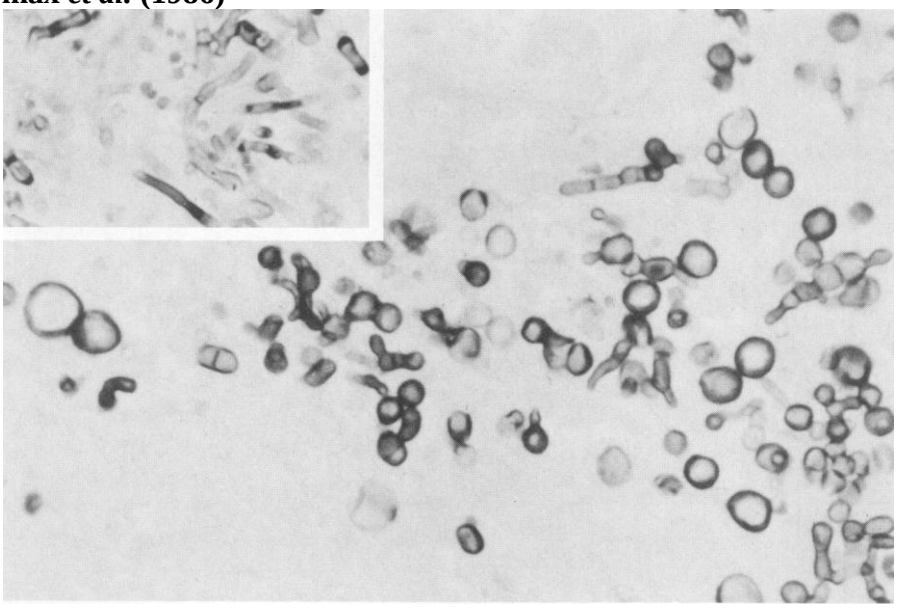
left tibia revealed septate, irregularly branched hyphae, swollen cells, and ovate-to-spherical cells divided by a transverse septum. The majority of the fungal elements were hyaline, but a few had lightly pigmented cell walls that had a greenish yellow tint. The presence of melanin in the cell walls of the hyphae and especially in their septa was verified by the use of the Fontana-Masson silver stain. P. obovatum formed moist, off-white-to-ochraceous, spreading colonies with a characteristic green pigment on their reverse side. The pigment was more prominent in cultures grown at 37 degrees C than in those grown at 25 degrees C. The isolate also grew at 40 degrees C. The dog isolate produced characteristic adelophialides without conspicuous collarettes and also basal septa from the creeping vegetative hyphae growing on the surface of the medium. The numerous obovate phialoconidia were smooth and one-celled.



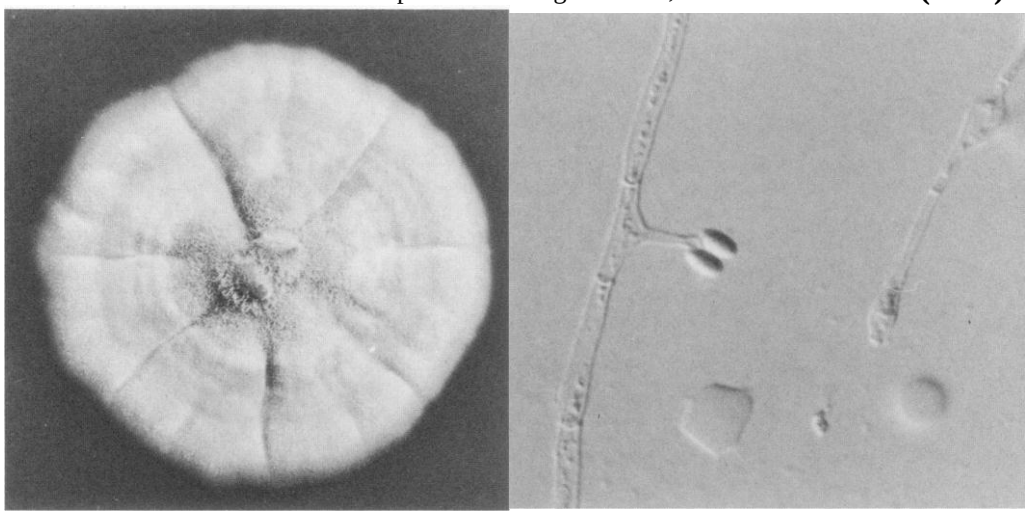
1. Lateral view of left tibia and femur. There is soft-tissue swelling, irregular periosteal proliferation, lysis of cortical bone, and increased medullary density in the mid-shaft region of the tibia. The distal metaphysis of the femur has periosteal proliferation and increased medullary density.2. Lateral view of the left radius and ulna showing overlying soft-tissue swelling and periosteal proliferation on both the radius and ulna. The mid-shaft region of the radius also has corticalysis and increased medullary density. **Lomax *et al.* (1986)**



3. Scattered and loosely aggregated hyphae of P. obovatum in a section of the tibial, 4.Budding yeastlike cells and pseudohyphae in the necrotic center of a granuloma. Arrows, Spherical-to-oval fungal cells, stained with Gomori methenamine silver, with a single, thin septation. Magnification, x700. **Lomax *et al.* (1986)**

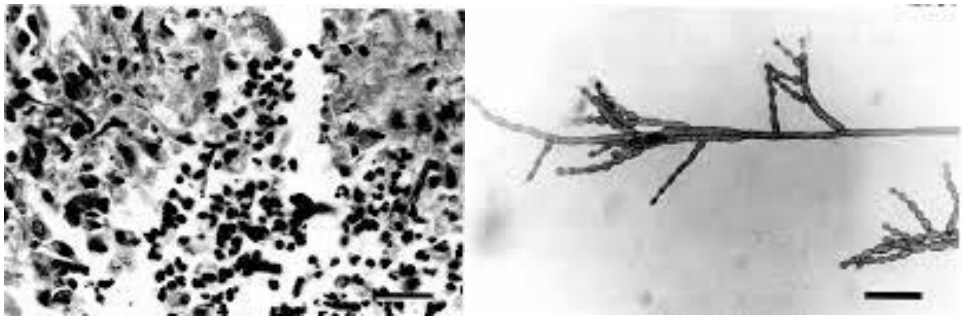


5. Pleomorphic fungal elements of P. obovatum, stained with Gomori methenamine silver, in a tibial abscess. Magnification, x700. Inset: Replicate section shows positive staining of some elements for melanin with the Fontana-Masson silver procedure. Magnification,x700. **Lomax *et al.* (1986)**



6. Colony of P. obovatum on Sabouraud dextrose agar after 2 weeks at 25°C.7. Photomicrograph showing an adelophialide and conidia of P. obovatum. Lactophenol cotton blue; magnification, **Lomax *et al.* (1986)**

Dillehay *et al*. (1987) reported Cerebral phaeohyphomycosis in two dogs. Case 1. A 4-month-old female pit bulldog had a 3 day history of seizures and a temperature of 105.0 F. The dog had not received any vaccinations and had been healthy. Because of neurologic abnormalities, it was euthanized. Case 2. A 6-month-old, male Dachshund mixed breed dog had a 3 week history of neck stiffness and pain upon manipulation. It had not received vaccinations. Treatment with antibiotics and steroids resulted in temporary clinical improvement. Cervical radiographs were normal. Analysis of Cerebrospinal fluid showed white blood cells, 26,4OO/cu mm; protein, 298 mg/dl; and glucose, 80 mg/dl. Cultures of cerebrospinal fluid were negative. The dog had several grand ma1 seizures and was euthanized. At necropsy, gross lesions were seen only in the brain. Meninges were diffusely opaque, congested and multifocally adherent to the cerebrum. Case 1 had a discrete, focal, green-brown lesion, 1.0-1.5 cm in diameter, in the right thalamus. Case 2 had a well circumscribed, red-green malacic lesion, 0.5-1 cm in diameter, on the dorsal surface of the right parietal lobe and multifocal, discrete to coalescing, green-brown foci varying in diameter from 0.1 to 0.5 cm in both white and gray matter of the dorsolateral area of the frontal lobe . Microscopically, lesions varied from abscesses, some of which were encapsulated by thick fibrous tissue, to less discrete and often coalescing foci of pyogranulomatous inflammation. Within epithelioid and multinucleated giant cells, and extracellularly, were moderate numbers of septate, pigmented hyphae 3-6 pm in width. Hyphae were light brown to basophilic in hematoxylin and eosin (HE)-stained sections and had parallel contoured walls with occasional branching. Hyphae were also within blood vessels, some of which were thrombosed. Both animals had multifocal suppurative meningitis. Phaeohyphomycotic lesions were not present in extraneural sites. ***C. bantianum*** was grown in culture.



Abscess containing pigmented hyphae, cerebrum, dog (Case 2). HE. Bar = 20 pm.. C. bantianurn grown in culture, branching hyphae with conidia. Lactophenol cotton-blue stain. Bar = 20 pm.

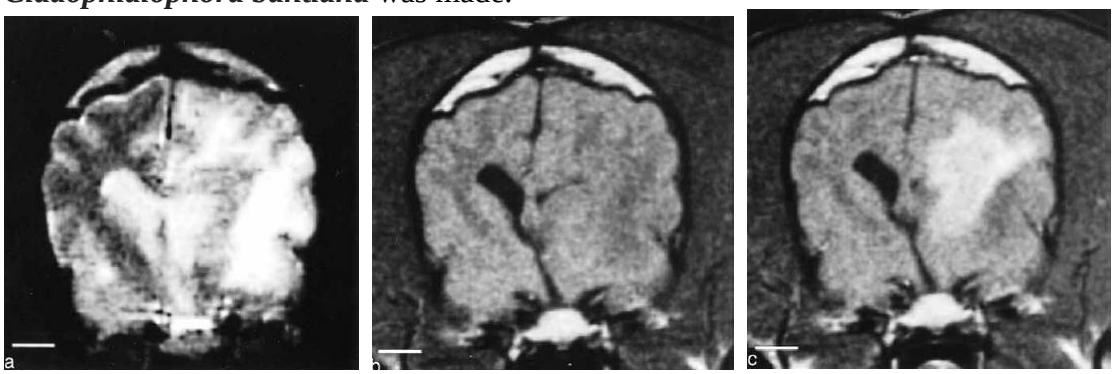
Migaki *et al.* (1987) diagnosed cerebral phaeohyphomycosis in a 9-year-old spayed dog that had a series of epileptic convulsions a day before death. About 6 weeks before her death, she had been treated for severe demodectic mange. During this period, persistent leukopenia, lymphocytopenia, and thrombocytopenia were found by blood analyses. At necropsy, multiple large pyogranulomatous lesions were found in the cerebrum and meninges. Dematiaceous fungi with brown, branching, septate hyphae and budding yeasts were found within tissue cells and in the necrotic areas.

Schroeder *et al.* (1994) presented an 8-year-old, Maltese-cross bitch with chronic neck and back pain and an acute onset of circling, hyperaesthesia and constant crying. Clinical examination revealed temporal muscle atrophy, an abnormal hanging reflex, cervical rigidity and severe hepatomegaly. Ultrasonography of the liver showed

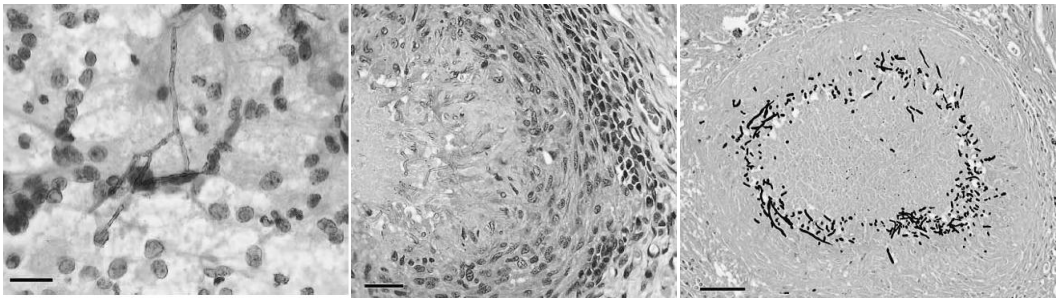
several disseminated, poorly demarcated, hypoechoic areas which on fine needle aspirates, contained large numbers of pigmented fungal hyphae. Cerebrospinal fluid examination revealed fungal hyphae and numerous Ehrlichia canis morulae. A diagnosis of systemic phaeohyphomycosis secondary to ehrlichiosis was proposed. Treatment was unsuccessful and the dog was euthanased. At necropsy, multiple yellowish-green to black, necro-granulomatous foci were found throughout the liver parenchyma and similar foci were present in the spleen, renal cortices and adrenal glands. Irregular, multifocal, grey to black foci of malacia were present in both the grey and the white matter of the brain. On histopathological examination pigmented fungal hyphae were demonstrated in the liver, spleen, kidneys, portal lymph node and adrenals, as well as in the brain. Cultures of various organs yielded a fungal organism identified as ***Xylohypha bantiana.***

Añor *et al.* (2001) reported an 8-year-old 18-kg castrated male Chow Chow dog generalized cachexia, abdominal distension, hepatomegaly, and a full, distended urinary bladder. On neurologic examination the dog was obtunded and severely tetraparetic. Based on the neurologic findings a multifocal central nervous system disorder affecting mainly the left cerebrum and the spinal cord at the level of the right cervical intumescence was suspected. Abdominal ultrasound demonstrated moderate hepatomegaly, small adrenal glands, corticomedullary rim sign involving the kidneys bilaterally, and urinary bladder sediment consistent with chronic cystitis. Magnetic resonance imaging (MRI) of the brain was done. Additional T1transverse images were acquired. On the PW and T2W images, a large, irregularly shaped region of increased signal intensity, affecting mainly the white matter of the left parietal and temporal lobes of the cerebrum, was observed. The right lateral ventricle appeared dilated.

Examination of a smear preparation from the biopsy sample revealed numerous pig mented fungal hyphae. The sample was hypercellular,with numerous macrophages, some neutrophils, reactive gemistocytic astrocytes, and large multinucleated giant cells. Histopathology of the biopsy sample revealed multifocal areas of malacia with dense aggregates of macrophages, and perivascular infiltrates of lymphocytes and plasma cells. Fungal hyphae, 7–9 _m wide, pigmented light brown to green, and septate were seen. A presumptive diagnosis of a **phaeohyphomycosis** due to ***Cladophialophora bantiana*** was made.

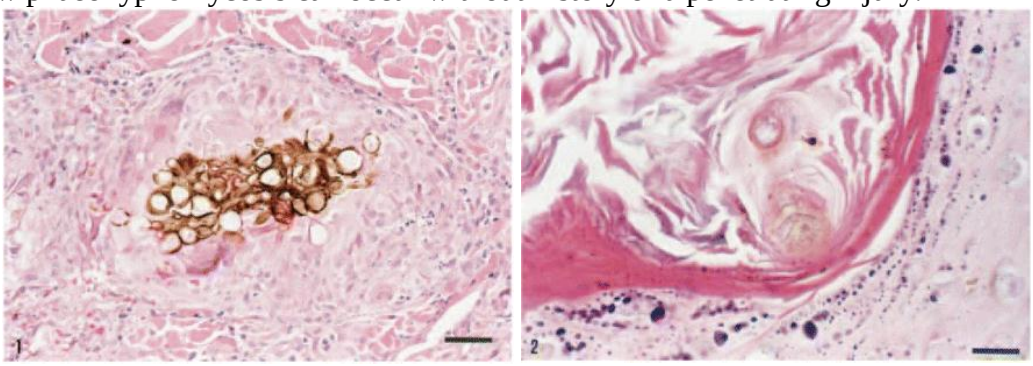


(a) T2-weighted transverse image of the brain of a dog with systemic phaeohyphomycosis. Note the irregular area of increased signal intensity in cerebral white matter extending from the falx to the left parietal and temporal lobes of the cerebrum. The large area of increased signal intensity in these lobes corresponds to the location of the fungal granuloma. A pronounced right shift of structures toward the midline is present, as well as a compression of the left lateral ventricle. Bar _ 12 mm. **(b)** Precontrast T1-weighted (T1W) transverse magnetic resonance image illustrating the same large, irregular area of decreased signal intensity, causing right deviation of the falx cerebri and loss of volume of the left lateral ventricle. Bar _ 12 mm. **(c)** Postcontrast T1W transverse magnetic resonance image illustrating the large hypointense, irregular lesion, which is nonuniformly contrastenhancing. Bar _ 12 mm. **Añor *et al.* (2001)**

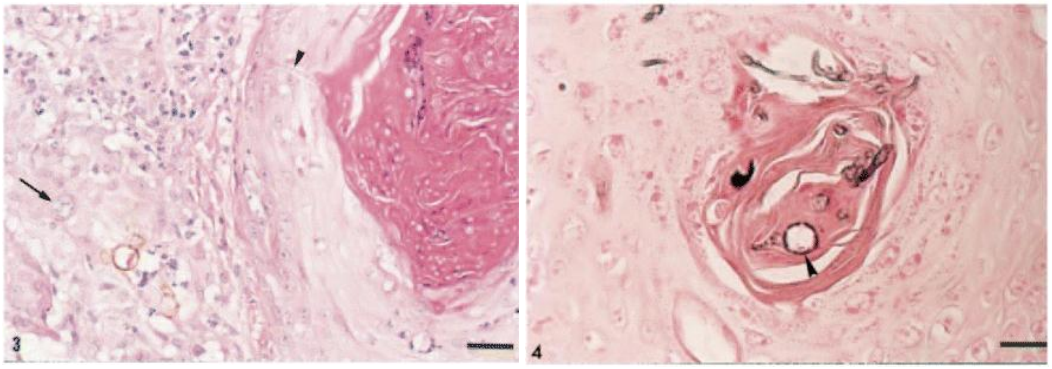


Large numbers of macrophages and multinucleated cells are observed. Fungal hyphae are branching, pigmented, and septate structures. Hematoxylin and eosin stain. Bar _ 14 _m., fungal granuloma with central necrosis. , fungal granuloma demonstrating the inner rim of viable fungal hyphae. Gomori methenamine silver stain.Bar _ 20 _m. **Añor *et al.* (2001)**.

Herráez *et al.* (2001) reported a 2-year-old female Boxer dog with a history of skin lesions that started 1 month after being given oral glucocorticoids for a neurologic problem. Clinically, the animal had focal areas of alopecia with papules and nodules often with ulceration overlain by crusts. Lesions were most common on the dorsum and the lateral aspects of the trunk and extremities. Histologic evaluation revealed pigmented fungal organisms within the lumina of hair follicles and throughout the dermis and subcutis. These organisms were associated with a multinodular, pyogranulomatous luminal folliculitis/furunculosis, dermatitis, and panniculitis. **Curvularia sp**. was isolated from the cutaneous lesions. The histologic identification of dematiaceous fungal organisms in the hair follicles may explain how phaeohyphomycosis can occur without history of a penetrating injury.



1.Skin; dog. Granulomatous lesion in the deep dermis. Central aggregate of brown, pleomorphic hyphae surrounded by epithelioid-like macrophages, multinucleated giant cells, and lymphocytes. HE. Bar = 70 µm. 2. Skin; dog. Dematiaceous fungus within the keratin of follicular infundibulum. HE. Bar = 20 µm, **Herráez *et al.* (2001)**



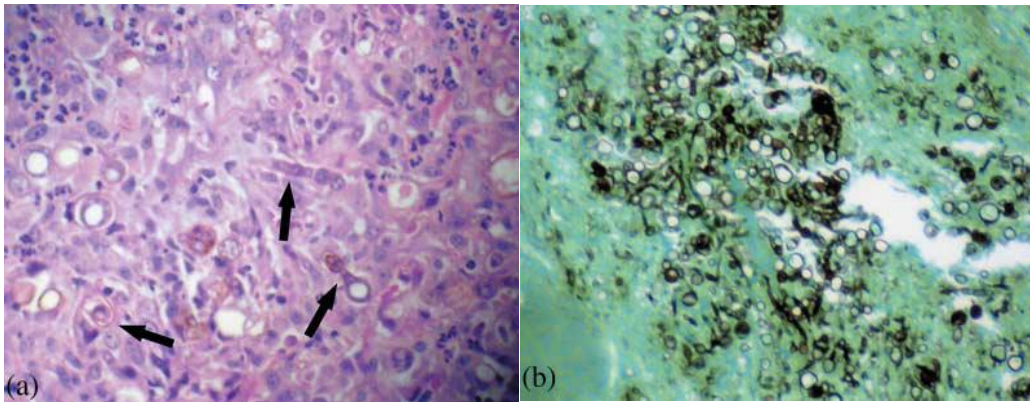
3.Skin; dog. Pyogranulomatous perifolliculitis. Numerous fungi are present in the follicular infundibulum. A septate hypha is present in the wall of the outer sheath (arrowhead). Notice the brown fungus near the hair follicle, with a dilatation similar to a chlamydospore. The organisms varied from dark brown to devoid of pigment (arrow). HE. Bar = 40 µm.**4.**Skin; dog. Intrafollicular fungi stained

black. Staining is limited to the cell walls of hyphae. The etiologic agent is pleomorphic, with dilated structures similar to chlamydospores (arrowhead). Fontana–Masson. Bar = 30 µm, **Herráez *et al.* (2001)**

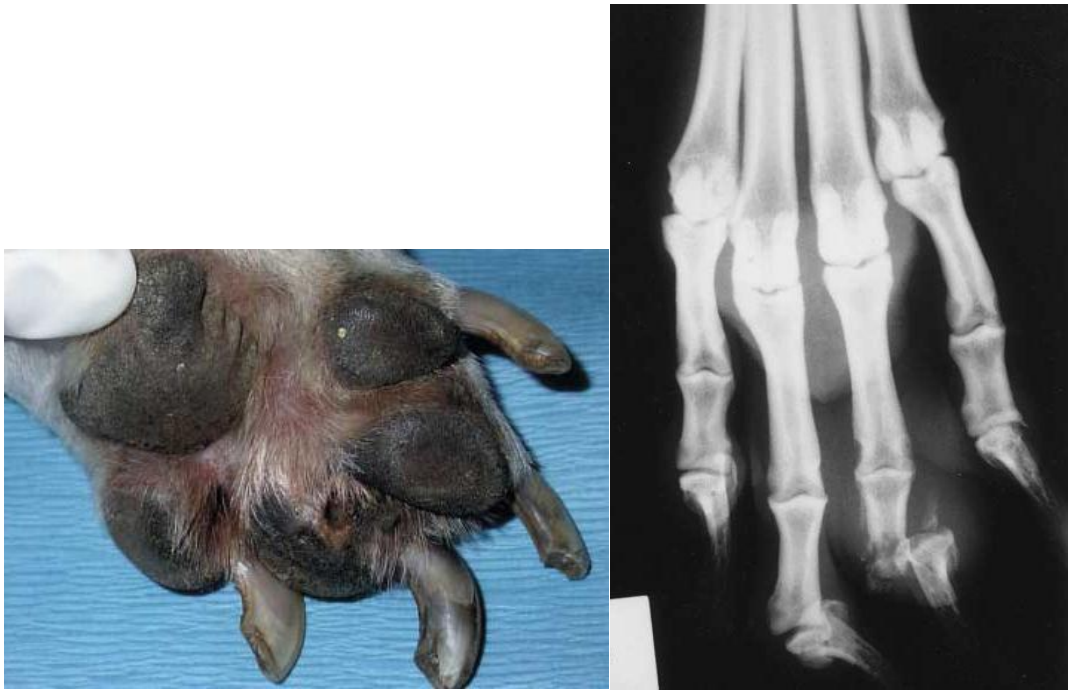
Singh *et al.* (2006) presented a 5-year-old Shetland Sheepdog with a history of weakness, ataxia, anemia, thrombocytopenia, and occasional seizures. The dog had been treated for 6 months with prednisone for inflammatory bowel disease. A positive titer for Ehrlichia canis was detected 6 months before referral. The initial physical examination revealed a weak, laterally recumbent dog with pale mucous membranes. Neurologic examination revealed multiple neurologic deficits. A complete blood cell count (CBC) revealed normochromic, normocytic, nonregenerative anemia; lymphopenia; thrombocytopenia; and neutrophilic and monocytic leukocytosis. Urinalysis revealed proteinuria, with a specific gravity of 1.045. The dog was unresponsive to treatment and died. At necropsy, there was severe serofibrinous peritonitis and pleuritis, with randomly scattered dark brown necrotic foci present in multiple organs, including liver, spleen, kidney, and pancreatic lymph node. Histologically, there were extensive regions of parenchymal necrosis surrounded by neutrophils admixed with epithelioid macrophages, lymphocytes, and pigmented fungal organisms. Numerous brown, 2 to 6 microm in diameter, septate, branching hyphae, subsequently identified as ***Ochroconis gallopavum*** (formerly Dactylaria constricta var. gallopava), were observed.

Swift *et al*. (2006) reported a 7-year-old castrated male Whippet that developed deep ulcerative skin lesions whilst receiving immunosuppressive doses of prednisolone and cyclosporine for the treatment of immune-mediated haemolytic anaemia. The lesions were determined to be a **phaeohyphomycosis**, caused by ***Curvularia lunata***. The dog was treated with a combination of systemic antifungals and weaning off immunosuppressants and made a complete recovery. To the authors' knowledge, this is the first case report of the successful treatment of disseminated cutaneous phaeohyphomycosis in a dog.

The distal limbs of a 7-year-old castrated male Whippet presenting with skin lesions 16 days after initiation of immunosuppressive therapy for immune-mediated haemolytic anaemia. Similar lesions were present on all four limbs, the neck, face and trunk. Swift *et al*. (2006)



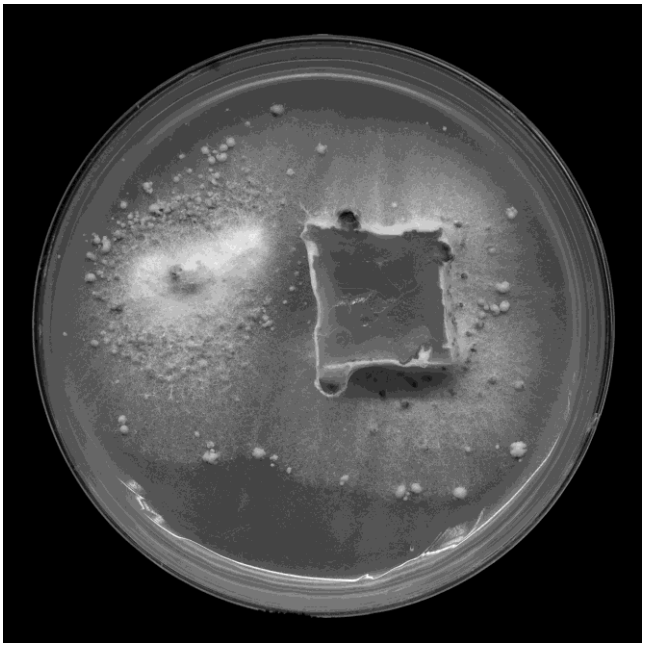
a. Histopathological section of skin biopsies taken from skin affected by phaeohyphomycosis, stained with haemotoxylin and eosin, demonstrating pyogranulomatous reaction, with fungal hyphae visible (arrows) within the tissue. b. A similar section of skin as in (a), stained with Gomori's methenamine silver stain, showing the fungal elements within the tissue. . Swift *et al.* (2006)



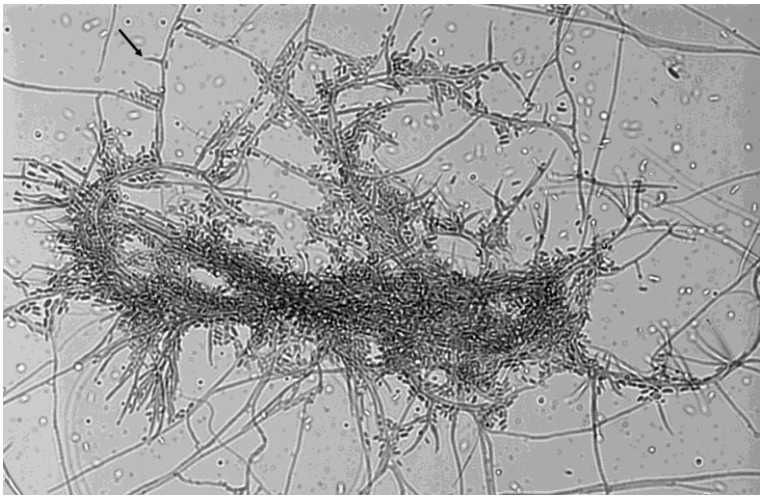
Plantar surface of right foot, day 60. A draining sinus is present under the third digit. Bone could be seen protruding from this lesion. Radiograph of right foot, day 60, demonstrating severe lysis of the distal second phalanx and proximal third phalanx of the third digit. A pathological fracture/luxation is present at this joint. Swift *et al.* (2006)

Photograph taken during toe amputation. Black fungal pigmentation in subcutaneous tissue can be seen along the incision line (arrow). a and b. Photographs of healed lesions of distal limbs, day 190. The extent of previous lesions can be clearly seen. Swift *et al*. (2006)

Sutton *et al*. (2008) reported ***Phialemonium curvatum***, as a new agent of **pulmonary phaeohyphomycosis** in a Standard Poodle dog.In vitro susceptibility data, for both human and animal isolates, suggests resistance to amphotericin B and susceptibility to the triazole agents itraconazole, voriconazole, and posaconazole.



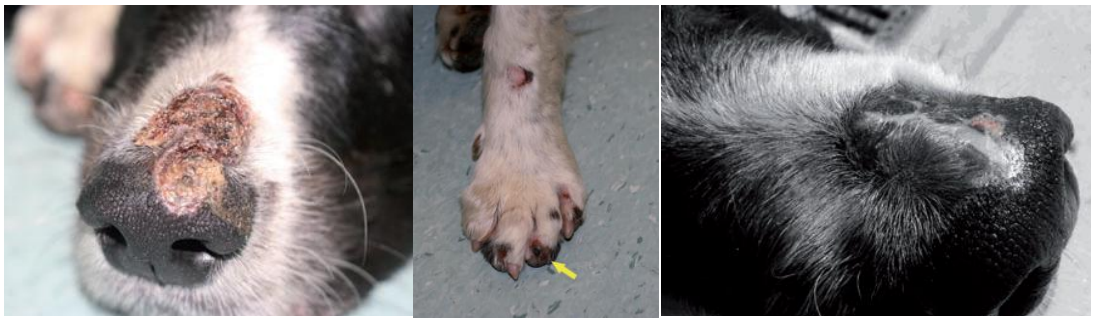
Potato flakes agar plate, 8 weeks at 25°C, showing area of slide culture preparation on the right, and an undisturbed colony on the left. Salmon to brownish-yellow, moist, raised sporodochial areas are seen throughout the culture. **Sutton *et al*. (2008)**



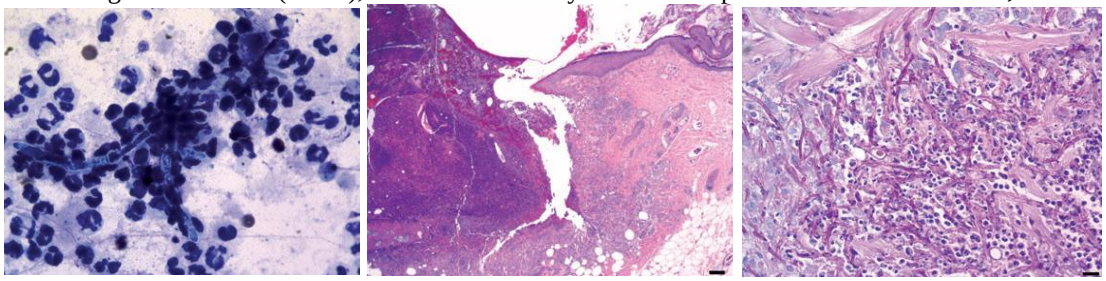
Microscopic morphology of a young, immature sporodochium after 7 days growth at 25°C on potato flakes agar. Figure depicts short adelophialides (reduced phialides lacking a basal septum), black arrow, as well as longer phialides delimited by basal septa as seen in *Acremonium* species, **Sutton *et al*. (2008)**

Dedola *et al.* (2010) reported a 4-year-old, ovariohysterectomized, English springer spaniel on immunosuppressive therapy for 3 months receiving 1.3 mg/kg

prednisolone and 2.6 mg/kg ciclosporin, both administered orally twice daily. Physical examination revealed hepatomegaly and multiple, purulent, crusting, erosive to ulcerative lesions over different body areas. Onychorrhexis had occurred on one digit and the underlying corium had blackened. There were two proliferative and one plaque-like lesions in the mouth. Thick walled fungal hyphae were detected in impression smears from all skin lesions and staining with periodic acid-Schiff's stain confirmed the presence of multiple fungal hyphae and spores in all biopsies examined. Fungal culture isolated a heavy, pure growth of an Alternaria sp. which was identified as ***A. infectoria*** by sequencing the internal transcribed spacer 1 region of the rRNA gene. The animal's condition prevented detailed investigation of the oral lesions. Withdrawal of the ciclosporin and reduction of the prednisolone dosage resulted in spontaneous resolution of the skin lesions within 40 days. Further gradual decrements in the prednisolone dosage to zero were carried out without recurrence of the immune-mediated haemolytic anaemia. After 12 months, there has been no recurrence of either the skin lesions or the anaemia. To the authors' knowledge, this is the first reported case of A. infectoria infection in a dog.



The largest skin lesion, covered with crust and draining a small amount of purulent exudate, The left forefoot showing a number of erosive and crusty lesions and, on the fourth digit, onychorrhexis and blackening of the corium (arrow), Nasal lesion 40 days after initial presentation**. Dedola et al., 2010**

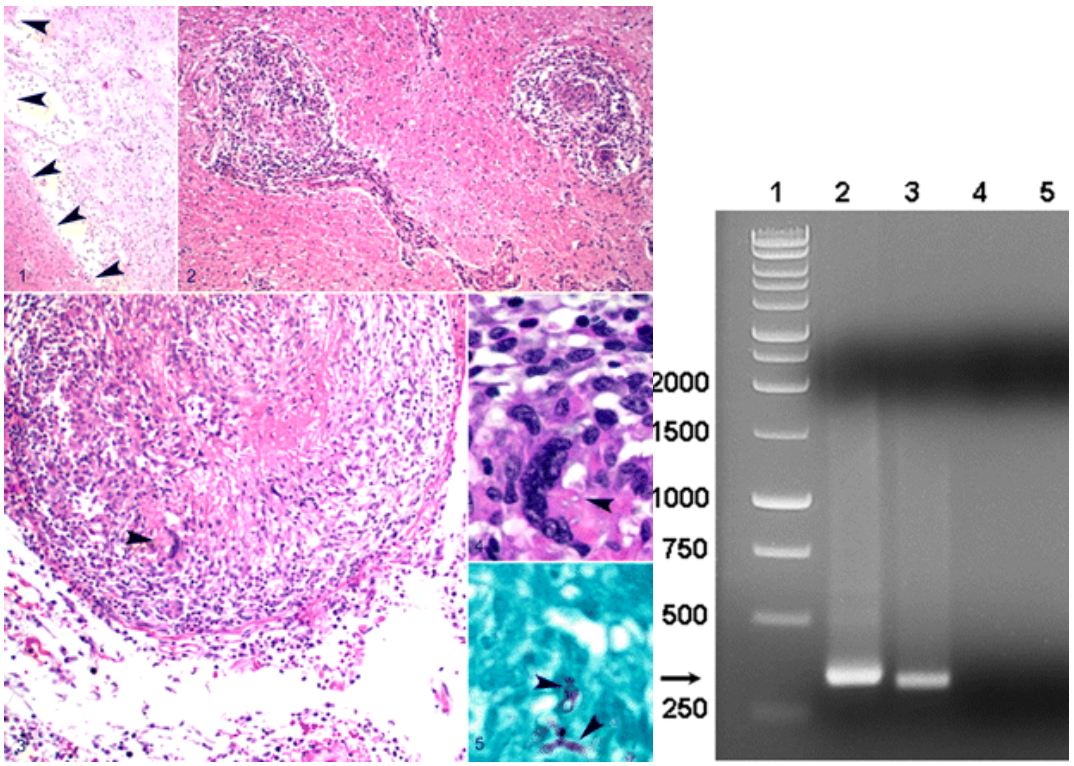


Cytology from the ulcer on the thigh showing thick walled fungal hyphae surrounded by degenerate neutrophils with intracellular cocci visible in some of them. Modified Wright ⁄ Giemsa stain, magnification · 1000., Histopathology from the lesion on the lateral left thigh.Note the extensive ulceration and the diffuse inflammatory infiltrate extending from the dermis down into the sub-dermal fat layer. Haematoxylin and eosin stain, magnification · 40. Bar = 100 lm., Histopathology from the ulcerated lesion on the lateral left thigh. Note the presence of many fungal hyphae and sporulating body-like forms in the section. Periodic acid–Schiff stain, magnification · 400. Bar = 20 lm. **. Dedola et al., 2010**

Bentley *et al.* (2011) reported a 12-month-old castrated male Boxer with signs of acute, progressive intracranial disease. Cytologic and histologic findings were consistent with an intracranial fungal granuloma in the right cerebral hemisphere. Fungal culture yielded a **Cladophialophora sp**. The granuloma was surgically debulked to remove infected brain tissue and the avascular purulent core. Postoperatively, the patient was treated with fluconazole (2.3 mg/kg [1 mg/lb], PO, q 12 h) for 4 months, followed by voriconazole (3.4 mg/kg [1.5 mg/lb], PO, q 12 h) for a further 10 months. The outcome was considered excellent on the basis of resolution

of neurologic signs and a lack of evidence of recurrence of the granuloma during magnetic resonance imaging and CSF analysis 8 months after surgery. Magnetic resonance imaging and CSF analysis 9 weeks after administration of antifungal medications was discontinued (16 months after surgery) confirmed resolution.

Giri *et al.* (2011) reported a case of **Bipolaris** infection in a dog with granulomatous **meningoencephalitis, nephritis, and vasculitis**. The clinical and histological features resembled those of the more common aspergillosis, thus warranting confirmation by molecular methods. Polymerase chain reaction and sequence analysis identified Bipolaris from the brain lesion, indicating its involvement in the disease. To the authors' knowledge, this is the first reported case of meningoencephalitis caused by this fungus in a domestic animal.



1. Cerebrum; dog. Focally extensive rarefaction in the neuropil has progressed to cavitation (arrowheads) in the cerebral gray matter. HE.**2.** Cerebrum; dog. Multiple discrete angiocentric granulomas focally replace cerebral parenchyma. HE.**3.** Cerebrum; dog. Leptomeninges are expanded by edema and numerous leukocytes. A meningeal vessel has mural infiltration by numerous macrophages, lymphocytes, plasma cells, and rare multinucleated giant cells (arrowhead). HE.**4.** Cerebrum; dog. Higher magnification of the vessel wall in Fig. 3. Numerous inflammatory cells include a multinucleated giant cell with cytoplasmic fungal hyphae (arrowhead). HE.**5.** Cerebrum; dog. Fungal hyphae (arrowheads) are in the inflammatory exudate in a vessel wall. Grocott's methenamine silv. **6.**Agarose gel electrophoresis of polymerase chain reaction–amplified fungal rDNA: ITS3 and ITS4. Lane 1, 1 Kb DNA ladder (numbers on the left are in kilobases); lane 2, test sample; lane 3, positive control (*Candida albicans*); lane 4, kidney from an age-matched dog with no history of fungal infection; lane 5, no template control. **Giri *et al.* (2011)**

6.6.2. Reports on Phaeohyphomycoses in cats

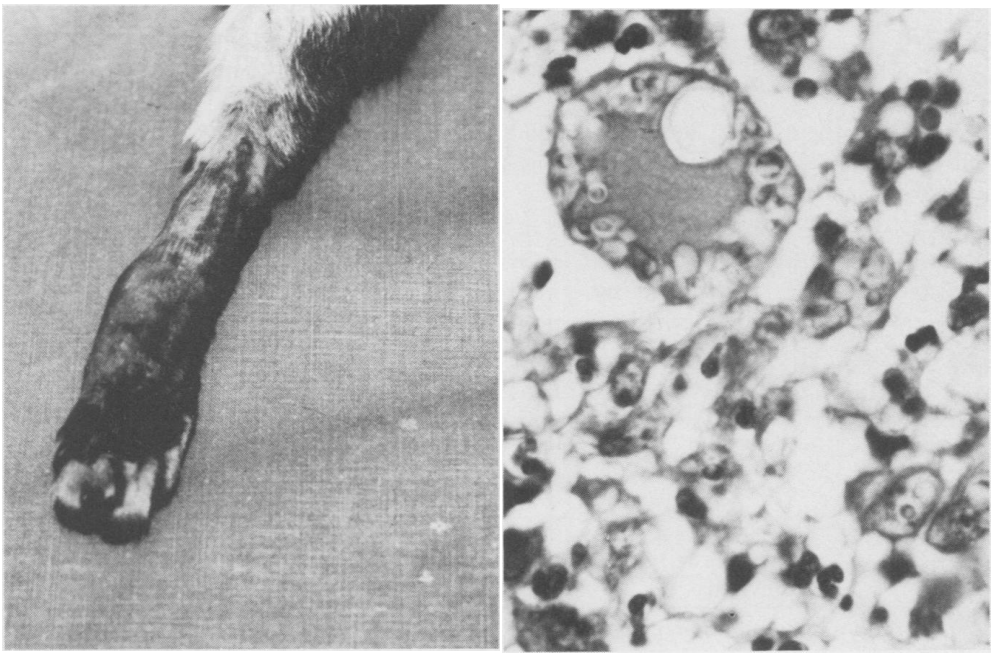
Muller *et al*. (1975) reported a slowly evolving subcutaneous mycosis in a 10-year-old domestic shorthair cat caused by ***Drechslera spicifera***, the imperfect state of ascomycete Cochliobolus spicifer. The cat had circular, nodular, granulomatous lesions over its sternum. Scattered individual and small groups of septate hyphae and chlamydospores were found in histologic sections. Many of the hyphae also had bizarre dilatations. Most of the fungal elements were hyaline; a few, however, were dematiacious. Because the fungus was not organized into granules in tissue, the disease could not be classified as a mycetoma. The preferred name for infections of this type was phaeohyphomycosis.

Haschek and Kasali (1977) described a case of severe focal granulomatous dermatitis in a 10-year-old neutered male domestic short-haired cat due to a dematiaceous fungus, ***Phialophora gougerotii*** . The infection was believed to be secondary to squamous cell carcinoma of the nasal septum.

Bostock *et al.* (1982) reported a rapidly growing subcutaneous nodule excised from the nose of an 8-year-old domestic shorthair cat. It was found to be a fungal granuloma caused by ***Exophiala jeanselmei***.

McKenzie *et al*. (1984) isolated ***Moniliella suaveolens*** in pure culture from histologically typical phaeohyphomycotic **granulomas** containing dematiaceous fungi in two cats. One cat had several slow-growing black lesions up to 2 cm in diameter in the abdominal subcutis. These lesions recurred after surgical excision was attempted. The second cat had a single black subcutaneous 0.5 X 1.5-cm lesion near one dewclaw. This lesion was successfully removed surgically without recurrence. M. suaveolens has not been isolated previously from lesions in animals including man.

Pukay and Dion (1984) treated 2 cats with phaeohyphomycosis, one infected with ***Phialophora verrucosa*** and the other with ***Exophiala jeanselmei***, with ketaconazole alone and in combination with 5-fluorocytosine after recurrence of the infections following surgical excision. The drugs were given orally at various doses and for various lengths of time, but were ineffective. Hepatocellular damage occurred in one cat.

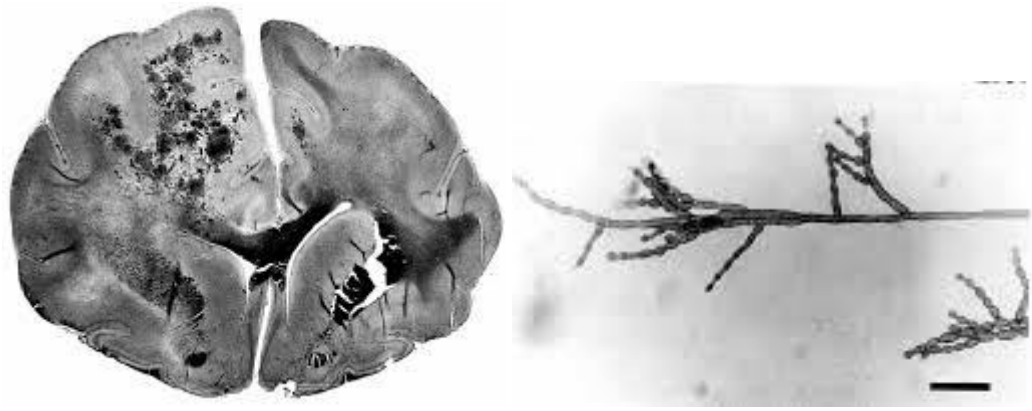


Subcutaneous nodule caused by Exophialajeanselmei.Cells of E. jeanselmei inside a giant cell. H & E. X250. **Pukay and Dion (1984)**

Sousa *et al.* (1984) reported subcutaneous phaeohyphomycosis caused by **Stemphylium sp** and **Cladosporium sp** in a cat.

Shinwari *et al.* (1985) reported a cat with cerebral phaeohyphomycosis associated with ***Cladosporium bantianum.***

Dillehay *et al.* (1987) reported Cerebral phaeohyphomycosis in a 6-year-old male castrated Persian cat had a 3 week history of intermittent ataxia, lethargy, and occasional episodes of circling to the left with right-sided hemiparesis. Antibiotics and steroids resulted in temporary improvement. Chest and cranial radiographs were normal. Electroencephalograms suggested encephalitis involving the left frontal lobe. During anesthesia to obtain cerebrospinal fluid, the cat died. At necropsy, gross lesions were seen only in the brain. Microscopically, lesions varied from abscesses, some of which were encapsulated by thick fibrous tissue, to less discrete and often coalescing foci of pyogranulomatous inflammation. Within epithelioid and multinucleated giant cells, and extracellularly, were moderate numbers of septate, pigmented hyphae 3-6 pm in width. Hyphae were light brown to basophilic in hematoxylin and eosin (HE)-stained sections and had parallel contoured walls with occasional branching. Hyphae were also within blood vessels, some of which were thrombosed. There was multifocal suppurative meningitis and diffuse pyogranulomatous ependymitis with hyphae in the lateral ventricles. Phaeohyphomycotic lesions were not present in extraneural sites. ***C. bantianum*** was grown in culture



Cerebral Phaeohyphomycosis a cat, brain with midline shift (edema) and extensive pigmented lesion in left frontal cortex. brown septate hyphae, 1-3 pm in width, and conidiophores bearing long, branched chains o fconidia 2.5 x 5-7 pm of *C. bantianum*, , **Dillehay *et al.* (1987) vet.sagepub.com**

Dhein *et al.* (1988) diagnosed phaeohyphomycosis caused by ***Alternaria alternata*** in a 6-year-old cat. A lesion in the nose resulted in enlargement of the dorsum of the nose. Similar appearing lesions had been removed from the dorsum of the nose 1 and 4 years earlier. The lesion recurred 3 months after surgical excision and irregular administration of ketoconazole. A second cytoreductive operation followed by 5 months' treatment with ketoconazole resolved the infection. Nasal trauma occurring at 8 months and at 5 years before initial examination may have predisposed the cat to development of the Alternaria infection.

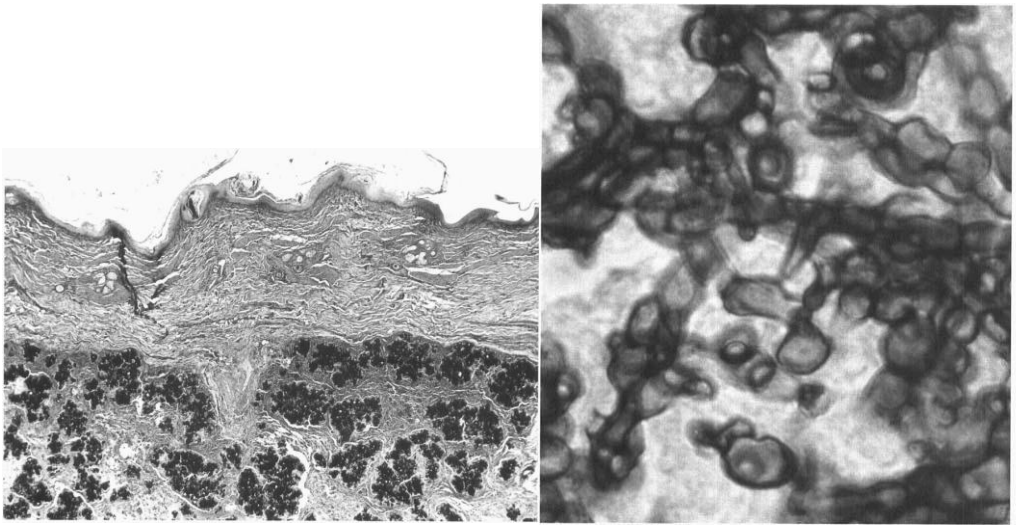
VanSteenhouse *et al.* (1988) isolated ***Scolecobasidium humicola*** from **granulomatous lesions on the tail and foot** of a cat. The paw lesion, of 2 years duration, had recurred after surgical debridement and antibiotic therapy. In tissue sections of the biopsy, S. humicola was observed in the form of broad, septate, dematiaceous hyphal elements and thick-walled, chlamydoconidium-like cells. The cat was successfully treated with ketoconazole and has since shown no signs of recurrence. This is the first record of S. humicola being an etiologic agent of phaeohyphomycosis in a mammalian host.

Kettlewell *et al.* (1989) isolated ***Exophiala spinifera*** from a **cutaneous** lesion on the paw of a male domestic shorthair cat and from the nasal exudate and abscess contents from a female domestic shorthair cat. Treatment with ketoconazole (10 mg kg-1 daily) resulted in improvement in the first cat but unfortunately this animal was subsequently lost to follow-up. The second cat was treated initially by the same regimen without apparent benefit. The dose of ketoconazole was subsequently increased but finally had to be discontinued when the cat developed signs of hepatotoxicity. At this stage treatment with flucytosine (150 mg kg-1 daily) was commenced. The cat improved and cultures of nasal exudate performed 8 and 16 weeks after initiation of 5-fluorocytosine therapy were negative for E. spinifera. However, the condition recurred with granulomatous tissue appearing in each nostril and abscess formation with subsequent rupture occurring on the bridge of the nose when therapy was withdrawn. These two cases constituted the first report of E. spinifera infection in animals and of this fungal infection in Australia.

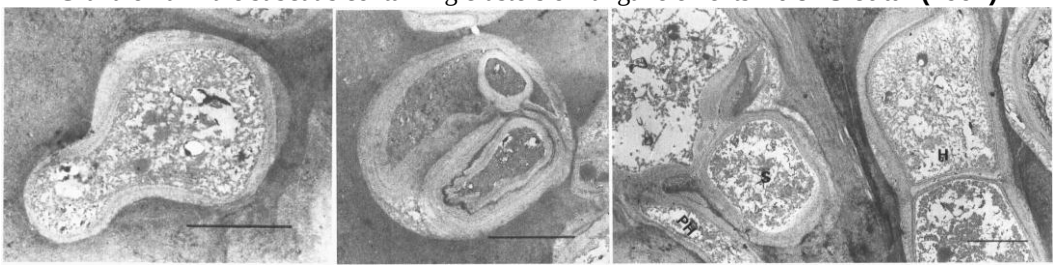
Roosje *et al*. (1993) reported the first case of phaeohyphomycosis in a cat caused by ***Alternaria infectoria***, which was isolated from several cutaneous nodules. The cat was treated with itraconazole .

Outerbridge *et al.* (1995) reported a 10-year-old, neutered male domestic shorthair with a small **cutaneous nodule** (approximately 5 mm diameter) on the inner aspect of the left pinna, near the lateral margin. The nodule was smooth, dark red-purple, turgid, and non-painful on palpation. The only other physical abnormalities noted were mild gingivitis, moderate dental tartar, and absence of the left upper canine tooth and several incisor teeth. Aspiration of the pinnal mass yielded a small amount of serosanguinous fluid. Cytological examination of air-dried preparations stained with May-Griinwald-Giemsa revealed inflammatory cells (predominantly degenerate neutrophils, monocytes, and macrophages, with occasional multinucleated giant cells and plasma cells) and fungal organisms. Fungal hyphae predominated and were irregular, septate, branched, and approximately 2 to 4 pm wide; fungal cells were round, thick-walled, and ranged from 15 to 30 pm in diameter. Some of the fungal organisms were present within macrophages. Cytological diagnosis was pyogranulomatous inflammation due to fungal infection of unknown identity with pathological hemorrhage. Most of the fungal organisms were brown to black on staining with Fontana-Masson The organism's identity was later confirmed as ***Alternaria alternata***, when the isolate sporulated lightly on tap water agar after 4 wk.

Fuchs *et al.* (1997) reported a 5.5-year-old, male, feline leucosis virus-positive cat that developed a concurrent dermatophytosis due to Microsporum canis and a subcutaneous infection due to ***Staphylotrichum coccosporum***. St. coccosporum caused mycetoma-like lesions. The fungal elements revealed features like those seen in phaeohyphomycosis. Until now St. coccosporum has been described to be non-pathogenic. The pathogenicity of St. coccosporum was corroborated by experimental infection.



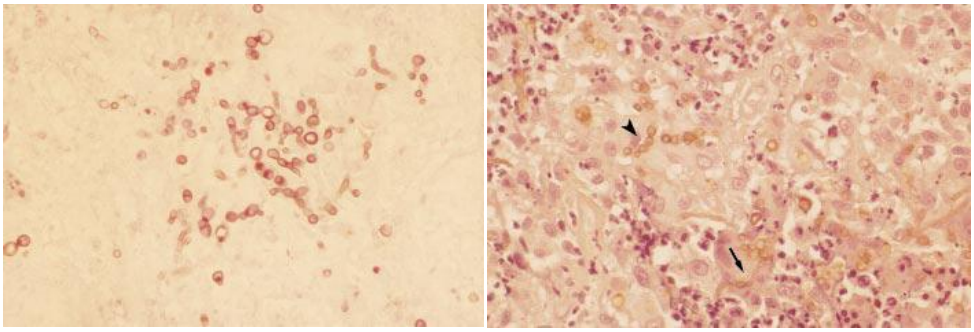
Granuloma in the subcutis containing clusters of fungal elements **Fuchs *et al.* (1997)**



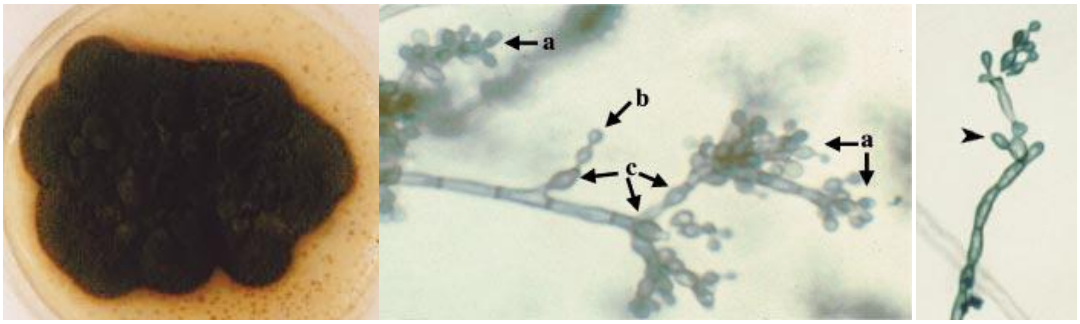
Budding spore, endospores, pseudohypha and septate hypha of *Staphylotrichum coccosporum*, Fuchs *et al.* (1997)

Fondati *et al.* (2001) reported the first report of a case of feline phaeohyphomycosis due to ***Fonsecaea pedrosoi*** in a cat. The lesion was confined to the skin and appeared as a firm swelling on the bridge of the nose. Diagnosis was based on histological examination of a cutaneous biopsy and fungal culture of a tissue sample on Sabouraud's dextrose agar. Further diagnostic tests failed to reveal an underlying immunosuppression. Two treatment cycles with itraconazole, at the oral dose of 5 mg kg-1 given twice daily, induced complete clinical remission, but relapses occurred.

Feline phaeohyphomycosis due to *Fonsecaea pedrosoi*. Swelling on the bridge of the nose. Photo reproduced from *A Practical Guide to Dermatology*, © 1999 Merial.

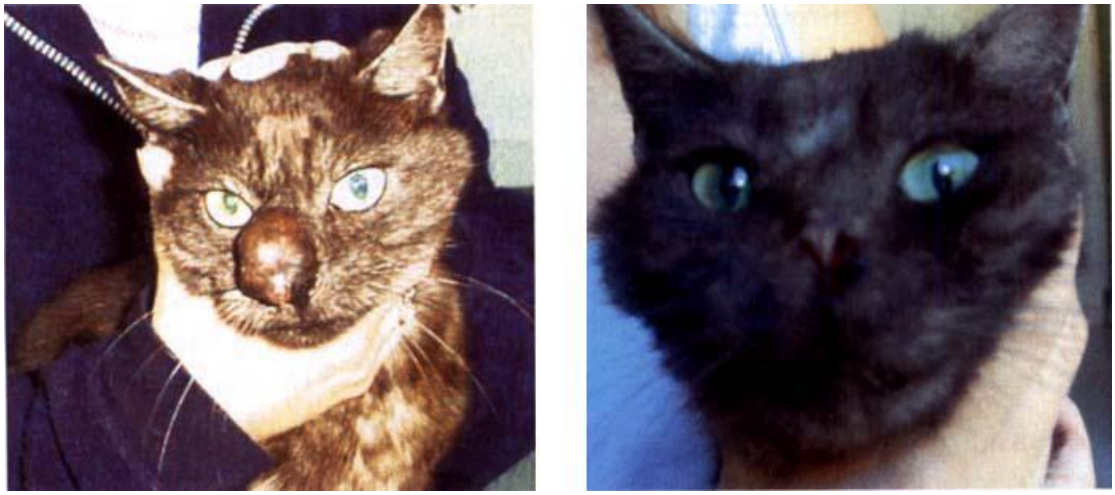


Histopathology. Numerous, slightly brown-pigmented, periodic acid Schiff (PAS)-positive fungal elements (PAS × 400).
Histopathology. Brown-pigmented, thick-walled yeastlike fungal cells (arrowhead) and irregularly septate hyphae (arrow) surrounded by macrophages, neutrophils, plasma cells and lymphocytes (H&E × 400).

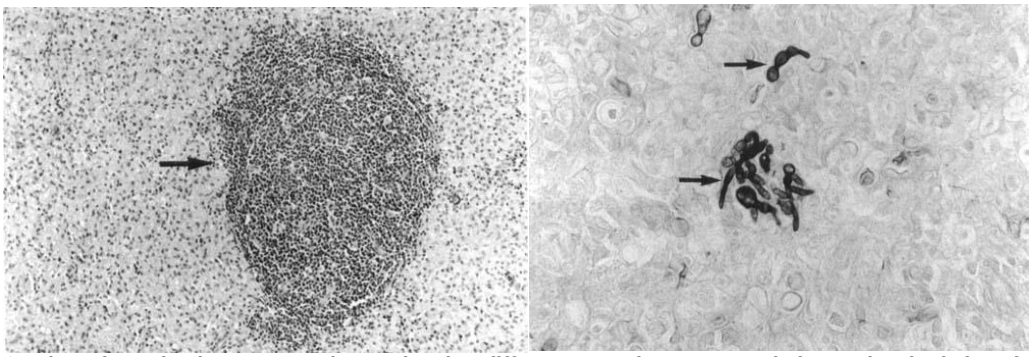


A velvety, olive green fungal colony of *Fonsecaea pedrosoi* cultured on Sabouraud's dextrose agar.
Slide culture of *Fonsecaea pedrosoi*. Conidial structures '*Rhinocladiella* type' bearing two rows of conidia, (a) one playing the role of conidiogenuos cell and (b) one conidial structure '*Cladosporium* type'. Phialides '*Phialophora* type' are shown in (c).
Slide culture of *Fonsecaea pedrosoi*. Flask-shaped phialide of *Phialophora* type (arrow).

McKay *et al*. (2001) presented a 10-year-old male domestic shorthaired cat with a chronic, slowly enlarging subcutaneous mass on the right side of its nose. The cat had been treated medically with various drugs. Oral itraconazole had been the most effective in reducing the size of the mass, but had caused hepatotoxicity and had to be withdrawn. The mass was finally removed surgically, and a diagnosis of granulomatous cellulitis caused by ***Alternaria alternata*** (phaeohyphomycosis) was established, based on histopathology and fungal isolation. There has been no recurrence of the lesion after 21 months and the cat remains clinically well at the time of writing.



Cat at the tlme of presentatlon to ENT Referrals (Aprlll999), Cat three months after surgery (colour variation la an exposure artefact)



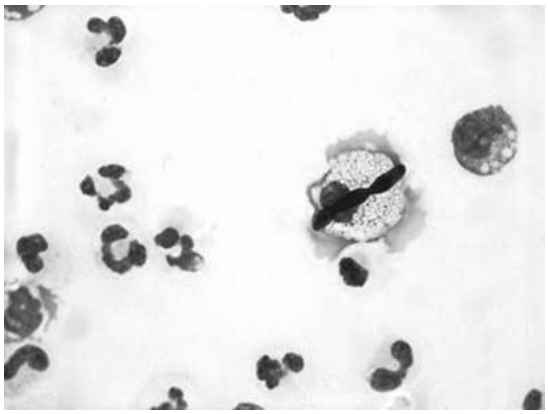
Sectlon of nasal subcutaneous tissue showing dlffuse macrophage accumulation and a single lymphold aggregate (arrow). Haematoxylln & eosln (H&E) x 140, Variably shed fungal hyphae have a surrounding capsule and globular expansions along their length (arrows). Grocott-Gomorl x *S60*

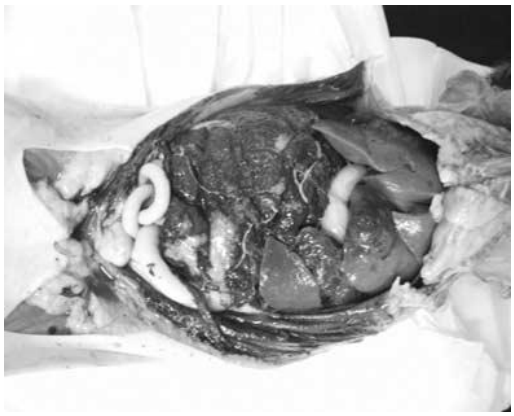
Abramo *et al.* (2002) described a case of feline **cutaneous phaeohyphomycosis** due to ***Cladophyalophora bantiana***. The cat was presented with breathing difficulty and a swollen, ulcerated nodule on the dorsal nose and left nostril. Histological examination of the nodule revealed a cystic granulomatous dermatitis characterised by neutrophils, macrophages and giant cells. Pigmented, yeast-like fungus cells and hyphal elements were easily identified in haematoxylin-eosin stained tissue sections. Cladophyalophora bantiana was isolated from a tissue specimen. This organism, primarily known to cause cerebral infection in humans and cats, only rarely causes cutaneous infection. Despite anti-fungal chemotherapy two relapses occurred. The cat was feline immunodeficiency virus- and feline leukemia virus-negative and even if the owner was unaware of trauma, the hypothesis of wound contamination is the most likely.

Bouljihad *et al.* (2002) necropsied a 6-month-old, castrated male domestic cat with progressive neurological signs of 2-3 weeks duration. Macroscopic findings were restricted to the brain and included irregularly shaped, well-delineated but unencapsulated areas of intense black pigmentation involving the rostral portion of both cerebral hemispheres. Microscopically, numerous brown, oblong, segmented branching hyphae and conidial-like structures and extensive pyogranulomatous inflammation were identified throughout the cerebral lesion and in adjacent blood vessels. Hyphae and oval conidia were best demonstrated with either Gomori methenamine silver or periodic acid-Schiff stain. Fungal infection in the brain of this cat was unrelated to any concurrent immunodeficiency syndrome or immunosuppressive treatment. This report deals with a case of cerebral phaeohyphomycosis from which a different species of dematiaceous fungus, ***Cladophialophora bantiana***, was isolated and identified.

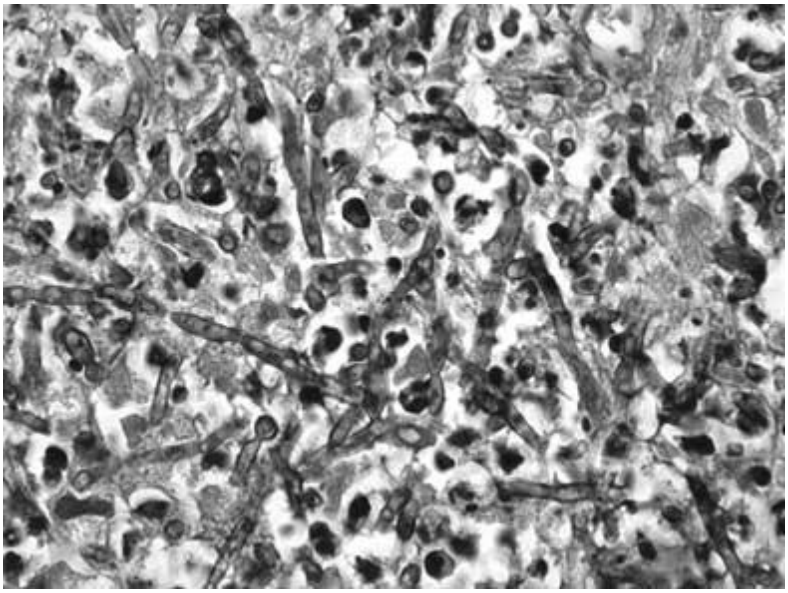
Mariani *et al.* (2002) presented two domestic shorthair cats presented for clinical signs related to multifocal central nervous system dysfunction. Both cats had signs of vestibular system involvement and anisocoria, and one had generalized seizure activity. Cerebrospinal fluid analysis revealed a neutrophilic pleocytosis with protein elevation in one cat and pyogranulomatous inflammation in the second. Electroencephalography and brain-stem auditory-evoked potentials in the first cat confirmed cerebral cortical and brain-stem involvement. Euthanasia was performed in both cats, and postmortem diagnoses of phaeohyphomycosis secondary to Cladosporium spp. were made based on histopathology and fungal culture in both cats.

Elies *et al.* (2003) reported a case of fatal systemic mycosis in a 9-year-old cat. Diagnosis of phaeohyphomycosis was made by histology. Morphological and molecular identification of the fungus isolated from the lesions yielded the species ***Cladophialophora bantiana***. The lesions were widespread, distributed without the involvement of central nervous system. The origin of systemic manifestation is still unknown and no evidence of immunosuppression was found. It is the first feline case of C. bantiana infection reported in Europe.





Cytological aspect of abdominal fluid: non-degenerate neutrophils mixed with some macrophages. A single pigmented fungal hyphae is visible within a macrophage. MGG, 1 cm ¼ 13 lm. Abdominal cavity at necropsy: all serosal surfaces are covered by a black granular substance. Necrotic foci are scattered throughout the liver.



Liver: high power view showing numerous pigmented and septate hyphae in a necrotic area. HES, 1 cm ¼ 19 lm

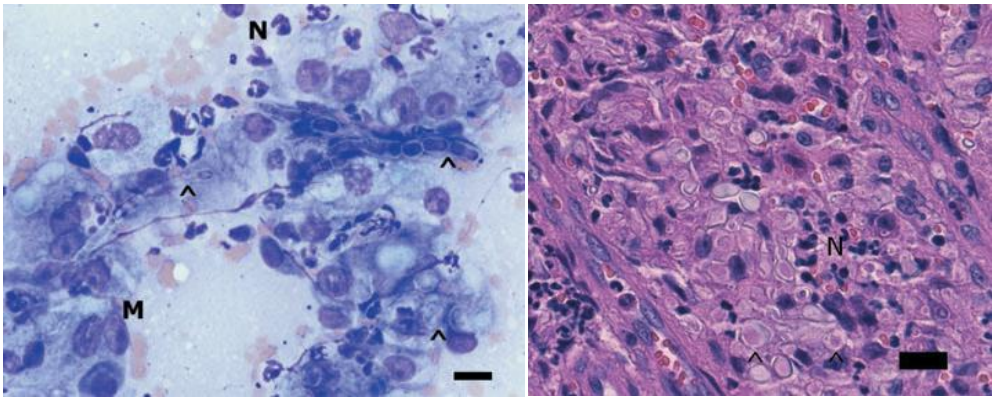
Beccati *et al.* (2005) reported a case of phaeohyphomycosis caused by ***Phialophora verrucosa*** as the first European case in a cat

Knights *et al.* (2008) reported a case of phaeohyphomycosis caused by **Ulocladium species** in a cat.

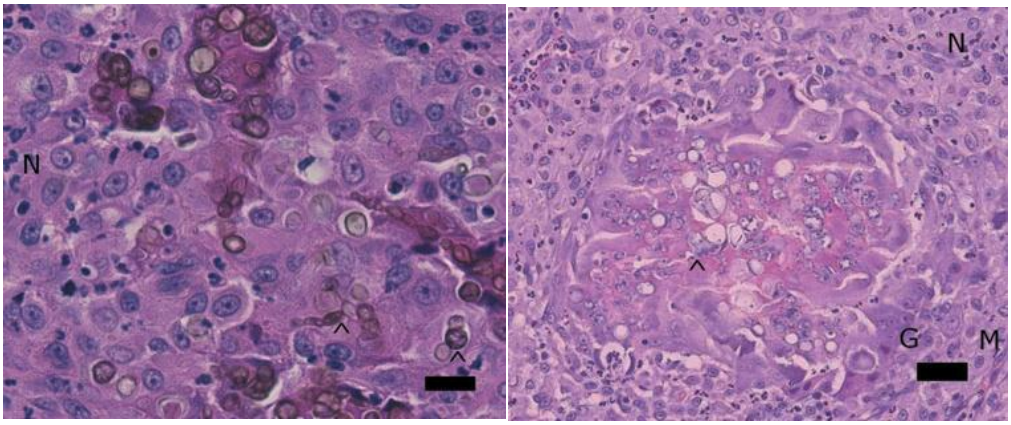
Maeda *et al.* (2008) demonstrated black nodule measuring 1 cm in diameter in the base of nail of an 8-year-old Japanese domestic male cat. Histological examination of the excised nodule revealed a granulomatous lesion extending from the epidermis to adjacent bone. The lesion consisted of diffuse infiltration of macrophages with epithelioid cells and multinucleated giant cells. These macrophages contained a few to numerous yeast-like brown pigmented fungus cells with a spherical shape and dark

thick wall. The PCR amplification with universal primers of the 28S ribosomal RNA gene yielded a 628-bp fragment and the direct sequence confirmed that the diagnosis of the lesion was phaeohyphomycosis caused by the pathogenic dematiaceous fungus, ***Exophiala jeanselmei***.

Miller (2010) investigated IDEXX Laboratories database of cases submitted from the UK between March 2005 and February 2008 (36 months) for feline nodular granulomatous skin disease associated with fungal infection. Cytological and/or histological slides were reviewed and the diagnosis was based on the microscopic pattern of the inflammatory response and morphology of the causative organism. Aetiological diagnoses were hyalohyphomycosis (64 of 77 cases), phaeohyphomycosis (five of 77 cases) and dermatophytic pseudomycetoma (eight of 77 cases). All cases of hyalohyphomycosis were suspected to be alternariosis based on common features including anatomical distribution of lesions (48 of 64 cases involved the nostril and bridge of nose, face and ears), pattern of histological changes, morphology of the causative organism and results of fungal culture (Alternaria three of 16 and 'saprophyte' nine of 16 cases). Cases of phaeohyphomycosis were demographically and histologically similar to those of alternariosis, except the causative organisms were deeply pigmented brown and had a variety of morphologies that were different from Alternaria. Dermatophytic pseudomycetomas had a characteristic histological pattern including the presence of fungal microcolonies or grains within the tissue. These occurred most often on the trunk (five of eight cases) and four of eight cases were in Persian cats. These findings indicate that alternariosis is by far the most common nodular fungal skin disease of cats in the UK.

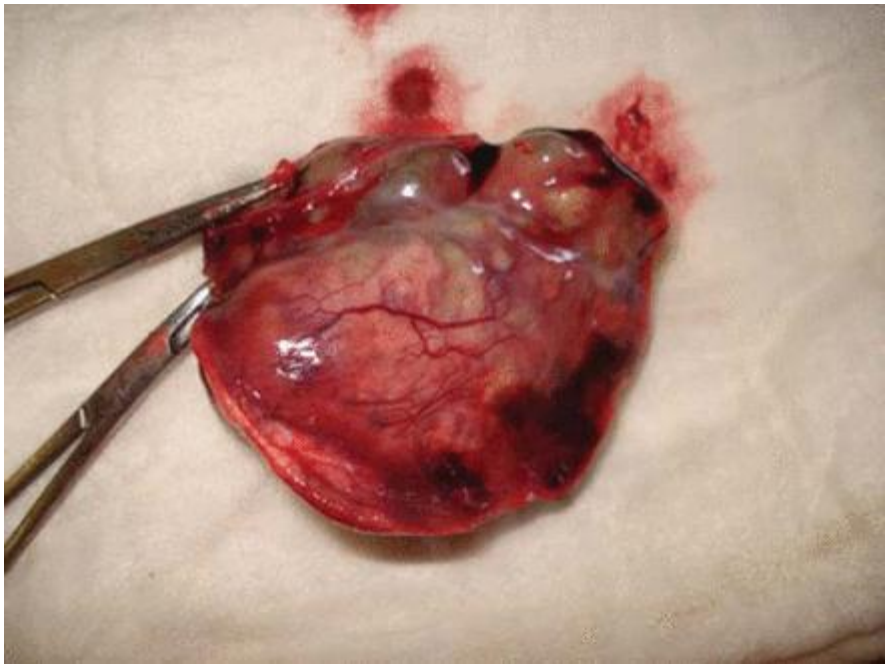


Photomicrograph: Cytology of hyalohyphomycosis. Neutrophils (N) and macrophages (M) containing pleomorphic fungal hyphae (^) are mixed with small numbers of erythrocytes. Wright–Giemsa stain. Bar = 20 lm.Photomicrograph: Histology of hyalohyphomycosis. Numerous macrophages contain nonpigmented fungal organisms showing filamentous and spherical forms (^). Small numbers of neutrophils (N) are also present. H&E. Bar = 30 lm. **Miller (2010)**



Photomicrograph: Histology of phaeohyphomycosis. Numerous macrophages and giant cells contain pigmented fungal organisms showing filamentous and spherical forms (^). Small numbers of neutrophils (N) are also present. H&E. Bar = 30 lm. Photomicrograph: Histology of dermatophytic pseudomycetoma. A fungal microcolony (^) embedded in Splendore-Hoeppli reaction is surrounded by giant cells (G) with neutrophils (N) and macrophages (M) in the periphery. H&E. Bar = 60 lm. **Miller (2010)**

Evans *et al.* (2011) reported a 12-year-old cat with a history of insulin-dependent diabetes mellitus with a pulmonary granuloma caused by ***Cladophialophora bantiana***. Thoracic radiographs revealed consolidation of the right caudal lung lobe and cytology confirmed the presence of mycotic pneumonia. Results of clinical investigations showed no evidence of extra-pulmonary infection. A thoracotomy and lung lobe resection was performed. Histological examination of the mass revealed black pigmented fungal hyphae and pyogranulomatous inflammation. Cultures inoculated with portions of these tissues yielded a dark walled fungus consistent with an etiologic agent of phaeohyphomycosis and DNA sequencing confirmed the presence of Cladophialophora bantiana. The cat was treated with itraconazole for 4 weeks post-operatively and then with posaconazole for 7 months but was euthanized 13 months after initial diagnosis due to a hepatocellular carcinoma. On post-mortem examination there was no evidence of recurrent fungal infection. This is the first report of localized pulmonary C. bantiana infection in a cat.



Resected lung lobe with black pigmented edges. **Evans *et al.* (2011)**

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7. Eumycetoma in cats and dogs

Eumycetoma is a rare form of fungal infection in humans and animals as well. The lesions are characterized by tumefaction, draining sinuses and the presence of grains, which are composed of fungal hyphae, differentiating this disease from actinomycetoma, where the grains are made of filamentous bacteria.

Eumycetoma is rare in dogs. It was reported in the United States, Korea, Australia, India, South Africa, Israel and France. The pathogens are diverse, including Curvularia geniculata, C. lunata, Scedosporium apiospermum, Aspergillus terreus, Madurella mycetomatis and Cladophialophora bantiana.

Reports:

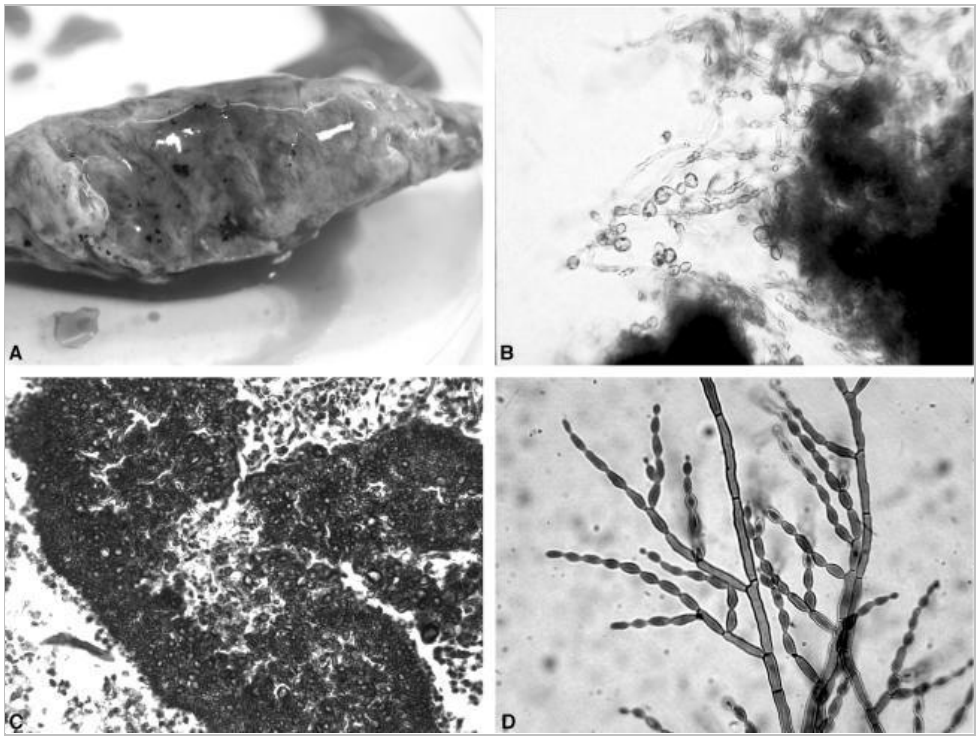
Allison *et al.* (1989) diagnosed an abdominal eumycotic mycetoma caused by ***Pseudallescheria boydii*** in a 3-year-old male Siberian Husky. The dog was examined because of weight loss and signs of depression. Initially, pyrexia was the only clinical finding. Antibiotic and corticosteroid treatment was ineffective. Two weeks later, the dog's appetite had decreased, it had vomited a few times, and the caudal portion of the abdomen was sensitive to palpation. Hematologic and serum biochemical abnormalities consisted of anemia, leukocytosis, hypoglycemia, hypoalbuminemia, hyperglobulinemia, and high alkaline phosphatase activity. One week later, the dog's condition continued to worsen, and testicular swelling was observed. The dog was castrated. Microscopic examination of specimens obtained at surgery revealed pyogranulomatous periorchitis with mycetoma granules. Ketoconazole treatment was

initiated and continued until the dog died one month later. Necropsy revealed multifocal duodenal ulcers, with transmural pyogranulomatous enteritis, pancreatitis, and peritonitis. This case is unique because the etiologic agent apparently entered via the intestinal tract rather than by contamination of an external wound.

Elad *et al.* (1991) cultured ***Curvularia lunata*** from black granules found in granulomatous tumefactions excised from the subcutis of a three year old Medium Schnauzer dog. Draining sinuses were present in some of the tumefactions. Accordingly the diagnosis of eumycotic mycetoma was made. This diagnosis was confirmed by histopathological examination. During the four years following the first surgical intervention, several more similar tumefactions were excised on three different occasions. The dog died of chronic renal failure at the age of 8 years. There was no bone involvement or visceral diffusion of the fungus. The granules were examined by scanning electron microscopy. Immunoglobulins in the dog's serum, assessed by a qualitative test, proved to be equal to immunoglobulins in the serum of a control dog. Precipitating antibodies against C. lunata were not found. The dog was treated for 150 days with itraconazole. In spite of good initial results, recurrence of the fungal lesions were observed after the treatment's interruption. Further treatment with itraconazole for 45 days proved ineffective. No side effects of the drug were observed.

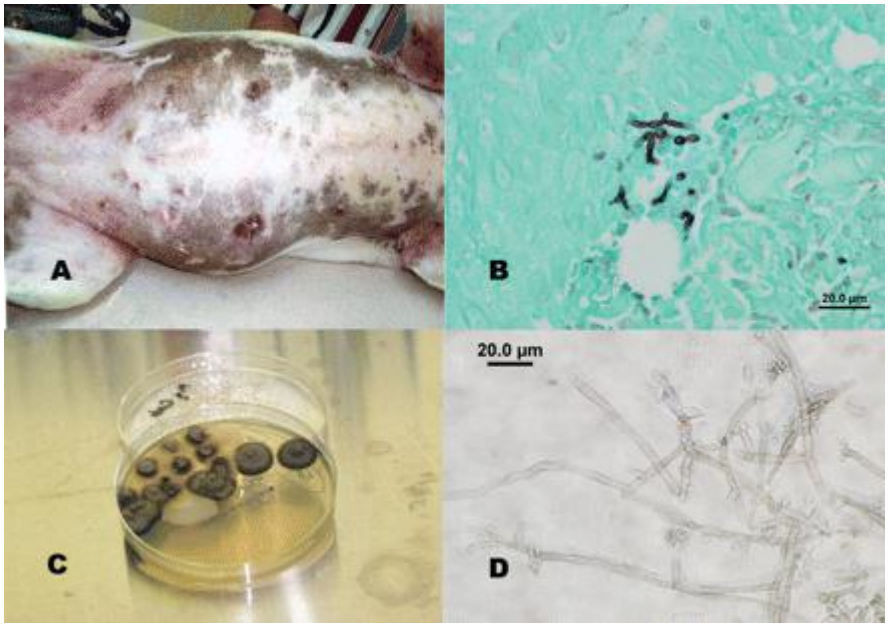
Lambrechts *et al*. (1991) removed a uterine stump granuloma surgically from a sterilized bitch. Histopathology and fungal culture revealed ***Madurella mycetomatis*** eumycetoma. Infection may have occurred through a cesarean wound dehiscence. Long-term fluconazole therapy was instituted but failed to arrest and eliminate the infection.

Guillot *et al.* (2004) reported a case of eumycetoma due to ***Cladophialophora bantiana*** in a 3-year-old male Siberian Husky. The dog presented a tumefaction on the thorax and deformity of the second and third subjacent ribs, which were surgically removed. Macroscopic black granules were visible on the ribs, and direct microscopic examination revealed their fungal origin. Cultures yielded pure colonies of C. bantiana. The identification of the causative agent was confirmed after amplification and sequence analysis of fungal internal transcribed spacers 1 and 2 and 5.8S ribosomal DNA regions. Surgery and antifungal treatment with oral itraconazole associated with flucytosine allowed apparent cure after a 10-month follow-up. Envenomation with pine processionary caterpillars (Thaumetopoea pityocampa) and subsequently intensive corticotherapy were considered as possible predisposing factors. This is, to the best of our knowledge, the first case in which C. bantiana is identified as the causative agent of eumycetoma.



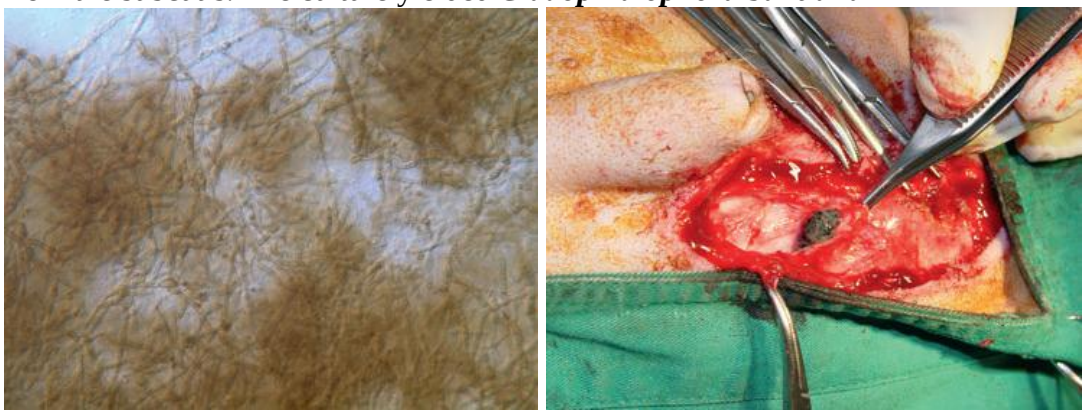
(Top left) One of the two ribs surgically removed. Note the deformity of the bone and the presence of numerous black grains. (Top right) Direct examination of one grain in Amann lactophenol. Brown septate hyphae with vesicles, toruloid filaments, and swollen granular thick-walled and budding cells are visible. (Bottom left) Hematoxylin and eosin stain showing a fungal grain in histological section of the bone. Fungal hyphae growing toward the periphery of the black grain and vesicles are clearly seen. (Bottom right) Microscopic aspect of *C. bantiana*. Lateral and terminal conidiophores of various sizes. Unicellular long chains of smooth, lemon-shaped conidia are produced. **Guillot *et al.* (2004)**

Rajeev *et al.* (2006) described a phaeohyphomycotic condition of the skin caused by ***Fonsecaea pedrosoi*** is described in a dog. The dog had lesions on the ventral abdomen. Tissue sections stained with Grocott-Gomori methenamine silver stain showed hyphae characteristic of dematiaceous fungi. Macro and microscopic features of colonies grown on Sabouraud dextrose agar were identical with that of ***Fonsecaea pedrosoi***.

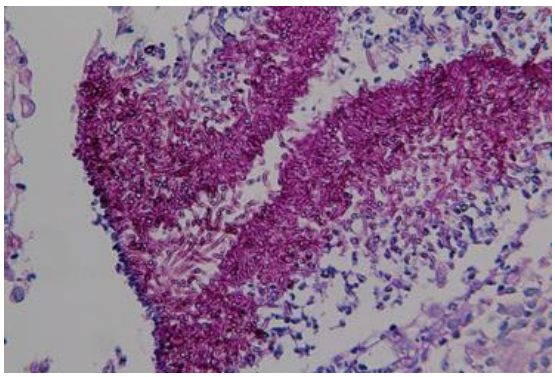
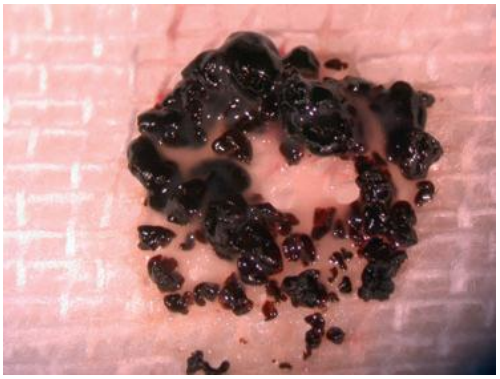


A, dog presented with lesions on the ventral abdomen. **B**, tissue sections showing the presence of hyphae Grocott-Gomori methenamine silver stain. 400X. **C**, fungal colonies growing on Sabouraud dextrose agar. **D**, lactophenol cotton blue stain of the fungal isolate. 400X **Rajeev *et al.* (2006)**

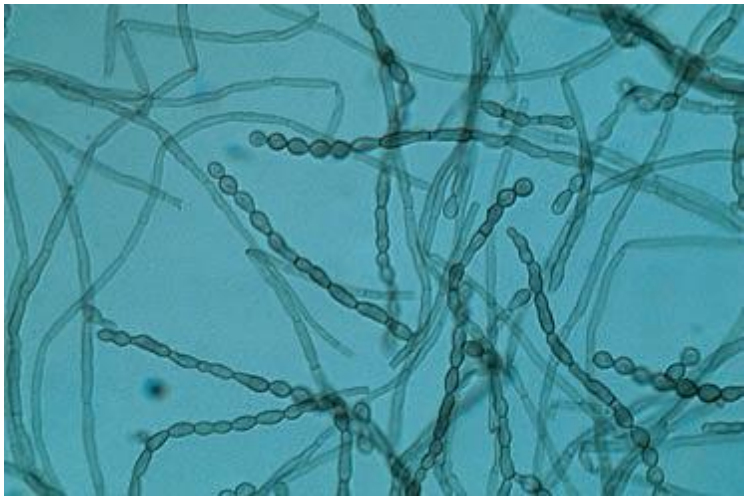
Sun *et al.* (2013) reported a 5-year-old castrated male Maltese with multiple subcutaneous nodules measuring ca. 1–2 cm with draining fistulae on the abdomen. Small black grains were drained from the fistulae. Medical history of the Maltese showed that he had received surgeries for an intestinal foreign body obstruction in 2007 and an umbilical hernia and castration in 2009. The diagnosis of eumycetoma was made. The patient received liquid nitrogen cryotherapy every week and oral antifungal of itraconazole 5 mg kg). The nodules persisted after 3 weeks of treatment. The grains were collected by a sterile swab and subjected for microscopic examination and culturing.The grains were composed of a dense mat of septate brown hyphae and chlamydospores by microscopy. The oral itraconazole of the same dosage was continued for another 4 months. Due to persistence of the lesions, surgical excision of the abdominal lesion was performed under general anaesthesia. During the operation, bouts of soft tissue mixed with small black grains emerged multifocally from the subcutis. The culture yielded ***Cladophialophora bantiana***



The grain was composed of a dense mat of septate pigmented, hyphae and chlamydospores, Bouts of soft tissue mixed with small black grains emerged from the subcutis of the abdominal wall during operation, **Sun *et al.* (2013)**

septate hyphae and large chlamydospores arranged in an arcuate manner with PAS stain (400 ·). The grains taken out from the abdominal wall were black, fragile and rough-surfaced by stereoscopym **Sun *et al.* (2013)**



The fungus had ovoid conidia with an inconspicuous scar in coherent chains born terminally or laterally from vegetative hyphae (400 ·). **Sun *et al.* (2013)**

Zambelli and Griffiths (2015) described a 6-year-old neutered male feline immunodeficiency-positive cat with repeated abdominal and thoracic effusions. The cat was diagnosed with and treated for lymphosarcoma but remission was short-lived and, on re-evaluation, a fungal peritoneal exudate was noted. Cytology of the organisms is described and the culture elucidated ***Cladosporium carrionii***, an important cause of chromoblastomycosis. Treatment with itraconazole was unsuccessful in this case.

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7. Zambelli AB, Griffiths CA. South African report of first case of chromoblastomycosis caused by Cladosporium (syn Cladophialophora) carrionii infection in a cat with feline immunodeficiency virus and lymphosarcoma. J Feline Med Surg. 2015 Apr;17(4):375-80.

8. Pythiosis in cats and dogs

Pythiosis is a chronic pyogranulomatous infection of the gastrointestinal tract or skin caused by Pythium insidiosum. Pythium insidiosum is an oomycete pathogenic to mammals. The infection occurs mainly in tropical and subtropical areas, particularly in horses, dogs and humans. Infection is acquired through small wounds via contact with water that contains motile zoospores or other propagules (zoospores or hyphae). The disease has been described since 1884. Depending on the site of entry, infection can lead to different forms of pythiosis i.e. a cutaneous, vascular, ocular, gastrointestinal and a systemic form, which is rarely seen. The infection is not contagious; no animal-animal or animal-human transmission has been reported so far. Therapy includes radical surgery, antifungal drugs, immunotherapy or a combination of these therapies. The prevention to contract the disease in endemic areas is difficult. Avoiding stagnant waters could be of help, although the presence of P. insidiosum on grass and soil in enzootic areas renders this practice useless (Gaastra *et al.*, 2010)

a. Forms of reported pythiosis

i. **Gastrointestinal pythiosis: (Miller,1985, Fischer *et al.*, 1994, Helman and Oliver, 1999, Graham *et al.*,2000, Liljebjelke *et al.*, 2002, Mendoza *et al.*, 2005, Rakich *et al.*, 2005,** Berryessa *et al.*, 2008, **Pereira *et al.*,2010, Hummel *et al.*, 2011, Connolly *et al.*, 2012, Fernandes *et al.*, 2012, Martins *et al.*, 2012, Schmiedt *et al.*, 2012, Pereira *et al.*, 2013, Aeffner *et al.*, 2015)**

ii. **Cutaneous/Subcutaneous pythiosis**: (**Bentinck-Smith *et al.*,1989, Howerth *et al.*,1989, Dykstra *et al.*, 1999, Hensel *et al.*, 2003, Thieman *et al.*,2011, Martins *et al.*, 2012, Oldenhoff *et al.*, 2014**)

iii. **Prostatic pythiosis**: **(Jaeger *et al.*, 2002, Liljebjelke *et al.*, 2002)**

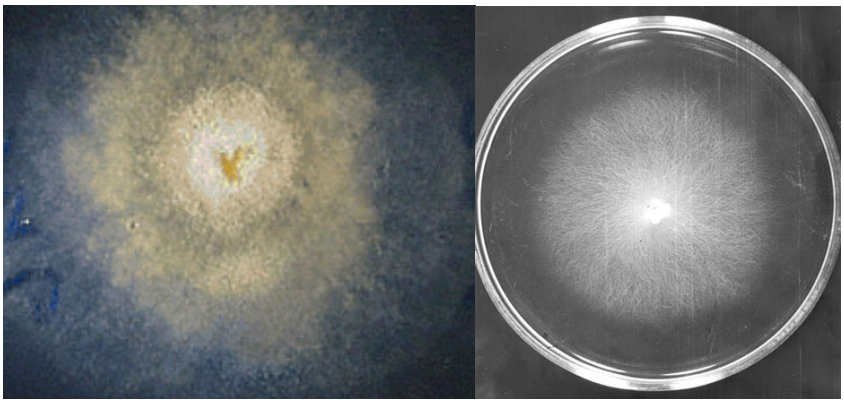
b. Aetiology

Pythium insidiosum De Cock, L. Mendoza, A.A. Padhye, Ajello & Kaufman, Journal of Clinical Microbiology 25 (2): 345 (1987)

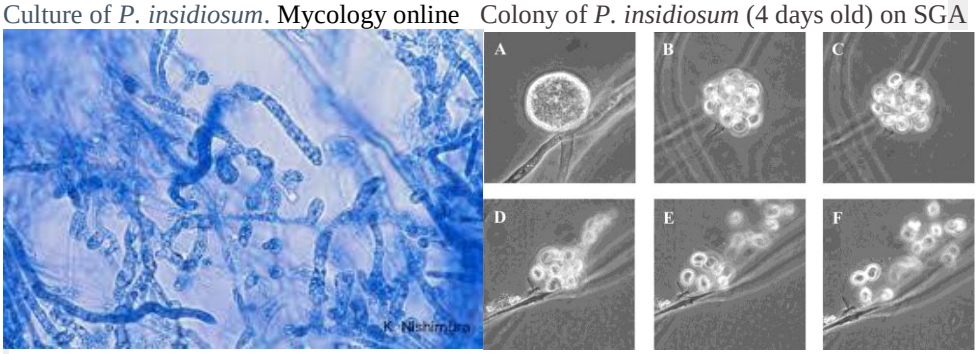
Synonym:
=Hyphomyces destruens C.H. Bridges & C.W. Emmons, Journal of the American Veterinary Medical Association 138 (11): 588 (1961)

Classification
Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae, Pythium

Description
Cultures (CMA) expanding, white, flat, submerged. Hyphae 4-6 ?m wide, irregularly branched (branches 2.5-4.0 ?m diam), sparsely septate in wider hyphae, locally disarticulating. Club-shaped appressoria present. Zoosporangia undifferentiated, filamentous, with two lateral flagella. Sexual organs (Oogonia) intercalary, subspherical, 23-30 ?m wide. Antheridia produced from adjacent hyphae, clavate, terminally up to 10 ?m wide.Optimal development at 35°C, maximum growth temperature 45°C.



Culture of *P. insidiosum*. Mycology online Colony of *P. insidiosum* (4 days old) on SGA



(A to C) Zoospore production inside the zoosporangium at time intervals of 3 min. (D to F) Rapid release of zoospores. **dailyparasite.blogspot.com** .

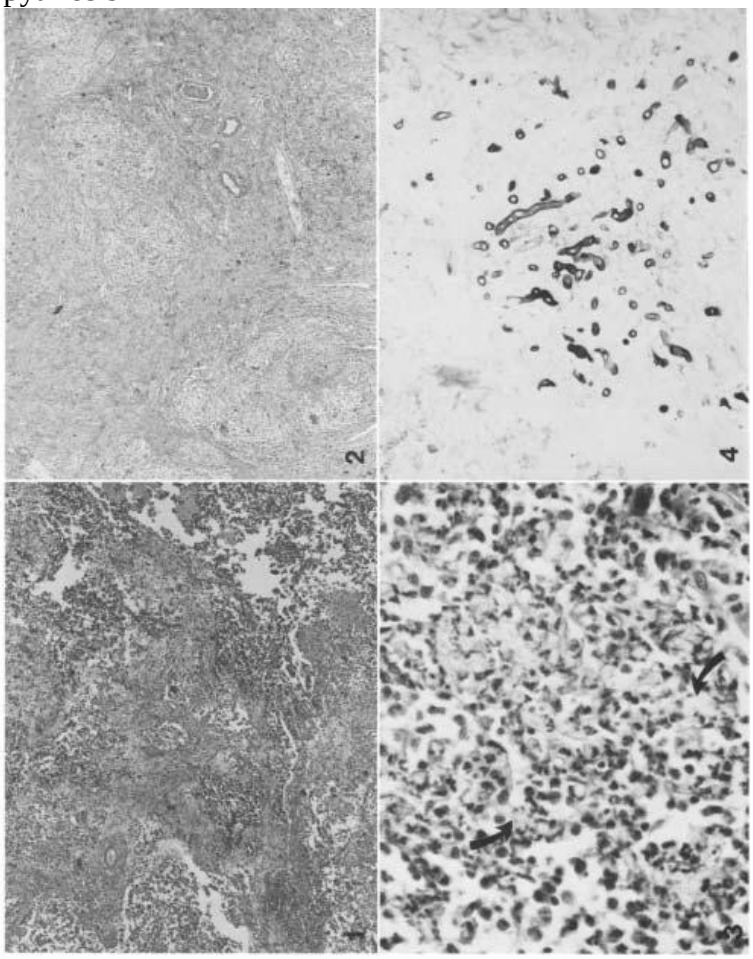
c. Reports

Miller (1985) studied 63 cases of canine **gastrointestinal phycomycosis**, of which 60 were determined to have pythiosis and 3 to have entomophthoromycosis. In pythiosis, male, large-breed dogs less than or equal to 3 years old were most commonly affected. Clinical signs usually included vomiting and weight loss and these were associated with lesions of the stomach and small intestine. Histologically, the causative organisms were found in necrotic regions of diffuse or discrete granulomas in the submucosa or muscularis mucosae. Entomophthoromycosis was diagnosed by finding wide eosinophilic sleeves intimately surrounding thin-walled hyphae. Less than 5% of the dogs were alive 3 months following diagnosis.

Bentinck-Smith *et al.* (1989) isolated ***Pythium insidiosum*** from the subcutaneous tissue of a 1-year-old tan crossbreed dog and from the intestinal tract of an 18-month-old Samoyed male. Gomori's methenamine silver stain was superior to hematoxylin and eosin in demonstrating the organism in tissue sections. The agent was identified

as P. insidiosum by zoospore formation in an aqueous yeast extract solution containing grass blades. Exoantigens produced in culture were shown to be identical to known P. insidiosum antigens by microimmunodiffusion.

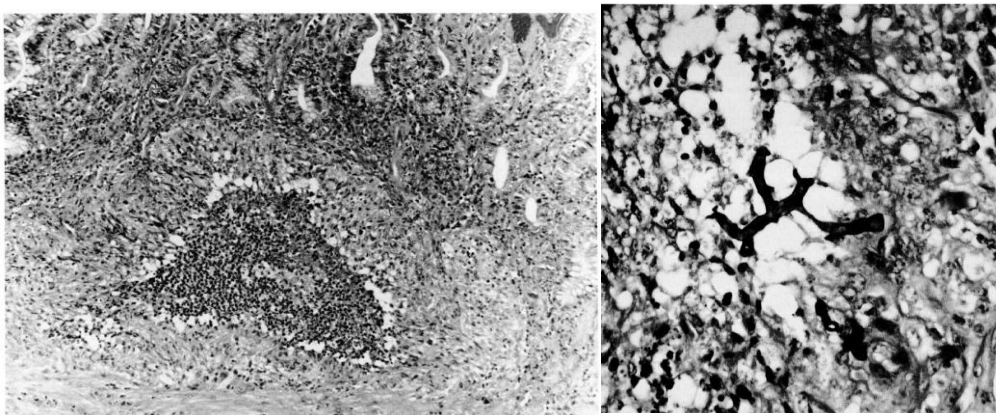
Howerth *et al.* (1989) reexamined a 2-year-old, female, walker hound because a non-healing cutaneous lesion on the left lateral thorax was nonresponsive to antibiotic therapy. The lesion, which was 15 cm in diameter and had a central draining tract, had been progressive for 1 month. A 4-cm-long elliptical. sional biopsy was obtained from the lesion. A portion of, biopsy was fixed in 10% buffered formalin for histopathology and a portion was frozen for fungal culture. The case was diagnosed as subcutaneous pythiosis



subcutaneous pythiosis in a dog**, Howerth *et al.* (1989)**

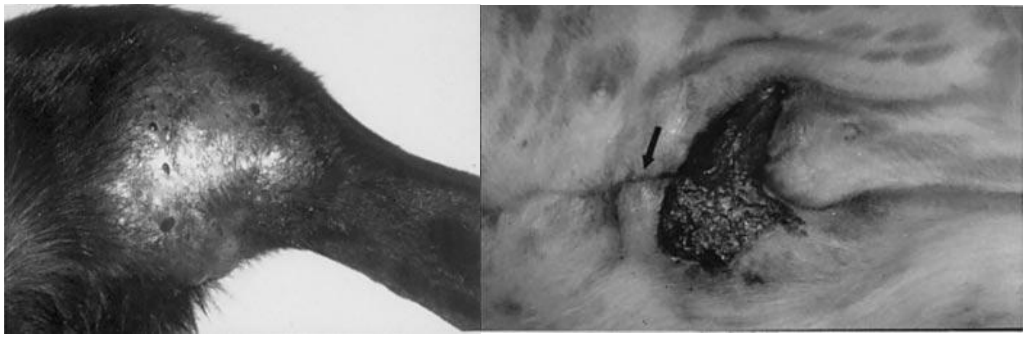
Fischer *et al.* (1994) examined formalin-fixed tissue samples from 11 dogs with gastrointestinal pythiosis. The average age of the dogs was 2.5 years, 8 of 11 were female, and several large and small breeds were represented. Clinical histories were typified by chronic anorexia, weight loss, vomiting, and diarrhea. The tissue samples were collected during exploratory celiotomy or necropsy and were identified by the submittors as masses or neoplasms of the gastrointestinal tract or associated tissues. Microscopic examination of hematoxylin and eosin (HE)- stained tissue sections revealed severe multifocal pyogranulomatous inflammation in the mucosa, submucosa, and/or both. The diagnosis was confirmed by an indirect immunoperoxidase technique specific for *Pythium*. Rabbit serum containing primary

antibody specific to *Pythium* antigen was applied to formalin-fixed tissue sections, which were subsequently stained using an avidin-biotin immunoperoxidase technique and examined microscopically. Negative controls with no primary antibody were run with all samples. Follow-up investigation of these dogs revealed that all had died within 2 months of the onset of clinical signs.

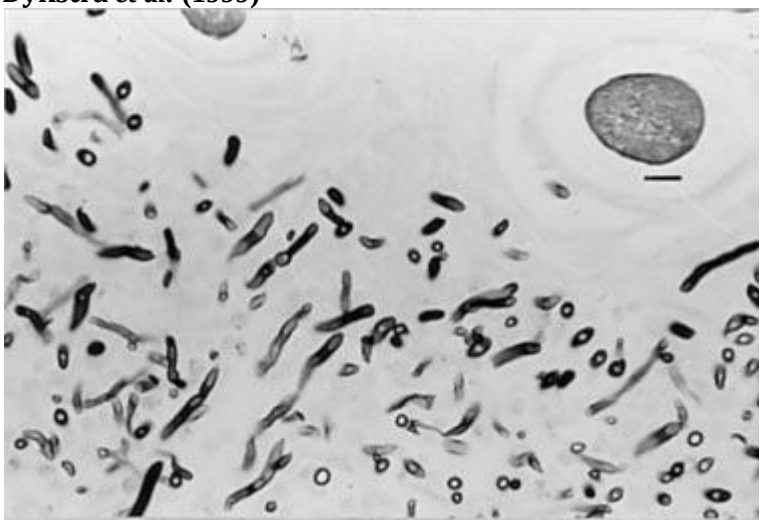


Pyogranulomatous inflammation in the small intestine of a dog with gastrointestinal pythiosis
Branching hyphae of *Pythium insidiosum* within the center of a pyogranuloma in the small intestine of a dog with gastrointestinal pythiosis. Grocott's methenamine silver, HE counterstain **Fischer *et al.* (1994)**

Dykstra *et al.* (1999) retrieved information regarding signalment, duration of clinical signs, history of swimming, results of CBC and serum biochemical analyses, biopsy findings and mycological results, together with treatments and outcome from the medical records of 15 dogs with a diagnosis of pythiosis made between 1985 and 1995 at the Colleges of Veterinary Medicine, North Carolina State University and the University of Florida. Most of the dogs were young (median age 22 months) and represented larger breeds (> 20 kg). Lesions were characteristically chronic, ulcerated, and nodular with multiple draining tracts on the limbs, thoracic wall or perineal regions. The median duration of these lesions was 3 months with a range of 2 weeks-6 months. Seven dogs had a history of swimming. Peripheral eosinophilia was observed in 14 of the dogs. Cytological evaluation of discharge, aspirates, or impression smears made from biopsy specimens revealed hyphae in five of 11 dogs (45%). Histopathological evaluation using the Gomori Methenamine-Silver (GMS) stain was the most useful test for providing presumptive evidence of cutaneous pythiosis. Immunotherapy or antifungal therapy using either amphotericin B, liposomal nystatin, itraconazole, or ketoconazole were all unsuccessful. The only dog to survive underwent amputation of the affected limb; thus, the prognosis for cutaneous pythiosis in the dog is poor.



Lesion on the medial aspect of the surface of the left pelvic limb of dog c2. The lesion was erythematous, with multiple sites of serum exudation. Thoracic lesion from dog c1, showing the larger mass with an ulcerated surface on the right cranioventral thorax. The scar from the previous surgery is visible (arrow). **Dykstra *et al.* (1999)**



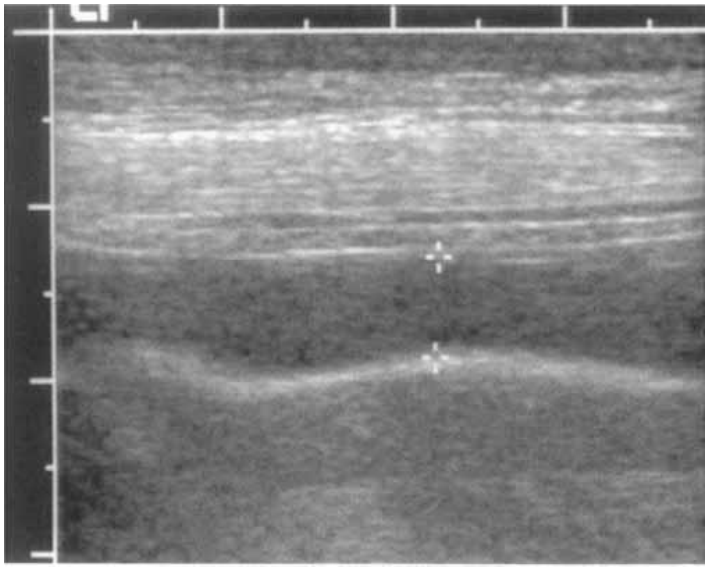
Gomori Methenamine-Silver (GMS) stained section of haired canine skin showing numerous hyphae in skin (note hair follicle in upper right). Size bar near follicle equals 10 mm; 330_. **Dykstra *et al.* (1999)**

Helman and Oliver (1999) diagnosed **enteric pythiosis** in nine dogs in Oklahoma. Eight dogs had anorexia and weight loss. Two of these dogs had diarrhea; two dogs exhibited vomiting and diarrhea; and one dog had vomiting. One dog presented with dysphagia. Seven dogs had either a palpable or radiographically visible abdominal mass. These seven dogs had localized regions of mucosal ulceration and thickened gastric or intestinal walls with some involvement of the adjacent mesentery or omentum. Two dogs had enlarged regional mesenteric lymph nodes. One dog that presented with dysphagia had an oropharyngeal mass involving the larynx and cranial esophagus. Microscopically, there was transmural chronic sclerosing and granulomatous to pyogranulomatous inflammation with arteritis. Pythium spp. were identified in all specimens by immunohistochemistry.

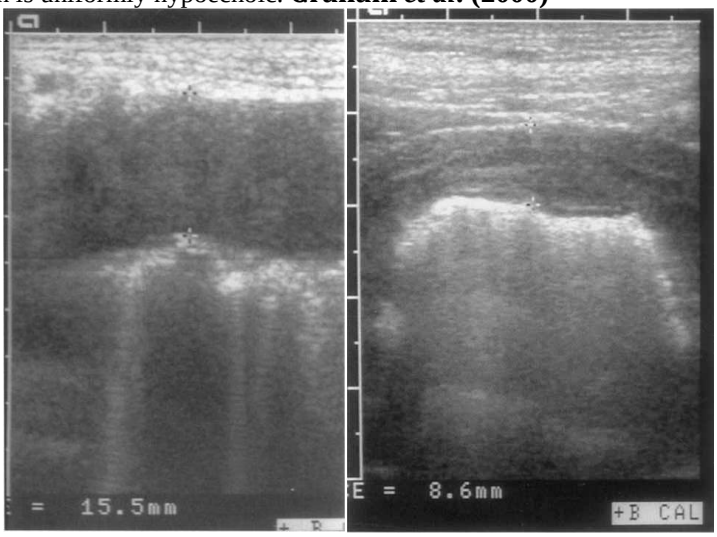
Willard and Radlinsky (1999) performed a retrospective study to determine whether endoscopic examination of the choanae resulted in diagnosis of various diseases in 91 dogs and 27 cats with signs of respiratory tract disease. Medical records were reviewed for endoscopy findings and results of examination of biopsy or cytologic specimens. 34 animals had neoplasia in the choanal region; in 26 animals, diagnosis was confirmed by evaluation of specimens obtained by endoscopy. Five dogs with neoplasia had an erroneous diagnosis of rhinitis made on the basis of evaluation of specimens obtained by endoscopy. Six dogs and 2 cats had foreign objects in the choanae; 7 foreign objects were removed endoscopically, whereas 1 required nasal flushing. Results of endoscopy and biopsy of the choanae provided diagnosis of cryptococcosis and aspergillosis, but did not aid in the diagnosis of pythiosis or nasal mites.

Graham *et al.* (2000) reported the ultrasonographic features of nine dogs with **gastrointestinal pythiosis**. The stomach, duodenum, jejunum or colon were affected. All dogs had thickening of the gastrointestinal wall and areas with obliteration of the normal layered appearance. In one dog an eccentric mass was found arising from the serosal surface of the wall of the colon with mild diffuse wall thickening. Regional

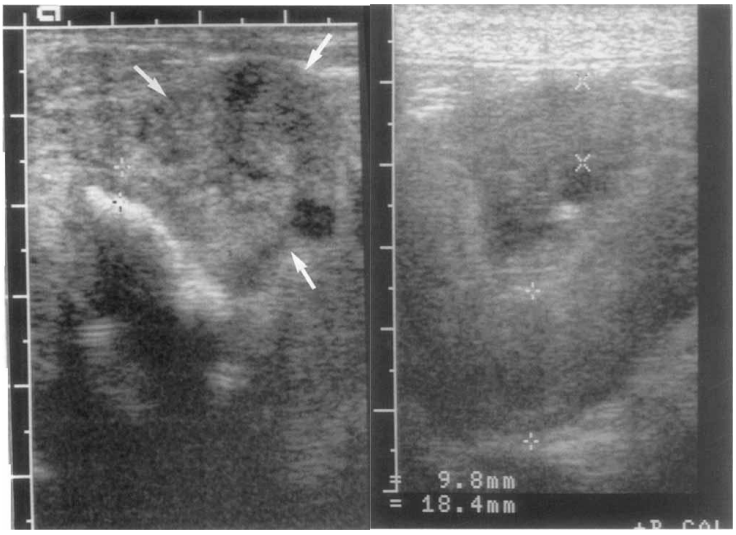
lymph node enlargement was seen in seven of the nine dogs. One dog had invasion of the pancreas and signs compatible with extrahepatic biliary obstruction. When compared to previous reports of gastrointestinal neoplasia, the features of wall thickening, loss of layering and regional lymphadenopathy are not considered specific for gastrointestinal pythiosis. Histological examination of tissue specimens is required for diagnosis.



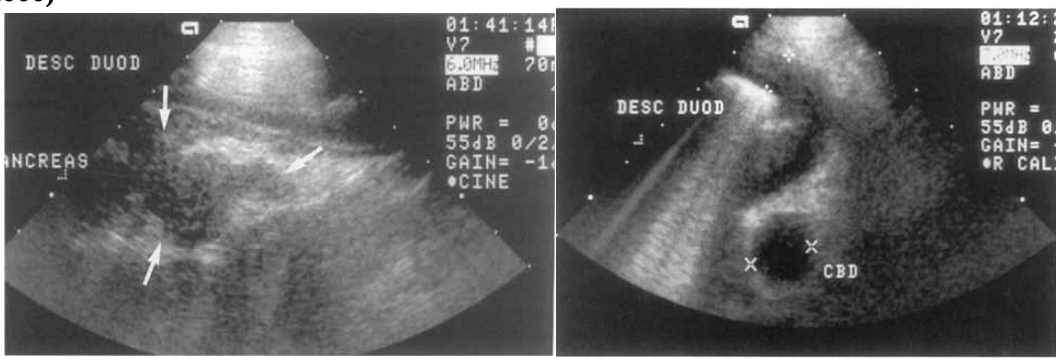
Sagittal plane image of the body of the stomach of dog five. There **IS** mild thickening of the stomach wall ***(6*** mm) which is uniformly hypoechoic. **Graham *et al*. (2000)**



Sagittal plane image of the body of the stomach of dog nine. The stomach wall is moderately thickened (+ = 15.5 mm) and the normal layers are obliterated. Transverse image ***of*** the distal descending colon of dog six. The wall of the colon is moderately thickened, measuring 10 mm in places. In this area, the layers are blurred but still visible. **Graham *et al*. (2000)**

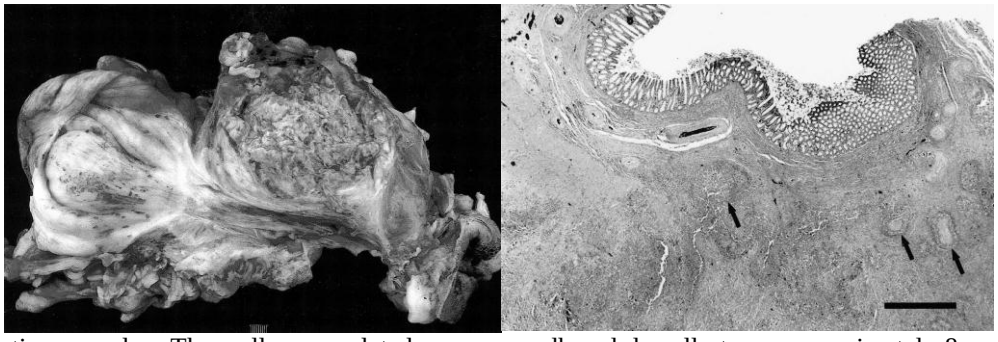


Transverse image of the distal descending colon of dog two. An eccentric mixed echogenic mass (arrows) is seen arising from the wall of the colon. The wall of the colon is mildly thickened with intact albeit blurred layers except in the area from which the mass arises. Sagittal plane image of the py,oric antmm of the stomach of dog four. The wall is circumferentially moderately to severely thickened, ranging from 9.8 mm to 18,4 mm in thickness in this image, The layers are obliterated. **Graham *et al*. (2000)**

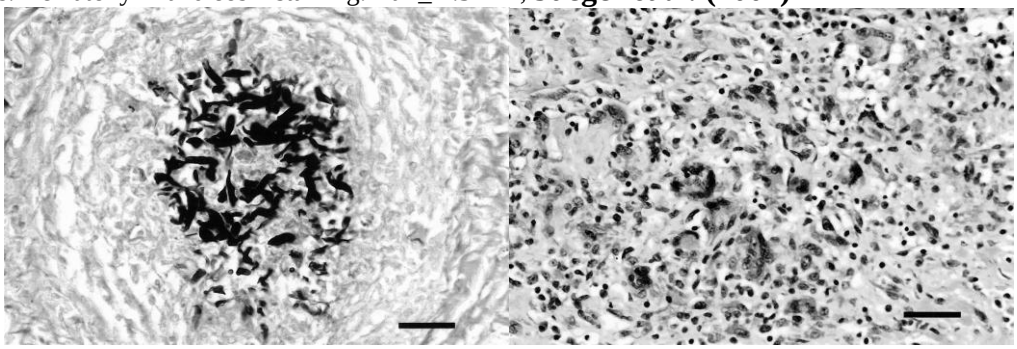


Sagittal plane image of the descending duodenum and pancreas of dog four. The pancreas is enlarged, hypoechoic and irregularly marginated (arrows). The peripancreatic fat is hyperechoic, Transverse image of the descending duodenum and common bile duct of dog four obtained using an intercostal approach. The wall of the duodenum is moderately thickened (+ = 9 mni) and no layers are visible. The common bile duct is dilated **(X** = 12 mm). **Graham *et al*. (2000).**

Jaeger *et al.* (2002) reported a case of prostatic pythiosis in a dog with a well-encapsulated mass, which was adhered dorsally to an approximately 8-cm area of the colon but did not involve the adjacent urinary tract. The colon showed multifocal granulomas, separated by layers of fibrous connective tissue, which expanded and replaced a focally extensive area of the wall of the colon. The inflammatory process did not extend to the mucosal surface. Deep to this area, the chronic inflammatory process was contiguous with the prostatic mass. The granulomas presented intralesional hyphae of the oomycete *Pythium insidiosum.* The hyphal structures were irregularly branching, non-septate, non-parallel walls and often have smudgy, indistinct outlines in histologic preparations.



Prostatic mass; dog. The well-encapsulated mass was adhered dorsally to an approximately 8-cm area of the colon but did not involve the adjacent urinary tract (left). Bar _ 1 cm. Colon; multifocal granulomas (arrows), separated by layers of fibrous connective tissue, expand and replace a focally extensive area of the wall of the colon. Note that the inflammatory process does not extend to the mucosal surface. Deep to this area, the chronic inflammatory process is contiguous with the prostatic mass. Hematoxylin and eosin staining. Bar _ 1.5 mm, **Jaeger *et al.* (2002)**



Detail of one of the granulomas, with intralesional hyphae of the oomycete *Pythium insidiosum.* The hyphal structures have irregularly branching, nonseptate, nonparallel walls and often have smudgy, indistinct outlines in histologic preparations. Gomori's methenamine silver staining. Bar _ 40 _m.
Histologic section demonstrating granulomatous prostatitis with multinucleated giant cells (Langhans' type). Hematoxylin and eosin staining. Bar _ 50 _m. **Jaeger *et al.* (2002)**

Liljebjelke *et al.* (2002) reported a 4-year-old castrated male Irish Setter with prostatomegaly and chronic progressive tenesmus of 8 months' duration. One week before presentation to the NCSU-VTH, stranguria and hematuria were noted in addition to tenesmus. Abdominal radiographs and abdominal ultrasonography before referral identified an enlarged prostate (8 by 8 by 15 cm) in the pelvic canal extending into the abdominal cavity. Multiple areas of spondylosis of the caudal thoracic and lumbar spine also were present. On abdominal ultrasonography, the prostatic capsule was thickened, and multiple cavitations were observed within the prostate gland without evidence of mineralization. Fine-needle aspirates of the prostate gland obtained under ultrasound guidance showed chronic suppurative inflammation, including moderate numbers of degenerate neutrophils and necrosis with numerous non-septate fungal hyphae present. Cytologic samples stained with Gomori's methenamine silver (GMS) were positive for fungal elements. Portions of resected prostatic tissue were submitted for fungal culture and histopathology.The final diagnosis was intestinal pythiosis.

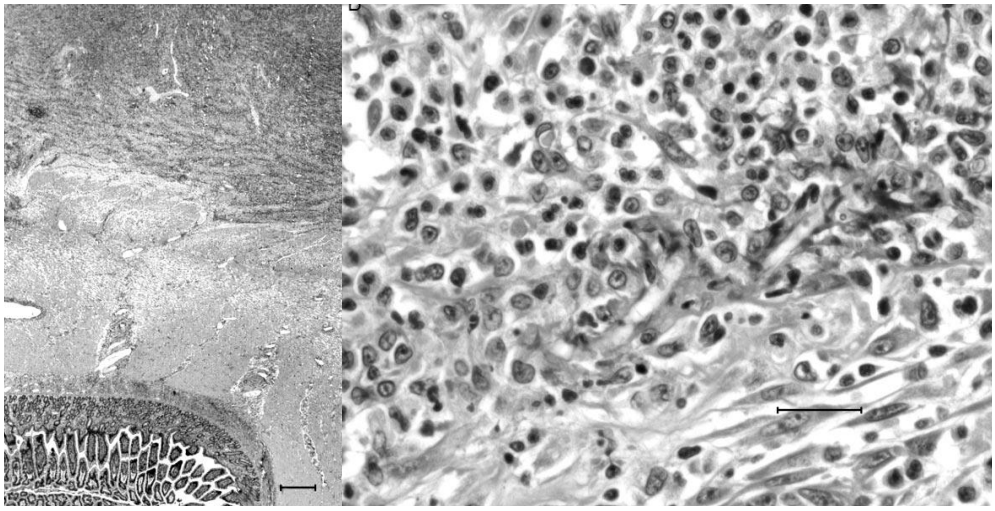
Hensel *et al.* (2003) reported a 4-year-old Labrador Retriever with 2 ulcerative nodular cutaneous lesions. One lesion was located on the medial aspect of the right carpus; the other was located on the medial aspect of the left tarsus. The dog had spent its entire life in the southeastern part of the United States and approximately half of its time outdoors with free access to a nearby lake. Histologic examination of

full-thickness wedge biopsy specimens from both lesions revealed severe, multifocal, puruloeosinophilic to pyogranulomatous deep dermatitis with intralesional filamentous structures, fibroplasia, and neovascularization. Examination of sections stained with Gomori methenamine silver stain revealed a moderate number of wide, bulbous, irregularly septate, branching hyphae. Results of an immunodiffusion test and an ELISA for anti-Pythium insidiosum antibodies were positive. Amputation was eliminated as a treatment option because lesions involved 2 limbs. Long-term systemic antifungal treatment was also rejected because of the cost, lack of therapeutic effect in many cases, and potential for adverse effects. The dog was treated with 2 doses of an anti-P insidiosum vaccine administered 2 weeks apart. One month later, the lesions were nearly completely healed, and values obtained via the immunodiffusion test and ELISA had decreased. Results of the immunodiffusion test and ELISA were negative 1 year later, and the dog had not had any recurrences.

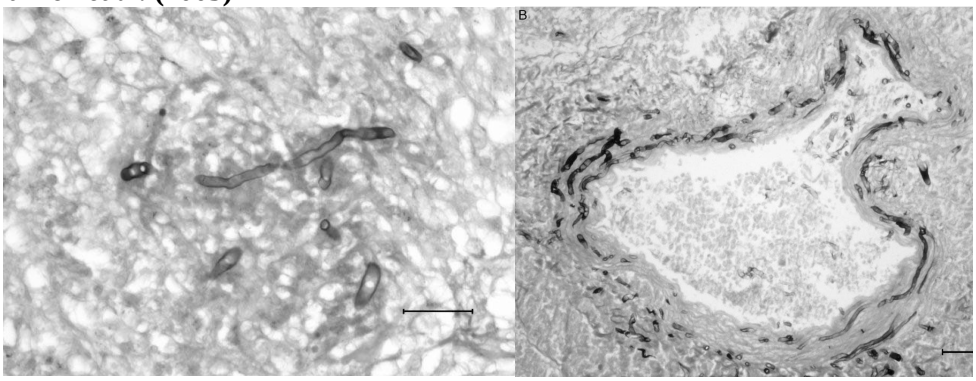
Mendoza *et al.* (2003) evaluated the immunotherapeutic properties of a new Pythium insidiosum-vaccine formulation (PIV) in 6 dogs with proven pythiosis from different enzootic areas in the United States. Dogs with chronic disease (greater than two months) did not responded to immunotherapy. The finding of eosinophils, mast cells, IgE and precipitin IgG during pythiosis suggested that a T helper 2 (Th2) subset is in place during this disease.

Mendoza *et al.* (2005) reported an 11-months-old mixed Terrier male originally from Venezuela with signs of depression, anorexia, vomiting and diarrhea. The illness had begun 1 month earlier. Despite antibiotic chemotherapy and vitamins, the disease progressed. Radiological exams showed involvement of the small intestine. Histopathological studies of tissue samples taken during surgical intervention revealed eosinophilic areas in the center of which, abundant eosinophils, histiocytes and giant cells were observed. Silver stained cross-sections of the small intestine showed slender sparsely septate hyphae within the necrotic areas. Attempts to isolate the etiologic agent in pure culture were fruitless. The dog died without a definitive diagnosis. Fixed tissue samples of the small intestine were later investigated using specific fluorescent antibodies for pythiosis and molecular tools. These exams indicated that the hyphae in the infected tissues belong to ***Pythium insidiosum***.

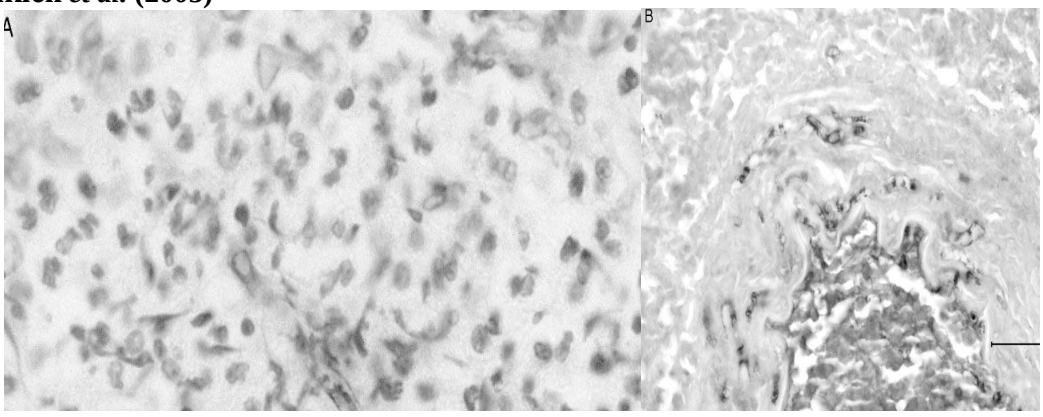
Rakich *et al.* (2005) reported 2 young adult male Domestic **Shorthair cats** living in the southeastern United States with a palpable **abdominal** mass in each animal. Exploratory laparotomy revealed a large extraluminal mass involving the ileum and mesentery with adjacent mesenteric lymphadenopathy in cat No. 1 and an abscessed mass in the distal duodenum in cat No. 2. Mass resection and intestinal anastomosis were performed in both cats. Histologic evaluation indicated that the intestinal lesions involved primarily the outer smooth muscle layer and serosa and consisted of eosinophilic granulomatous inflammation with multifocal areas of necrosis. In Gomori methenamine silver-stained sections, broad (2.5-7.5 microm), occasionally branching, infrequently septate hyphae were observed within areas of necrosis. A diagnosis of Pythium insidiosum infection was confirmed in both cats by immunoblot serology and by immunoperoxidase staining of tissue sections using a P. insidiosum-specific polyclonal antibody. Cat No. 1 was clinically normal for 4 months after surgery but then died unexpectedly from an unknown cause. Cat No. 2 has been clinically normal for at least 9 months after surgery and appears to be cured on the basis of follow-up enzyme-linked immunosorbent assay serology.



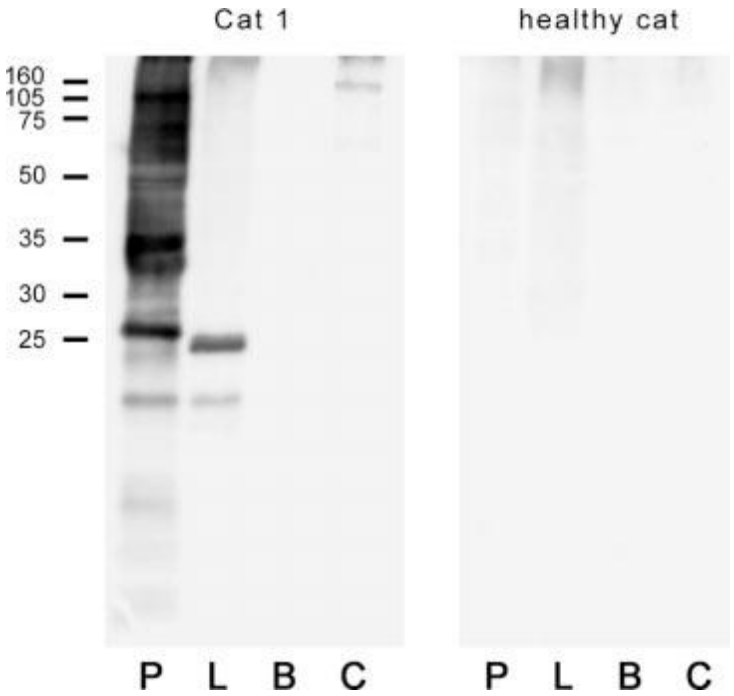
Intestinal mass; cat No. 1. **A**, small intestine with an inflammatory mass that extends from the serosal surface. The mucosa, submucosa, and inner muscular layer of the tunica muscularis are normal. HE. **B**, a focus of eosinophilic inflammation surrounds necrotic debris and hyphal ‘‘ghosts.’’ HE. Bar 5 25 mm. **Rakich *et al.* (2005)**



Intestinal mass; cat No. 1. **A**, hyphae within areas of necrosis are contorted, have nearly parallel walls, measure 2.5–7.5 mm in diameter, are occasionally branching, and have rare septa. GMS. Bar 5 15 mm. **B**, cat No. 2, numerous hyphae are present with the wall of a medium-sized artery. GMS. Bar 5 25 mm. **Rakich *et al.* (2005)**

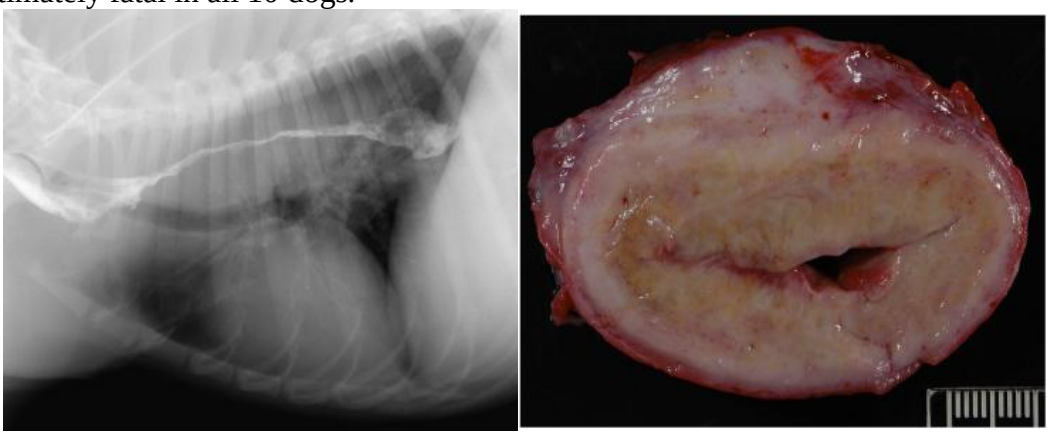


Intestinal mass; cat No. 1 **A** and cat No. 2 **B**. Hyphae of *Pythium insidiosum* are well delineated with the immunohistochemical stain. Numerous hyphae are present within the wall of an artery (**B**). Avidin–biotin–peroxidase method with polyclonal anti–*P. insidiosum* antibody. Mayer hematoxylin counterstain. Bar 5 25 mm. Intestinal mass; cat No. 1 **A** and cat No. 2 **B**. Hyphae of *Pythium insidiosum* are well delineated with the immunohistochemical stain. Numerous hyphae are present within the wall of an artery (**B**). Avidin–biotin–peroxidase method with polyclonal anti–*P. insidiosum* antibody. Mayer hematoxylin counterstain. Bar 5 25 mm. **Rakich *et al.* (2005)**



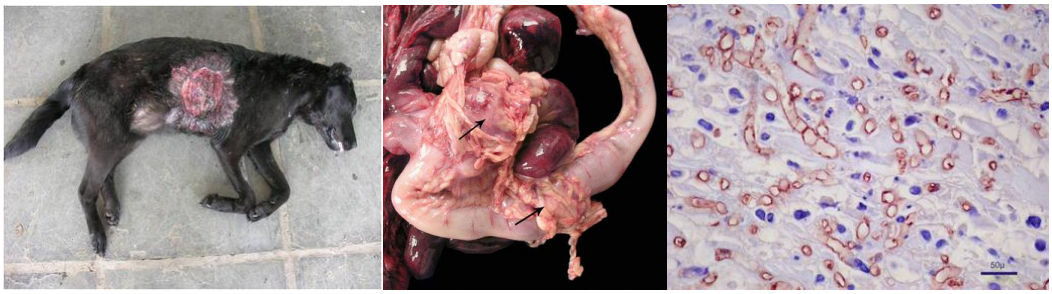
Immunoblot analysis demonstrating the ability of serum from cat No. 1 as well as from a healthy cat to recognize antigens of *Pythium insidosum* (P. in), a canine pathogenic *Lagenidium* species (L. sp.), *Basidiobolus ranarum* (Bas), and *Conidiobolus coronatu*s (Con). Markers on left indicate molecular weight in kilodaltons. **Rakich *et al.* (2005)**

Berryessa *et al.* (2008) conducted a study to describe the clinicopathologic and epidemiologic findings associated with GI pythiosis in 10 dogs from California. Dogs were initially identified on the basis of supportive clinical findings and routine histology. Pythiosis was confirmed in each dog with at least one of the following: immunoblot serology, enzyme-linked immunosorbent assay serology, immunohistochemistry, and culture followed by species-specific polymerase chain reaction, rRNA gene sequencing, or both. Between September 2003 and December 2006, GI pythiosis was confirmed in 1 dog from central California and 9 dogs that lived within a 30-mile radius of Davis, CA. Seven of 8 dogs for which environmental data were available had frequent access to flooded rice fields or other water sources. Esophageal lesions were present in 2 of 10 dogs. Common laboratory findings included eosinophilia (7/9), hypoalbuminemia (9/9), and hyperglobulinemia (8/9). Median survival time was 26.5 days (range, 0-122 days), and the disease was ultimately fatal in all 10 dogs.


Esophagram showing an esophageal mass and resultant stricture in a dog with esophageal pythiosis.

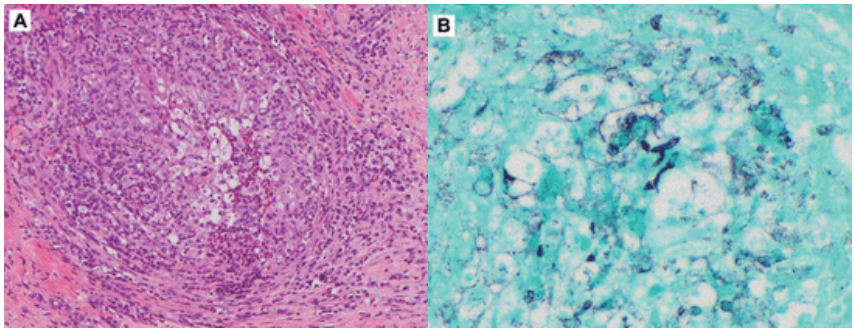
Esophagus. Gross photograph of transverse section. There is severe, circumferential, mural thickening with subsequent narrowing of the esophageal lumen. The mucosal surface is ulcerated, **Berryessa *et al.* (2008)**

Pereira *et al.* (2010) reported a case of concurrent cutaneous and gastrointestinal pythiosis in an 18-month-old female Labrador. This dog had an ulcerative cutaneous lesion on the right thoracic region for 12 months that was unresponsive to itraconazole and terbinafine therapy. Two months prior to death and concurrent with the cutaneous lesion, the dog became anorexic with frequent vomiting and bloody stools. At necropsy, a cutaneous lesion that extended subcutaneously into the intercostal muscles was observed. Additionally, the large intestine contained two lesions that caused luminal narrowing. Organs were collected, routinely processed and stained using hematoxylin and eosin and Gomori methenamine silver. Histological examination of the lesions in the large intestine and on the skin revealed areas of necrosis surrounded by a pyogranulomatous infiltrate. Occasionally, black, septate, branching hyphae were detected following staining with Gomori methenamine silver. The diagnosis of pythiosis was confirmed using immunohistochemical methods. This report describes the occurrence of concomitant gastrointestinal and cutaneous lesions in a dog and highlights the therapeutic difficulties encountered with this disease



Cutaneous pythiosis in Labrador. It was observed a well delimited, ulcerated, alopecic area in right lateral thoracic region, Intestinal pythiosis in Labrador. It was observed two masses in intestine wall (arrows), Intestinal pythiosis in Labrador. Hyphae strongly immunomarked. Immunohistochemical procedure using polyclonal antibody anti-*Pythium insidiosum*. Bar = 50 µm **Pereira *et al.* (2010)**

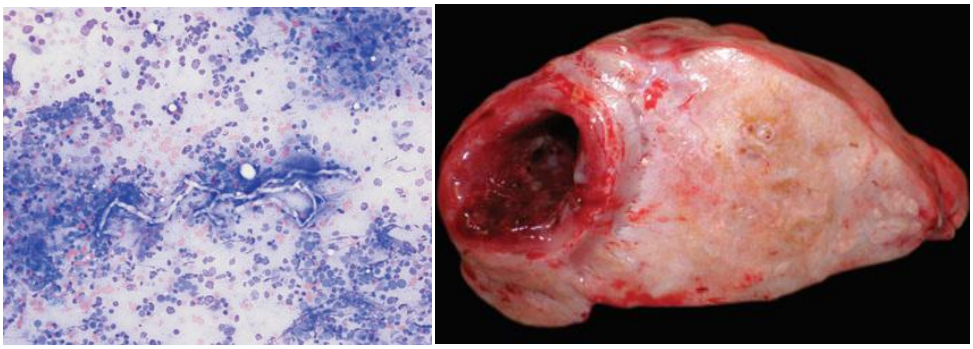
Hummel *et al.* (2011) described a case of canine **gastrointestinal** pythiosis in which lesions were resolved through the administration of itraconazole, terbinafine, and the agricultural fungicide mefenoxam. No substantial adverse effects occurred in association with administration of the latter compound. Additional studies are needed to evaluate the pharmacokinetics of mefenoxam and to further assess its tolerability and potential efficacy for the treatment of pythiosis in dogs.



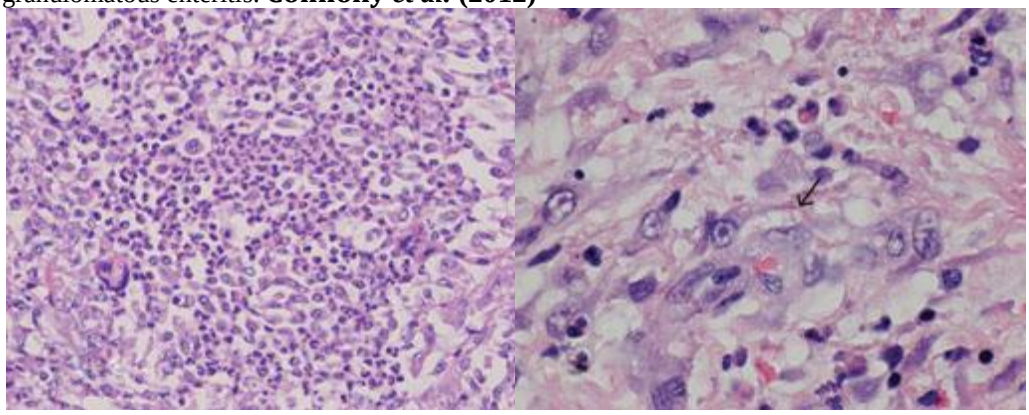
(A) Granuloma formation with extensive inflammatory infiltration of tunica muscularis in the duodenum (hematoxylin and eosin stain, original magnification × 200). (B) Hyphae with non-parallel walls that lack septation and exhibit infrequent branching (Gomori methenamine silver stain, original magnification × 600), **Hummel *et al.* (2011)**

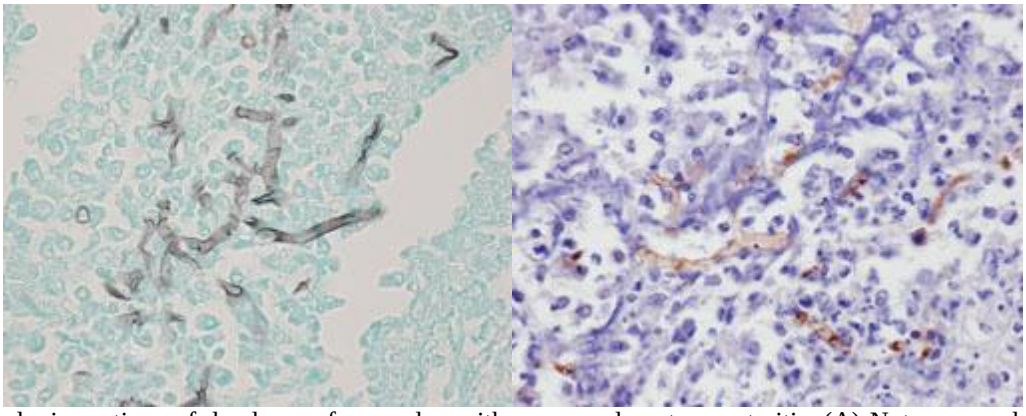
Thieman *et al.* (2011) reported a 4-year-old spayed female Boxer with a cutaneous mass of approximately 10 cm in diameter located on the dorsum. The mass had been present for 6 weeks and was increasing in size. Computed tomography of the abdomen and the mass were performed and revealed a contrast-enhancing soft tissue mass of the dorsum and enlarged intra-abdominal lymph nodes. The dog underwent surgical excision of the cutaneous mass, including 5-cm skin margins and deep margins of 2 fascial planes. The mass was completely excised on the basis of results of histologic examination of surgical margins. The dog received itraconazole and terbinafine by mouth for 3 months following surgery. Recheck examination at 20 months postoperatively showed no signs of recurrence of pythiosis at the surgical site.

Connolly *et al.* (2012) reported a 4-year-old male neutered Labrador Retriever with severe gastrointestinal signs and multifocal pyogranulomatous gastritis, enteritis, and lymphadenitis with intralesional hyphae and multifocal pyogranulomatous pneumonia with intralesional yeast. Based on cytologic evaluation, histologic examination with special stains, and immunohistochemical analysis of tissues collected antemortem or at necropsy, dual infections with ***Pythium insidiosum*** and Blastomyces dermatitidis were detected and are reported for the first time.

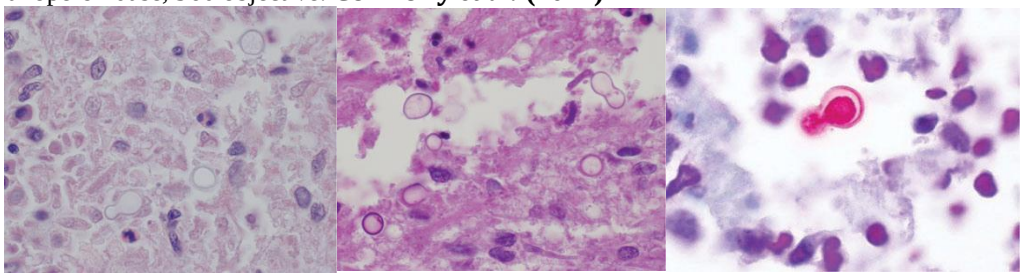


Fine-needle aspirate of a gastric lymph node from a dog, showing parallel-walled, negative-staining hyphae consistent with Pythium insidiosum or Lagenidium spp. Modified Wright–Giemsa, 920 objective. Markedly thickened ventral wall of the duodenum found at necropsy of a dog with pyogranulomatous enteritis. **Connolly *et al.* (2012)**





Histologic sections of duodenum from a dog with pyogranulomatous enteritis. (A) Note a granuloma with many neutrophils, few macrophages, and low numbers of multinucleated giant cells. H&E, 940 objective. (B) Rare nonstaining hyphal structures with blunt rounded ends (arrow) are present. H&E, 9100 objective. (C) Silver staining highlights more hyphal structures. Gomori methenamine silver, 960 objective. (D) Hyphae are immunoreactive for anti-Pythium insidiosum antibody. Avidin-biotin immunoperoxidase, 960 objective. **Connolly *et al.* (2012)**



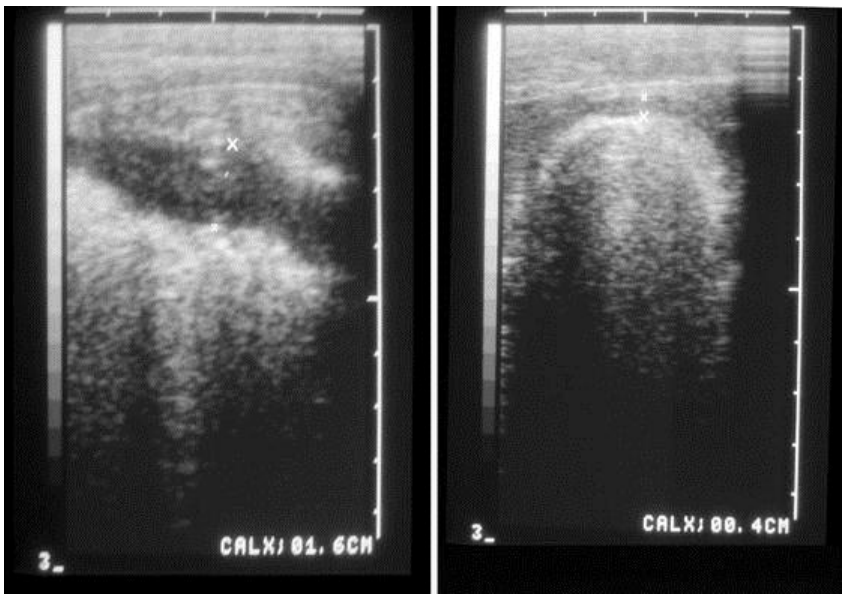
Histologic section of lung from a dog with pyogranulomatous pneumonia. 9100 objective. Note yeast structures consistent with Blastomyces dermatitidis in sections stained with H&E (A) and periodic acid-Schiff (B). (C) Organisms are immunoreactive for anti-Blastomyces dermatitidis antibody. Alkaline phosphatase red and hematoxylin, **Connolly *et al.* (2012)**

Fernandes *et al.* (2012) described the symptoms, pathological changes and diagnosis methods of **gastric pythiosis** in a three-year-old female German shepherd with vomiting and recurrent diarrhea of 30 days of duration. A palpable mass in the abdomen filling the left epigastric region was identified in the clinical examination. Simple and contrasted radiological examination and ultrasound of abdominal cavity were performed. The animal was referred for exploratory laparotomy for the removal of the mass. The extent of the mass prevented from the excision and the animal was euthanized. Samples of the tumor mass were collected and sent for morphological study and immunohistochemistry. The changes observed in imaging studies were consistent with gastric pythiosis. In cytology and histopathology, non-septate hyphae were identified, and in immunohistochemistry a strong positivity of anti-Pythium antibodies was observed, confirming the diagnosis of pythiosis.

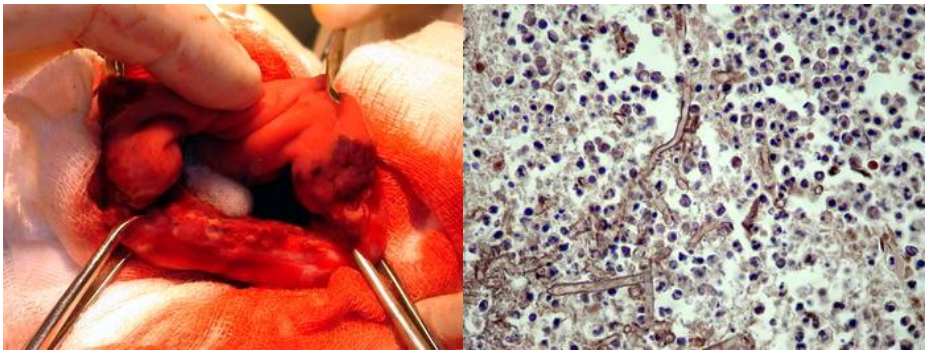
Martins *et al.* (2012) reported 9 cases of pythiosis in dogs. The aetiology in all cases was confirmed by immunohistochemistry. Data related to the clinical course and outcome and localization of the lesions were obtained from pathology reports. Affected dogs had **gastrointestinal** and/or **cutaneous lesions** with either or both of a granulomatous/pyogranulomatous or necrotizing eosinophilic inflammatory reaction. The number of intralesional hyphae, the distribution of hyphae, the presence of angioinvasion and the nature of the local inflammatory reactions were associated with the different types of lesions observed.

Schmiedt *et al.* (2012) examined a 1.5-year-old mixed-breed dog because of a 1-month history of anorexia, vomiting, diarrhea, and weight loss. The dog was very thin on physical examination. Results of all diagnostic tests were within reference limits except intestinal thickening and lymphadenopathy were identified on abdominal ultrasound examination. During exploratory laparotomy, thickening at the ileocecal-colic junction and within the transverse colon and mesenteric lymphadenopathy were identified, and the ileocecal-colic junction was resected. Histopathologic evaluation of the ileocecal-colic junction and full-thickness biopsy specimens from other sites as well as results of a serum ELISA were diagnostic for gastrointestinal Pythium insidiosum infection. Pythiosis was initially treated medically with administration of itraconazole and terbinafine by mouth, but the colonic lesion was progressive with this regimen. Two months after diagnosis, a subtotal colectomy was performed; marginal excision (0.6 cm) was obtained at the aboral margin. The dog was treated with 3 doses of a pythiosis vaccine beginning approximately 2 weeks after surgery and was continued on itraconazole and terbinafine for 5 months. Parenteral and enteral nutrition as well as considerable general supportive care were required postoperatively. Six months after treatment, the dog had a normal serum ELISA titer. Two years after treatment, the dog had returned to preoperative weight and was clinically normal.

Pereira *et al.* (2013) described a 2.5-year-old male beagle initially showed sporadic vomiting episodes, and this symptom became more frequent 5 months after the onset of clinical signs. Celiotomy procedure found thickness of the stomach wall extending to the pylorus and duodenum. A biopsy was performed, and the diagnosis of pythiosis was made by mycological, histopathological analyses and molecular identification. Therapy was based on an association of terbinafine plus itraconazole during 12 months and immunotherapy for 2.5 months. The healing of the dog reported here allows us to propose the use of immunotherapy associated with antifungal therapy to treat canine gastrointestinal pythiosis. However, additional studies should be performed on a larger number of patients to establish a standard treatment protocol for canine pythiosis.



Ultrasonography of gastrointestinal pythiosis in a beagle dog. *Left image* examination before starting the dog's treatment. Notice the stomach wall thicknesses (1.6 cm). *Right image* examination performed 4 months after ending the treatment. Observe the thickness difference between the stomach wall with normal standard (0.4 cm) (*right image*) and altered stomach wall (*left image*), **Pereira *et al.* (2013)**



Gastrointestinal pythiosis in a beagle dog. Exploratory laparotomy demonstrating an intense thickening of the wall of the pyloric region of the stomach. Immunohistochemistry assay was performed using a polyclonal anti-*P. insidiosum* antibody, and the immunostained *P. insidiosum* hyphae were strongly distinguished, **Pereira *et al.* (2013)**

Oldenhoff *et al.* (2014) reported the clinicopathological findings associated with **cutaneous pythiosis** in two dogs from a Northern temperate climate zone. The first dog was a 3-year-old intact male Chesapeake Bay retriever with an ulcerated soft-tissue swelling over the left eye. The second dog was a 4-year-old spayed female German shepherd dog with a soft-tissue swelling overlying the right hock. Both dogs lived in northern latitudes (between 43 and 45°N) and neither had travelled outside of Wisconsin or Michigan's upper peninsula, USA. Histopathological examination and culture of affected tissues on specialized media, serology for anti-P. insidiosum antibodies, ***P. insidiosum***-specific PCR and ribosomal RNA gene sequencing were carried out. Histopathological examination revealed pyogranulomatous and eosinophilic inflammation associated with wide, poorly septate hyphae.

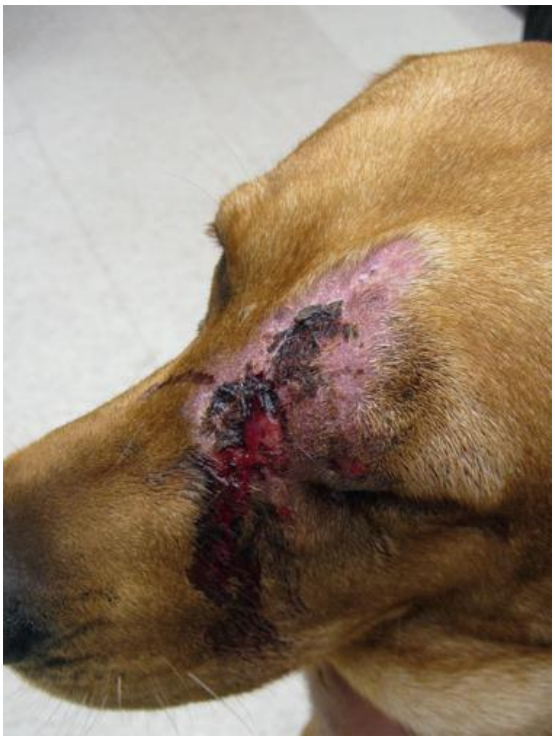
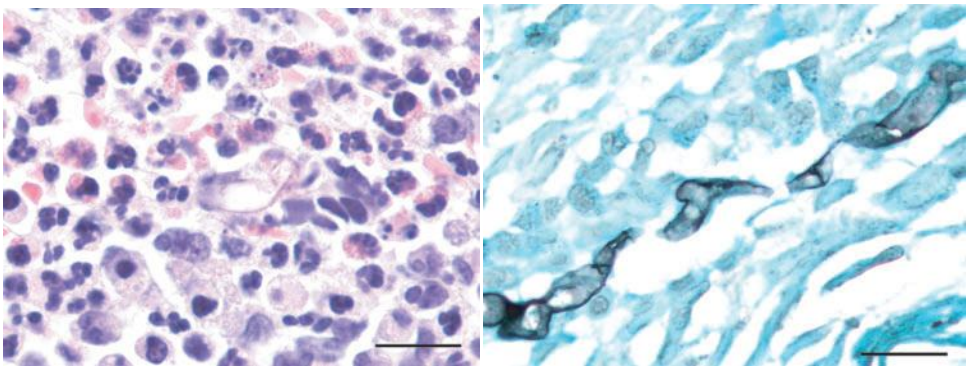
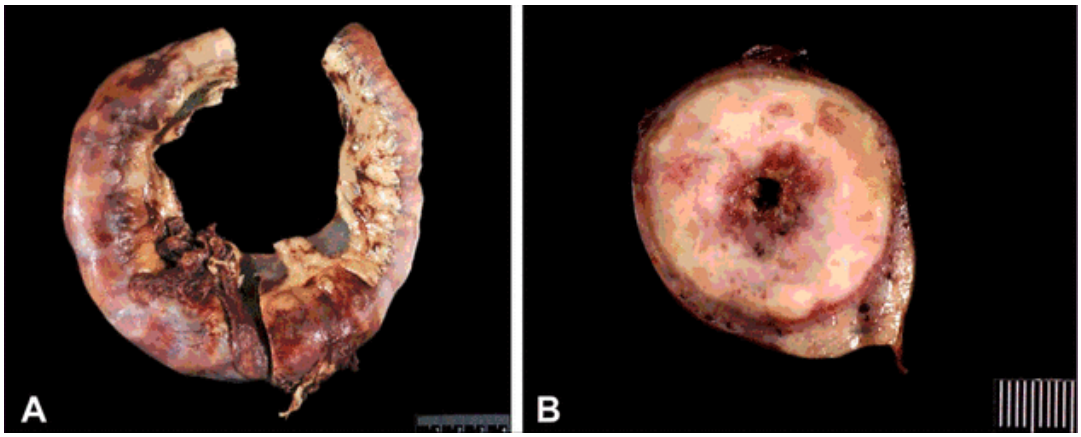


Image of case 1 dog pretreatment, showing soft-tissue inflammation over left side of face. **Oldenhoff *et al.* (2014)**



Histopathology showing pyogranulomatous and eosinophilic inflammation with rare hyphae. Haematoxylin and eosin stain. Histopathology showing broad hyphae with rare septa and rare short branching. Gomori methenamine silver stain. **Oldenhoff *et al.* (2014)**

Aeffner *et al.* (2015) reported a 7-year-old spayed female Labrador Reyriever with a mass in the jejunum as revealed by abdominal ultrasonography. Resection and anastomosis were performed and the resected segment showed a thickening of the wall with diminished non-occluded lumen. The case was diagnosed as **intestinal pythiosis.**



A:Portion of the jejunum of a dog,B: Cross section of the jejunum showing severe thickening of the wall, expanded mucosa and white multiple focci, Aeffner *et al.* (2015)

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9. Zygomycosis in cats and dogs

Basidiobolus ranarum is a saprophytic fungus in the environment that also is a part of the endogenous microflora in the gastrointestinal tract of several vertebrates. These organisms may penetrate skin or muscosa of humans and other animals, causing granulomatous inflammation.

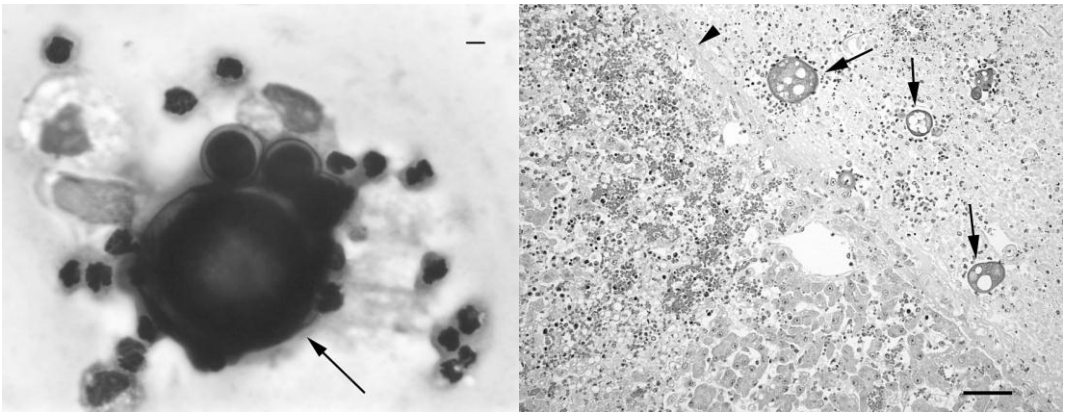
Reports:

Ravisse *et al.* (1978) mentioned that the pathologic examination of the brain of a pet cat, suspected of rabies, showed lesions of mucormycosis. The causal fungus, ***Mucor (Rhizomucor) pusillus*** was isolated and identified. The authors describe the lesions produced, the experimental pathogenicity for the rabbit and the morphologic and physiologic characteristics of the isolate.

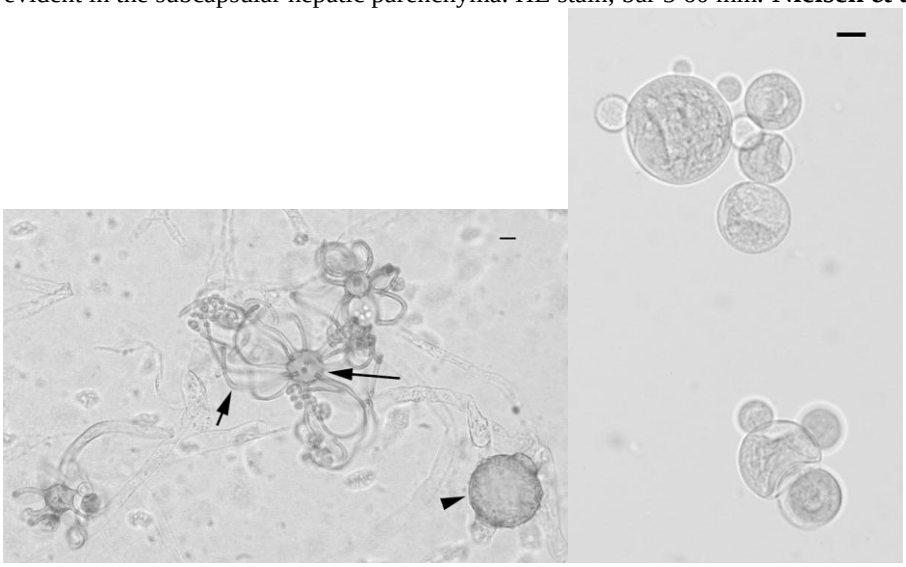
Greene *et al.* (2002) reported 2 dogs infected with ***B. ranarum*** with prolonged or repeated exposure to water or soil in their environment. One dog had progressive **subcutaneous** infection of all the limbs, and the other dog had recurrent coughing and dyspnea caused **by tracheobronchitis**. In both dogs, secondary bacterial infection of the lesions was evident. Treatment of the dog with subcutaneous infection involved cutaneous dressings and sequential use of enrofloxacin and itraconazole; however, this resulted in suspected liver damage without clinical improvement. Subsequent treatment with potassium iodide and a lipid formulation of amphotericin B was also unsuccessful, and the dog was euthanatized. The other dog was treated alternately with enrofloxacin and itraconazole. When the clinical signs and infection returned, combination treatment with both drugs was more effective; however, the dog developed liver damage. Subsequent treatment with enrofloxacin on an intermittent basis controlled the dog's coughing during a 3-year period.

Denzoin-Vulcano *et al*. (2005) reported a case of abdominal zygomycosis in a Doberman bitch. Clinical signs consisted of urinary incontinence and hard abdominal masses detected by palpation. The masses were surgically removed by exploratory laparatomy and had a tumoral-like appearance. A granulomatous reaction containing coarse and non septate hyphae was the main histological finding. Direct microscopic examination revealed the presence of fungal structures. On Sabouraud honey agar the fungus developed fluffy, greyish white colonies that were identified as ***Absidia corymbifera*** on the basis of their macro and microscopic morphology

Nielsen *et al*. (2005) isolated ***Cokeromyces recurvatus***, a zygomycete from the peritoneal fluid of a cat with jejunal perforation secondary to intestinal lymphosarcoma. This organism has not been recovered previously from a veterinary patient. The tissue form of C. recurvatus is morphologically similar to those of Coccidioides immitis and Paracoccidioides brasiliensis and may be misdiagnosed as 1 of these organisms on the basis of cytologic or histopathologic specimens, particularly in geographic regions where these organisms are not endemic.



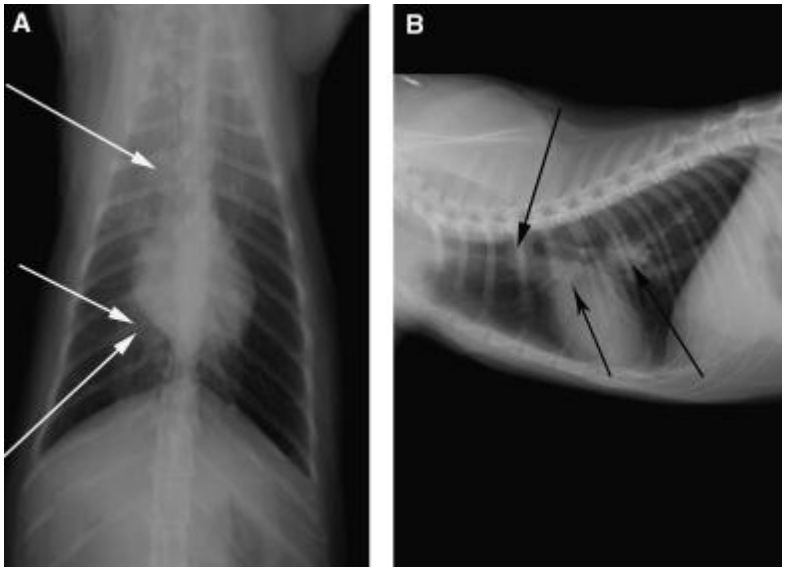
Photomicrograph of a direct smear of fluid obtained by means of abdominocentesis in a cat with fungal peritonitis. An extracellular, round to oval structure approximately 100 mm in width with a thick, double wall and basophilic interior [arrow] can be seen. The organism is surrounded by nondegenerate neutrophils. Wright–Giemsa stain; bar 5 10 mm. Photomicrograph of the liver at necropsy. Surface of the Glissons' capsule [arrowhead] is covered with a thick membrane of necrotic cellular debris and degenerate neutrophils admixed with several yeast-like organisms [arrows]. The organisms are 30–60 mm in diameter with 1-mm-thick eosinophilic wall surrounding amphophilic internal contents containing variably sized, clear, round vacuoles. Hepatocellular degeneration, hemorrhage and biliary stasis is evident in the subcapsular hepatic parenchyma. HE stain; bar 5 60 mm. **Nielsen *et al*. (2005)**



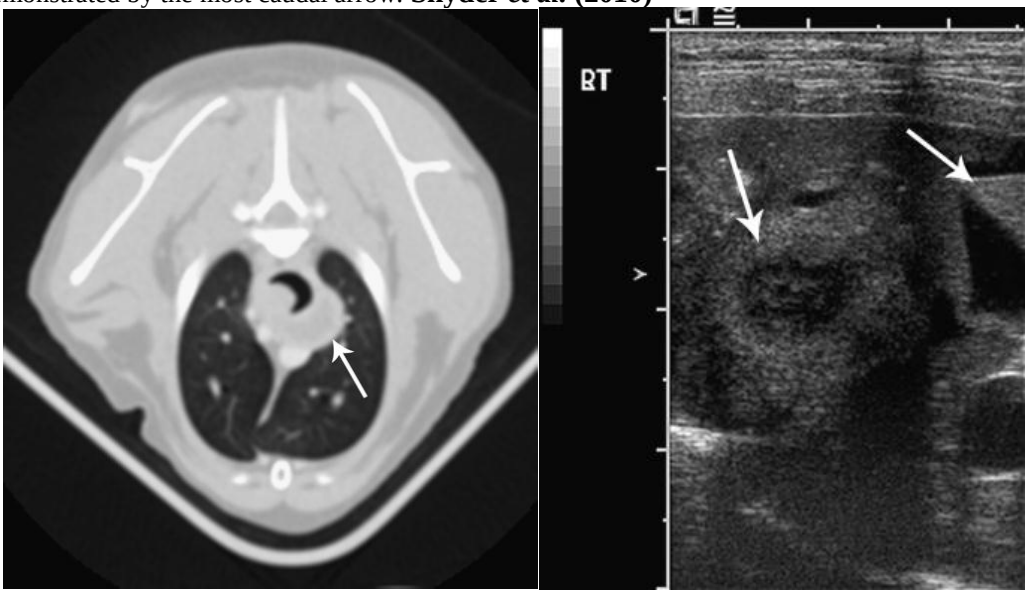
Photomicrograph of the microscopic features of *Cokeromyces recurvatus* when grown on PDA for 8 days at 25 C. Both asexual forms (the sporangiophore terminating in a fertile vesicle [long arrow] giving rise to the recurving sporangiole stalks [short arrow] and sporangioles containing sporangiospores) and the sexual zygospores [arrowhead] are shown. Lactophenol cotton blue stain; bar 5 10 mm Photomicrograph of the yeast form of *Cokeromyces recurvatus* grown on brain heart infusion agar with 5% sheep blood at 37 C for 2 days, demonstrating multiple buds resembling a ''Mariner's wheel.'' Lactophenol cotton blue stain; bar 5 10 mm **Nielsen *et al*. (2005)**

Wray *et al.* (2008) reported a 14-year-old neutered female domestic shorthair cat with a non-painful subcutaneous swelling of the nasal dorsum at the site of a scratch injury. Cytological evaluation demonstrated a granulomatous reaction and many variably shaped organisms consistent with yeasts/fungi. Subsequent biopsy and culture yielded a pure growth of a Mucor species. The cat was treated with the second-generation triazole antifungal agent posaconazole for 5 months. Complete resolution was seen with no recurrence 12 months after discontinuing treatment.

Snyder *et al.* (2010) described tracheobronchial zygomycosis in a cat with acute dyspnea. Radiographic, ultrasonographic, and computed tomography imaging allowed identification of tracheobronchial masses, with intraluminal and peribronchial involvement. Surgical removal was impossible and antifungal chemotherapy was ineffective.



Initial ventrodorsal (A) and right lateral (B) thoracic radiographs of the patient. On the VD view, arrows demonstrate M2 just cranial to the heart. The more caudal two arrows indicate the location of M3. On the lateral view, a soft tissue opacity is observed in the thoracic trachea (M1) outlined by gas opacity on its cranial and caudal borders. M2 is observed just caudal and ventral to M1, and M3 is demonstrated by the most caudal arrow. **Snyder *et al.* (2010)**

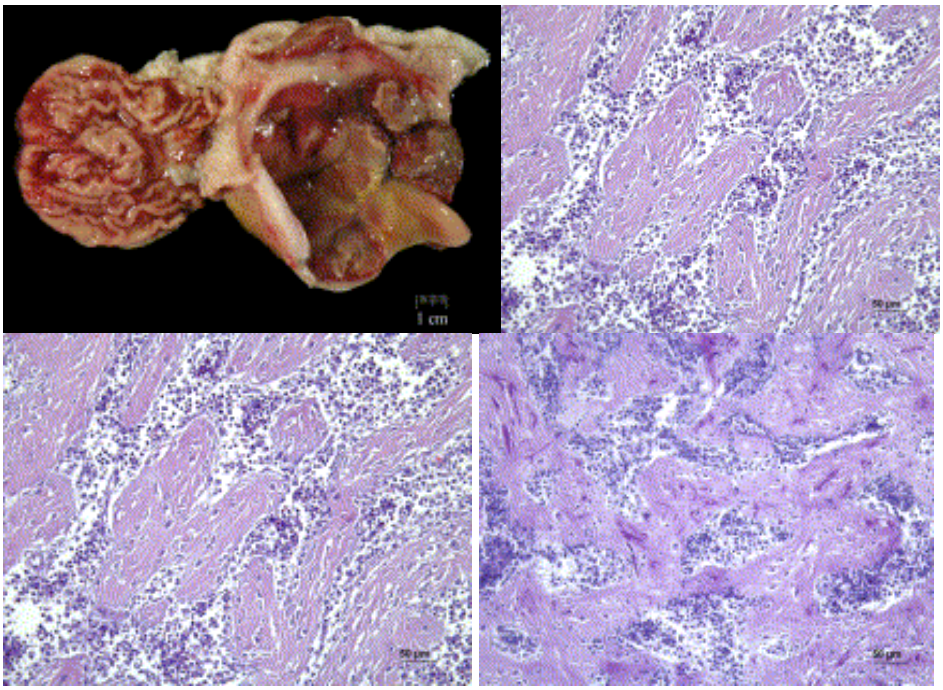


Computed tomography image of the affected lung. The intratracheal mass partially occludes the trachea and extends into the surrounding paratracheal tissue (arrow). Ultrasound image of the affected right caudal lung. There was consolidation of the right lung (right arrow), consistent with hepatized lung.
An irregular hyperechoic infiltrate/mass surrounded a cross section of the central hypoechoic right caudal bronchus (left arrow). This bronchus was dilated and contained hypoechoic fluid/infiltrate. **Snyder *et al.* (2010)**

Cunha *et al.* (2011) reported a 7-month-old female Persian cat with gastrointestinal (GI) necrosis and perforation caused by **Rhizomucor species**. Unfortunately, the cat

died of bacterial peritonitis and sepsis before a definitive diagnosis, based on histopathology and fungal culture, was achieved.

Grau-Roma *et al*. (2014) described gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) in a 9- month-old male Persian cat presented with a history of marked acute haematemesis. A mass (10 cm diameter) was detected within the pylorus and proximal duodenum and this was not surgically accessible. On necropsy examination the duodenal wall was seen to be markedly thickened with extensive mucosal ulceration. Microscopically, there were haphazardly oriented trabecular bands of dense eosinophilic collagen, separated by wide, clear areas containing variable numbers of fibroblasts, eosinophils, mast cells, neutrophils, macrophages, lymphocytes and plasma cells. Numerous pleomorphic, non-parallel walled, sparsely septate hyphae, characteristic of phycomycetes, were present within the collagen matrix. Colonies of gram-positive and gram-negative rods were also present within the lesion.



Grau-Roma *et al*. (2014)

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9.Rhinosporidiosis in cats and dogs

Rhinosporidiosis is caused by *Rhinosporidium seeberi*, an organism that was previously classified as a fungus but has been regrouped into the class Mesomycetozoa (family Rhinosporideacae). This class consists of several parasitic and saprophytic organisms, most of which infect fish and amphibians; only *R. seeberi* infects mammals. Rhinosporidiosis is endemic to India and Sri Lanka, although cases have been reported in Africa, the Americas, and Europe. Rhinosporidiosis is predominantly a human disease; however, it has been documented in many other species, including cats, dogs, cattle, and waterfowl. Equine cases are infrequent but have been reported from South Africa (Zschokke, 1913), the United States (Myers et al., 1964), South America (Londero et al., 1977, and in the United Kingdom (Leeming et al., 2007).

a. Aetiology

Rhinosporidium seeberi (Wernicke) Seeber, B. Aires (1912)

≡Coccidium seeberi Wernicke, Trat. Parasitol.: 62 (1903)
=Rhinosporidium kinealyi Minchin & Fantham, Quart. J. Microscop. Sci.: 521 (1905)
=Rhinosporidium equi Zschokke, Schweiz. Arch. Tierheilk.: 641 (1913)
=Rhinosporidium ayyari Allen & Dave, Indian Med. Gaz.: 376-395 (1936)

b. **Description in vivo**:

The fungus penetrates the mucosal epithelium. Spherical, 6-10 ?m wide cysts are formed in subepithelial tissue, prevalently in the nasal mucosa. The sporangia grow out to about 100 ?m diam; the up to 5 ?m thick wall has a chitinous outer layer and a cellulosic inner layer. After repeated nuclear division, cleavage of the cytoplasm occurs and the spherule swells to 250 ?m diam. Several thousands of spherical endospores 7-9 ?m diam, each containing multiple, indistinct, spherical bodies, are liberated through a rupture in the wall at a pre-formed thinner wall region. Liberated spores are encapsulated in mucoid, granular material and remain in the epithelium. They are surrounded by tissue, and the cycle is repeated. Massive growth of the fungus leads to the formation of large, friable, polyp-like structures. The polyps may

become polymorphous and pedunculated, and develop from pink to finally deep red. At close examination, the spherules can be seen macroscopically as whitish spots.

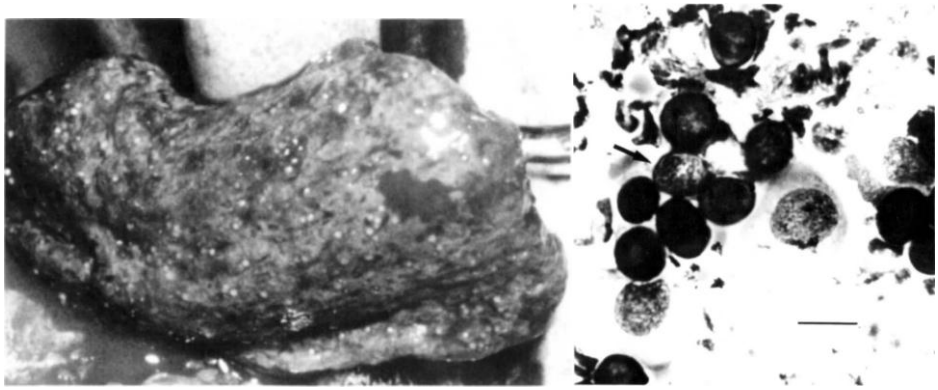
c. Differential diagnosis:

The sporangia stain with mucicarmine, unlike the spherules of Coccidioides immitis. The disease is chronic, and usually painless. The infrequent cases of infection of trachea and bronchus may be fatal due to obstruction of air passage. Dissemination is extremely rare

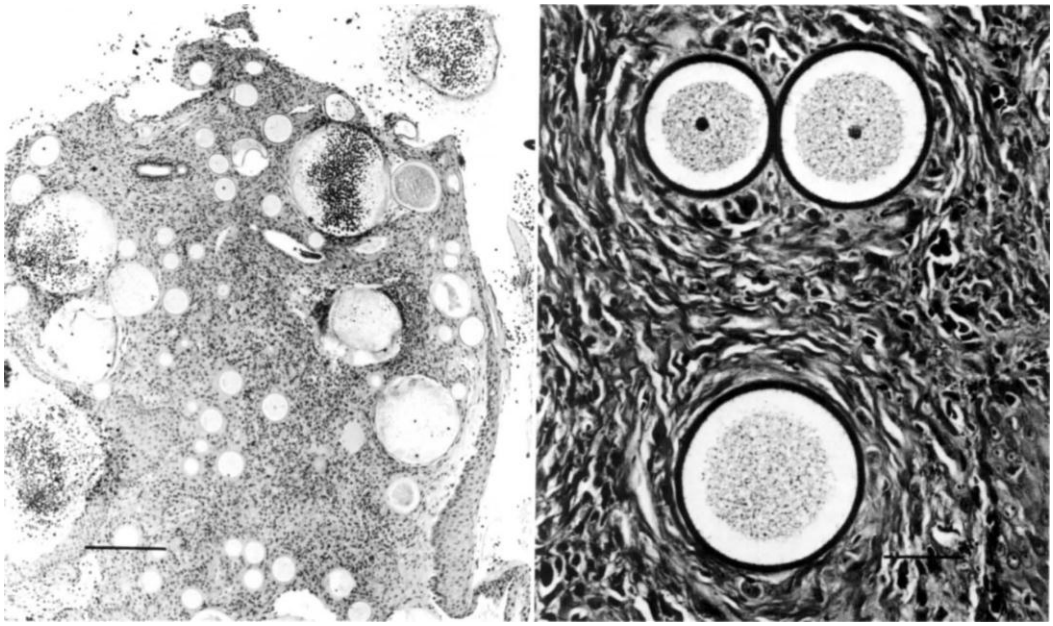
d. Reports on Rhinosporidiosis in dogs

Allison *et al.* (1986) diagnosed nasal rhinosporidiosis in 2 dogs. Cytologic criteria for diagnosis were the presence of 5- to 10-microns endospores and 50- to 1,000-microns sporangia. These findings made it easy to differentiate rhinosporidiosis from the more common nasal mycoses such as cryptococcosis. Treatment is principally surgical, but medical management can be performed if the lesion is inoperable or recurs despite multiple surgeries. Dapsone is one drug that has been used in such patients.

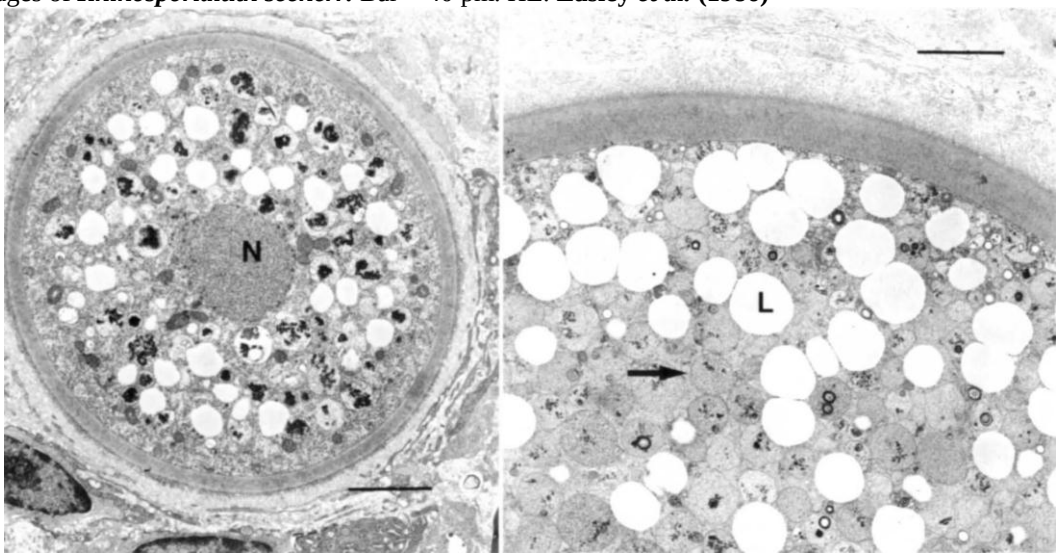
Easley *et al.* (1986) diagnosed rhinosporidiosis in six dogs from the southeastern United States. All six dogs had unilateral nasal polyps with multiple small white sporangia visible beneath the surface. Microscopically, the polyps consisted of organisms and fibrovascular tissue with a surface of columnar or squamous epithelium. Juvenile sporangia were unilamellar, 15-75 microns in diameter, nucleated, and accounted for about 65% of sporangia seen. Approximately 5% of the sporangia were in intermediate stages of maturation, were bilamellar, 100-150 microns in diameter, and contained immature endospores. Mature sporangia comprised about 30% of the total, were usually unilamellar, 100-400 microns in diameter, and contained a mixture of immature and mature endospores. The inner layer of the wall of the intermediate sporangia and the single wall of the mature sporangia were argyrophilic and carminophilic. Ultrastructurally, the earliest stage contained a nucleus and many ribosomes, lipid droplets, and phagolysosomes. Maturing sporangia contained discrete membrane-bound, round clevage products. These structures subsequently matured to spores, each of which had a wall and contained a nucleus and many lipid droplets. The organism from one dog was cultured and grown in vitro for 7 months and is the first successful cultivation of Rhinosporidium seeberi.



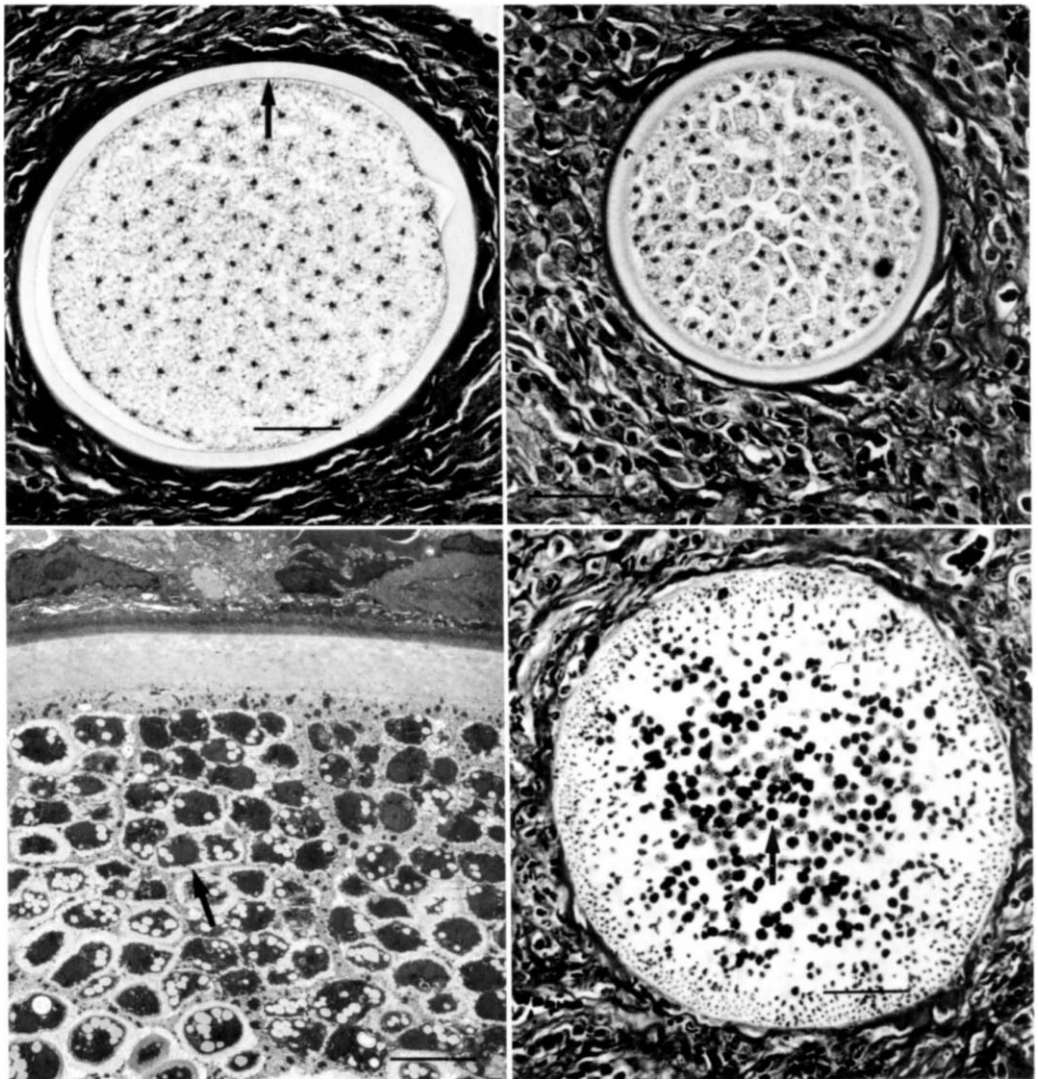
Nasal polyp at surgery. Miliary white foci on surface are sporangia of *Rhinosporidiutii seeberi.* Nasal exudate containing neutrophils. Epithelial cells and spores of *Rhinosporidium seeberi.* Internal "spherules" are evident in one spore (arrow). Bar = 10 Hm. Diff- Quick. **Easley *et al.* (1986)**



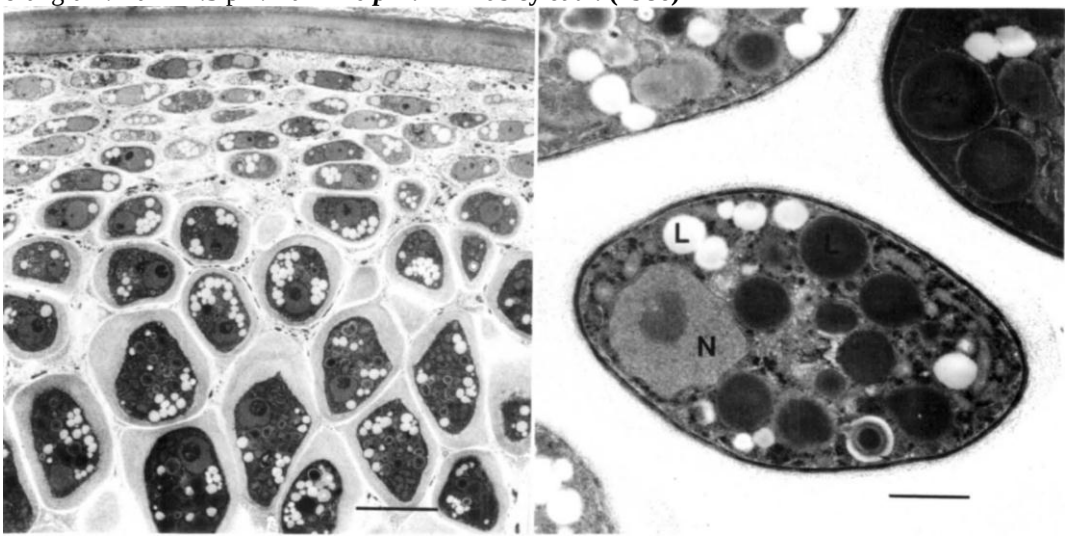
Nasal polyp containing many organisms in different stages of maturation. Bar = 200 pm. HE. Juvenile stages of ***Rhinosporidiutn seeherr.*** Bar = 40 pm. HE. **Easley *et al.* (1986)**



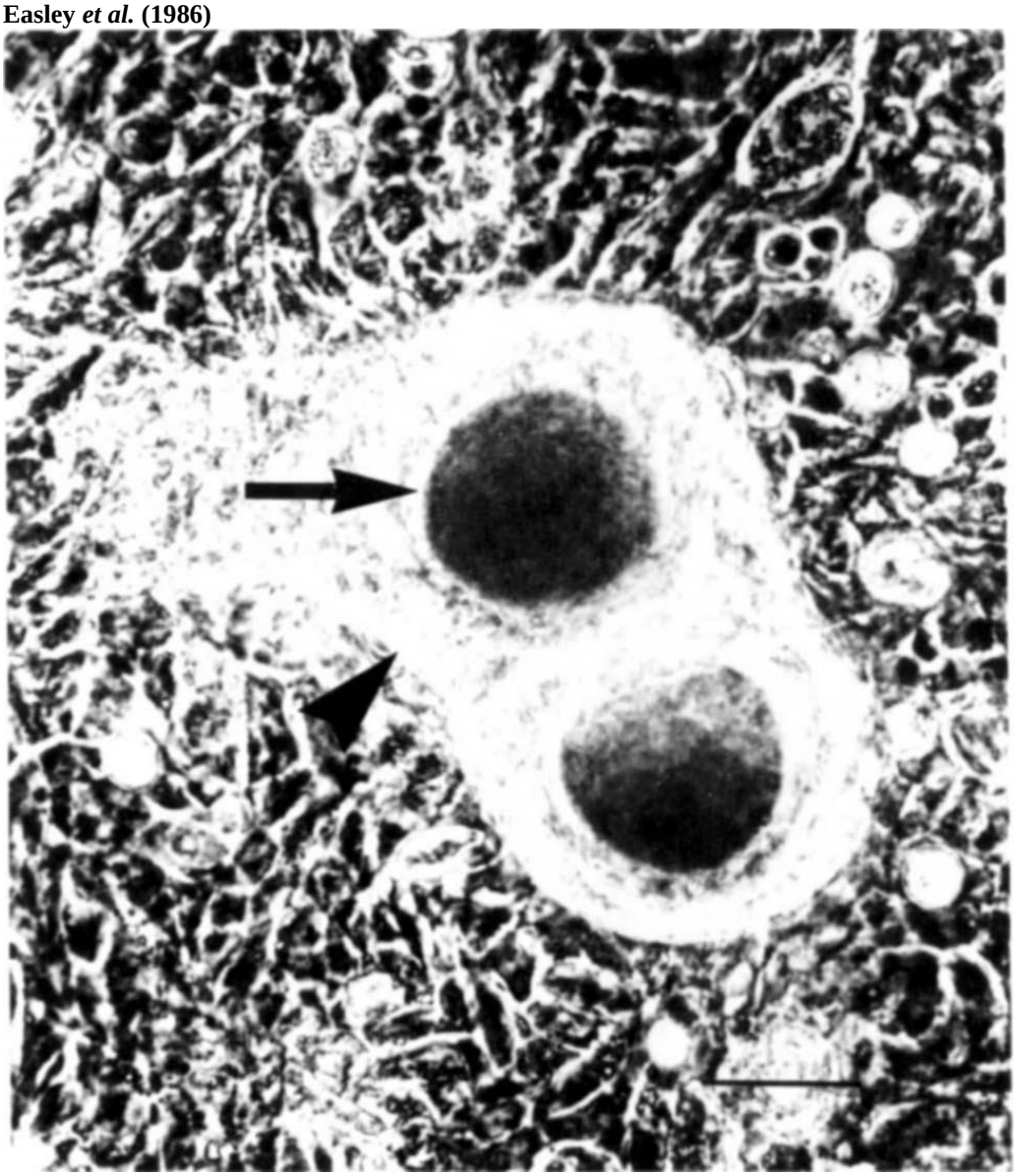
Nucleated (N) juvenile stage before sporulation. Bar = **3** pm. Portion of a juvenile sporangium after sporulation has begun. Lipid droplets **(L)** and cleavage products (arrows) are abundant. Bar = **4.5** pm. **Easley *et al.* (1986)**



Intermediate stage of sporangial maturation with thick bilaminar wall and multiple punctate granules. Thick, Later stage **of** sporangial maturation. Spores are discrete and wall is thick and bilaminar. Bar = 30 pm. HE., Electron micrograph, sporangium similar to that in Fig. 8. Note discrete spores (arrow) with internal lipid and Mature sporangium with mature spores (arrow) in the center and less mature spores toward the periphery. inner layer of wall (arrow). Bar = 30 pm. HE. bilarninar wall of sporangium. Bar = 4.5 pm. Bar = 40 ***prn.*** HE **Easley *et al.* (1986)**



Electron micrograph, mature sporangium similar to Fig. 10. Nuclei, nucleoli, and lipid bodies are evident. Bar = ***5*** rm., Electron micrograph of mature spores. Nucleus (N). lipid (L). Bar = 1.5 rm. **Easley *et al.* (1986)**



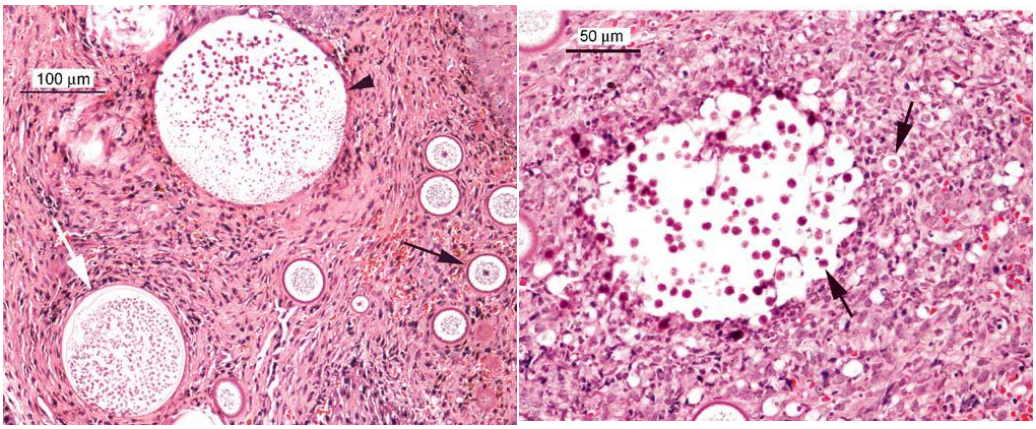
In vitro subculture, 2 months post-inoculation. Two sporangia (arrow) enveloped in a focal aggregate of proliferating tissue culture cells (arrowhead). Bar = 120 pm. **Easley *et al.* (1986)**

Jimenez *et al.* (1986) reported a one-year-old male Collie dog from Northeast Arkansas with rhinosporidiosis presenting as an intranasal polypoid mass.

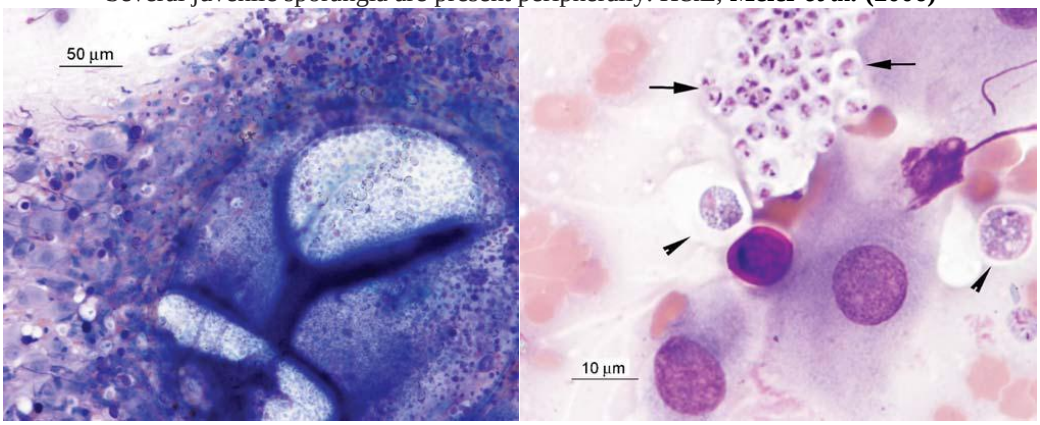
Caniatti *et al.* (1998) described four cases of canine rhinosporidiosis which occurred in Italy in 1994 and 1995. Four dogs with a history of exposure to the muddy environment of rice fields, developed respiratory signs. Rhinoscopy revealed nasal polypoid lesions with a characteristic gross appearance due to the presence of multiple, tiny, white-yellowish spots representing sporangia filled with spores. In cytological samples obtained by brushing, many spores were present in an inflammatory background. Histologically, the polyps consisted of fibrovascular tissue embedding sporangia in different developmental stages, and free spores which elicited a severe pyogranulomatous inflammation. All the dogs were treated surgically and the

condition did not recur in two cases during a year's follow-up and in the other two cases during two years.

Meier *et al.* (2006) reported an 8-year-old, intact, male Labrador Retriever with a 2-month history of severe sneezing episodes that resulted in epistaxis and bilateral sanguineous discharge. Rhinoscopy revealed a small polypoid mass, and specimens were obtained for histopathology. Microscopic examination of formalin-fixed tissue specimens revealed organisms consistent with ***Rhinosporidium seeberi***. The mass was surgically excised and impression smears were made for cytology examination. Smears revealed high numbers of endospores, typical of those previously described for R seeberi. In addition, numerous smaller structures, presumed to be immature endospores, were noted. The immature endospores were morphologically distinct from mature endospores and have not been described previously. Recognition of immature forms of Rhinosporidium may help prevent misidentification of the organism or misdiagnosis of a dual infection.

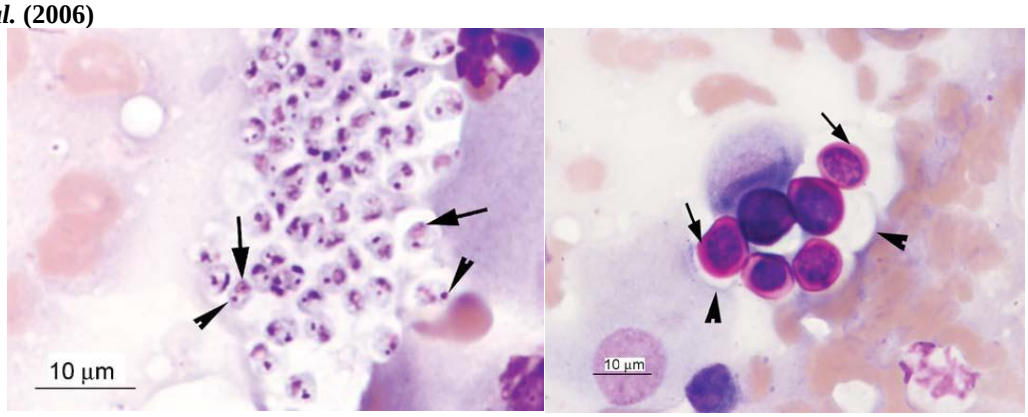


Histologic section of a nasal mass biopsy from a dog with rhinosporidiosis. (Top) Note the mature sporangium of Rhinosporidium seeberi containing both mature and immature endospores (arrowhead). An intermediate sporangium with only immature endospores is present at the lower left (white arrow). Both mature and intermediate sporangia lack an organized nucleus. Several juvenile sporangia containing central nuclei surrounded by abundant faintly basophilic, fibrillar material are seen (black arrow). H&E, bar5100 lm. (Bottom) Large numbers of mature endospores (arrows) free within the connective tissue stroma are surrounded by an intense pyogranulomatous inflammatory cell infiltrate. Several juvenile sporangia are present peripherally. H&E, **Meier *et al.* (2006)**



Impression smear of a nasal mass from a dog with rhinosporidiosis. A large sporangium contains, and is surrounded by, hundreds of variably sized endospores. Wright's-Giemsa. Impression smear of a nasal mass from a dog with rhinosporidiosis. A large sporangium contains, and is surrounded by, hundreds of variably sized endospores. Wright's-Giemsa. Impression smear from a dog with rhinosporidiosis. Two epithelial cells and 3 stages of developing endospores are shown. A single, mature, eosinophilic endospore is seen in the center. A group of smaller, immature endospores is at the

top of the image (arrows). Also seen are 2 intermediate spores (arrowheads). Wright's-Giemsa. **Meier *et al.* (2006)**



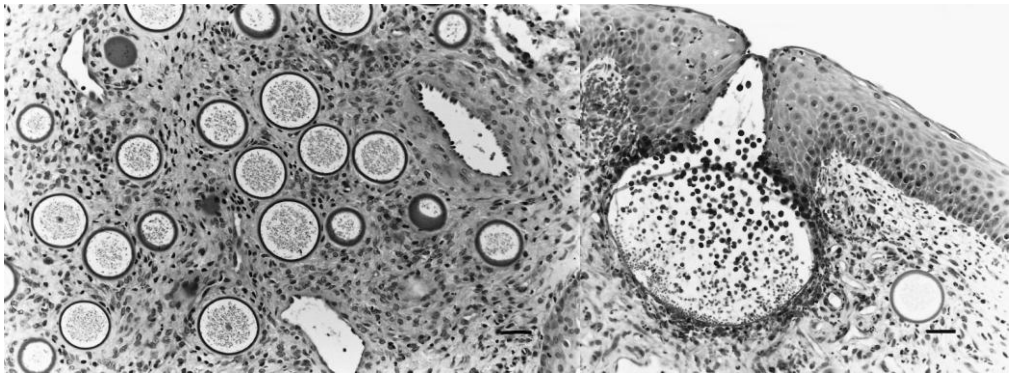
Immature endospores, approximately 2–4 lm in diameter and lightly basophilic, contain a relatively large pink-purple area thought to be nuclear material (arrows) as well as 1–2 smaller, dark-purple structures (arrowheads). Wright's-Giemsa. Mature endospores of Rhinosporidium. When the spores are well spread out, they appear eosinophilic with a thick cell wall (arrows) and are surrounded by a variably-sized, clear halo (arrowheads). Numerous eosinophilic globular structures can be seen within some mature endospores. Wright's-Giemsa. **Meier *et al.* (2006)**

Miller and Baylis (**2009**) reported a case of rhinosporidiosis in a dog native to the UK.

Hill *et al*. (1010) reported **Nasal rhinosporidiosis** in two dogs , 4 and 7 years of age. Physical examinations in both cases were within acceptable limits except for the presence of a single mass in the left nasal passage in the first case and left-sided nasal discharge in the second case. Rhinoscopy was used to visualize the nasal masses, and in both cases a single mass was surgically removed. Impression smears and histopathology submitted from each mass revealed lymphoplasmacytic and neutrophilic inflammation with spores typical of Rhinosporidium seeberi. These are the first reported cases of nasal rhinosporidiosis in two dogs native to the Upper Mississippi River Valley area with no travel history outside the region.

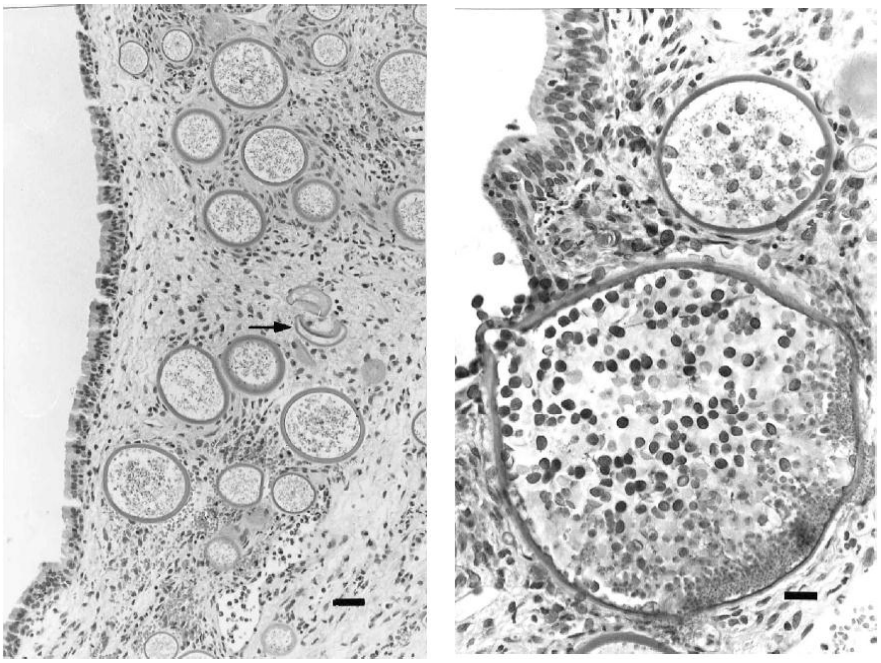
e. Reports on Rhinosporidiosis in cats

Moisan and Baker (2001) examined a polypoid nasal mass from an adult cat histologically and demonstrated the presence of sporangia and sporangiospores consistent with Rhinosporidium seeberi. Inflammatory infiltrates were moderate and pyogranulomatous to lymphohistiocytic and were associated with hyperplasia of the transitional nasal epithelium. Apparently, this is the first reported case of rhinosporidiosis in a cat.



Nasal submucosa; feline rhinosporidiosis. Pyogranulomatous to lymphohistiocytic rhinitis with multifocal immature sporangia of *Rhinosporidium seeberi.* HE. Bar 5 50 mm. Nasal mucosa; feline rhinosporidiosis. Pyogranulomatous to lymphohistiocytic and hyperplastic rhinitis with a mature sporangium. Note the mature and immature sporangiospores and prominent fistula. HE. Bar 5 50 mm. **Moisan and Baker (2001)**

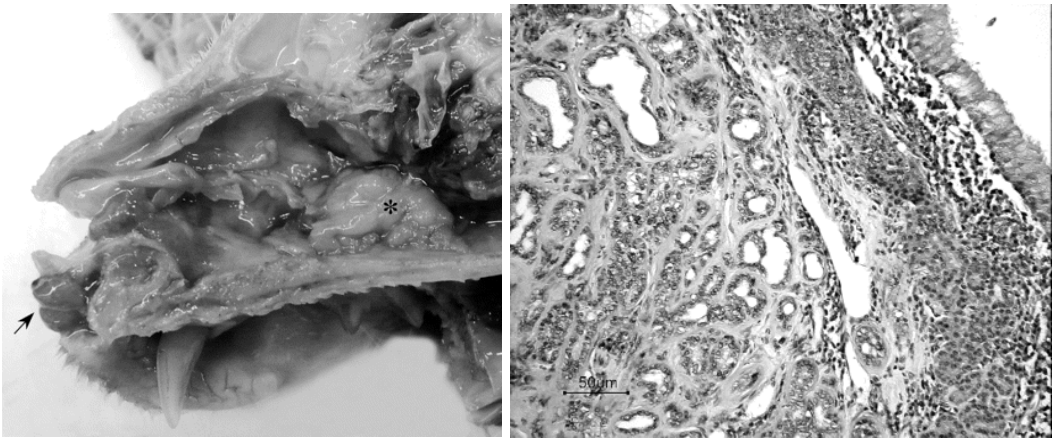
Wallin *et al*. (2001) diagnosed rhinosporidiosis in a domestic shorthair **cat** from a suburb of Washington DC, USA. The clinical presentation of protracted sneezing and epistaxis was associated with a polypoid lesion in the right nostril. Light microscopic examination revealed a polypoid lesion with numerous sporangia containing maturing endospores. Free endospores were present in the stroma of the polyp and lumen of the nasal cavity. Transmission electron microscopy revealed ultrastructural features typical of Rhinosporidium seeberi. The case was followed clinically for a total of 70 months and there were five attempts at surgical excision. This is the first reported case of rhinosporidiosis in a domestic cat.



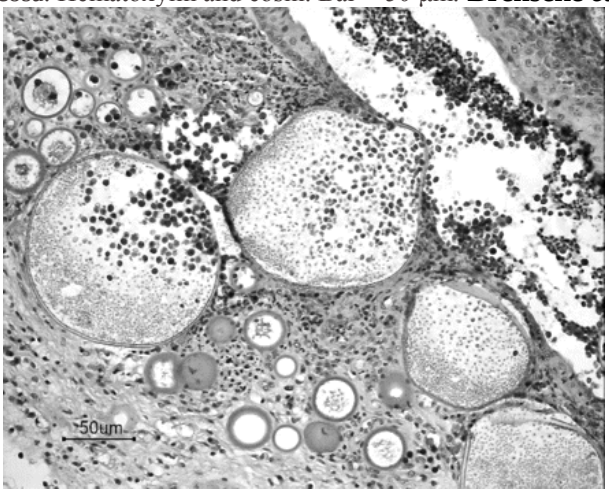
Nasal polyp of cat with maturing *R. seeberi* sporangia beneath intact nasal epithelium. Note fragments of sporangial walls (arrow). H&E stain, bar ˆ 40 m m. Nasal polyp of cat showing mature *R. seeberi* sporangium with nipple-like projection into lumen of nasal cavity and released mature endospores in lumen of nasal cavity. Note the zonary distribution of maturing endospores within the sporangium. H&E stain, bar ˆ 20 m m. **Wallin *et al*. (2001)**

Brenseke *et al.* (2010) reported a 10-year-old, neutered, male Domestic Shorthair **cat** with labored breathing, anorexia, and weight loss of several months duration. External

examination revealed distortion of the bridge of the nose and pink fleshy polyps protruding from each nostril. The cat was euthanized and submitted for postmortem examination. In addition to the external findings, the nasal cavity had extensive bone and cartilage loss and contained a tan firm mass in the caudal region of the nasal cavity near the cribriform plate. On histologic examination, the mass was a nasal adenocarcinoma, and the polyps were composed of hyperplastic nasal epithelium and submucosal stroma that contained sporangia consistent with Rhinosporidium seeberi.



Sagittal section of the head. Polyps caused by *Rhinosporidium seeberi* are present in the nares (arrow), and an adenocarcinoma is located in the caudal region of the nasal cavity (asterisk).Adenocarcinoma from the caudal region of nasal cavity. Neoplastic epithelial cells form tubular and tubulopapillary structures, which are supported by a thick collagenous stroma. Many lymphocytes and plasma cells are present beneath the mucosa. Hematoxylin and eosin. Bar = 50 µm. **Brenseke *et al.* (2010)**



Nasal polyp; feline rhinosporidiosis. Pyogranulo-matous and hyperplastic rhinitis with mature and immature sporangia. Note the release of endospores from mature sporangium into the nasal cavity. Hematoxylin and eosin. Bar = 50 µm. **Brenseke *et al.* (2010)**

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10. Pneumocystosis in cats and dogs

10.1. Introduction

- Pneumocystosis is a severe pneumonia in immunodepressed man and a wide variety of mammalian host species, including laboratory animals, wild animals, zoo animals and domestic animals caused mainly by *Pneumocystis carinii*
- Only a few cases of *P. carinii* pneumonia have been reported in dogs and cats, and most commonly among young animals.
- *Pneumocystis carinii* is an extracellular opportunistic pathogen of the lung with an uncertain taxonomic status. It was classified initially as a protozoan, but more recent DNA and RNA-based investigations relate it to a fungus.
- As a clinical disease, it often has had a fatal outcome.

a. Diagnosis

- The only part of the life cycle of the organism that is known is that involving the mammalian lung, in which 2 main developmental stages can be identified: the trophozoite and the cyst. Cysts may be found with up to 8 intracystic bodies (trophozoites) or be found ruptured and empty.
- Diagnosis of PCP is difficult due to the absence of any specific alterations in hematological or biochemical parameters or in thoracic radiography. Although serological methods have offered valuable information in epidemiological studies, they are not reliable for diagnosing PCP due to a possible underlying immunodeficiency. Recently, circulating *P. carinii* DNA has been identified during acute PCP, but the diagnostic usefulness of this finding has not been established.

- Definitive diagnosis of *P. carinii* is based upon direct visualization of the causative organism from respiratory fluids or biopsy specimens. Several histochemical stains are useful, and diagnostic immunohistochemical kits are available. When using immunohistochemistry it is notable that host species-specific antigenic variation has been demonstrated in *P. carinii.* The development of polymerase chain reaction (PCR) techniques has given a new diagnostic alternative for identification of the organism.

b. Aetiology

Pneumocystis carinii P. Delanoë & Delanoë, Compt. Rend. Hebd. Séances Acad. Sci., Sér. D: 660 (1912)

Pneumocystis carinii is classified in Eukaryota; Fungi; Dikarya; Ascomycota; Taphrinomycotina; Pneumocystidomycetes; Pneumocystidaceae; Pneumocystis

One of the major problems in *Pneumocystis* research is the absence of an in vitro culture system. Although many researchers have attempted to propagate the organism, both with and without feeder cells, the prolonged propagation of *Pneumocystis* is still not possible, nor the production of clonally derived stocks. Consequently, many fundamental aspects of the organism remain unknown, including its life-cycle. To date, all information on the life-cycle has come from studies using electron microscopy. Two distinct morphological forms have been identified: the single nucleated, thin-walled trophic form, and the cystic form which possesses a thick cell wall containing up to eight intracystic bodies. Another morphological form, termed the procyst, has been observed which has a range of morphological features and is thought to develop from the trophic form, with thickening of the cell wall and an increase in the number of nucle**i.**

c. Reports

Copland (1974) described one confirmed case and three clinically diagnosed cases of canine pneumocystis pneumonia in pedigreed Miniature Dachshunds. The main clinical sign were respiratory embarrasasment, loss of weight with out loss of appetite, normal temperature and low exercise tolerance. Two cases were fatal and post mortem findings of one revealed generalized red hepatisation in the lungs with marked interstitial pneumonitis and eosinophilic honeycombmasses of *P. carinii* in the alveoli. The general pathology was similar to that described for human pneumocystic pneumonia.

Botha and van Rensburg (1979) reported pneumonia caused by ***Pneumocystis carinii*** in two unrelated Miniature Dachshunds is reported. The clinical findings, gross- and histopathology and some diagnostic transmission and scanning electron microscopic features of the condition are described. Although pneumocystosis has been reported from a human and a domestic goat in the Republic of South Africa, these are probably the first reported cases of the canine disease in this country.

McCully *et al*. (1979) reported a case of pneumocytosis of an eight month old Dachschund from the Cape Province. Clinically it was an afebrile disease with signs limited primarily to the lower respiratory tract. The report consists of a short history, the histopathologic findings, evidence of the electron microscopic confirmation of the diagnosis and a brief discussion. It is believed to represent the first case of canine pneumocystosis in the Republic of South Africa.

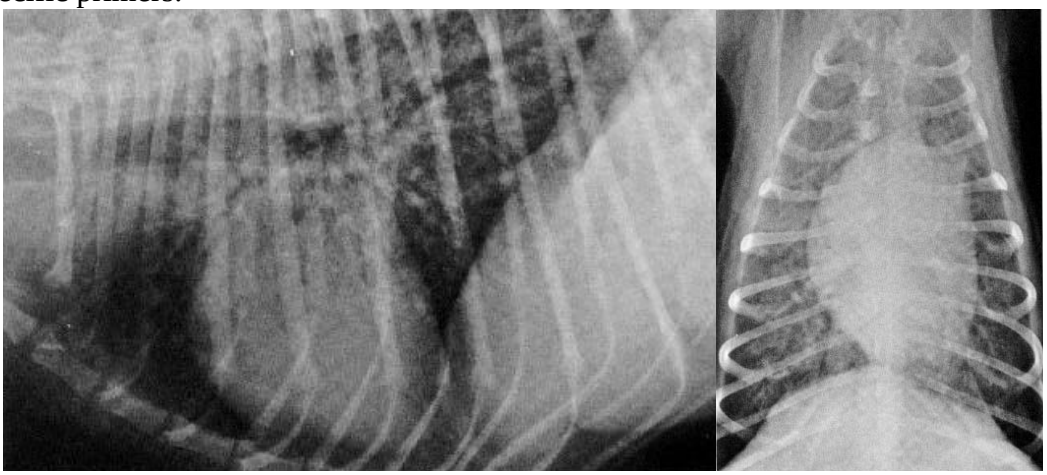
Settnes and **Hasselager (1984)** examined lungs from 106 normal dogs and 75 normal cats for ***Pneumocystis carinii*** by microscopy of toluidine blue O stained imprints. Pneumocysts were demonstrated in 1 dog and 3 cats. It is suggested that these animals may constitute a part of the natural reservoir for this parasite.

Shiota *et al.* (1990) administered corticosteroids to produce ***Pneumocystis carinii*** infection in cats. Six of 10 cats, injected intramuscularly for 97-141 days with 2 mg/cat twice weekly of betamethasone sodium phosphate, developed a light infection with P. carinii. Six of 7 cats, injected intramuscularly for 11-168 days with 10-25 mg/cat weekly of prednisolone acetate, also developed a light infection with P. carinii. There was no significant difference in the infection rate between the sexes and ages of the cats. Using Giemsa staining and Gomori's methenamine silver nitrate stain, P. carinii organisms were indistinguishable morphologically from human and rat P. carinii. The cysts and trophozoites were usually present singly or in small groups, and they always were adhering to the periphery of alveoli. The inflammatory changes were inconspicuous except for the fact that alveolar macrophages often were seen. Corticosteroid-treated cats should be useful in the study of experimental P. carinii infection. This is the first reported case of experimentally induced P. carinii infection in cats.

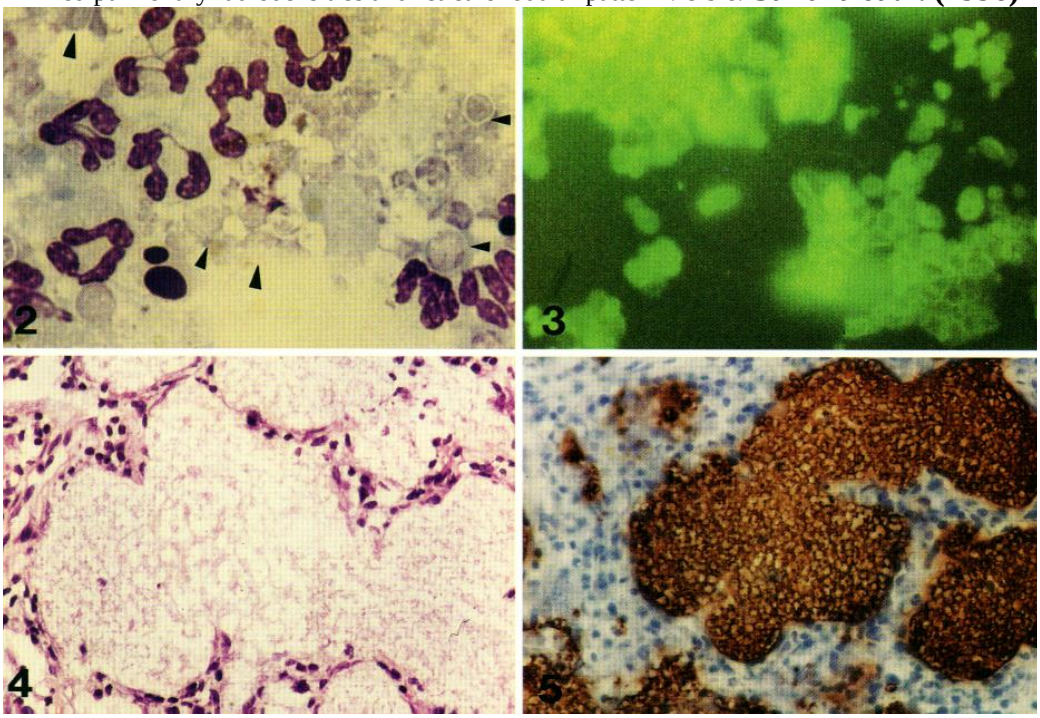
Lobetti *et ai.* (1996) reviewed ***Pneumocystis carinii*** and presented four cases in the miniature dachshund with hyperpnoea, tachypnoea and exercise intolerance. There were also clinical signs suggestive of immune incompetence in all the dogs. P carinii pneumonia was diagnosed in all four cases on transtracheal aspirate cytology. Immunological studies showed low globulin levels on serum electrophoresis, decreased lymphoblast transformation response (in the two cases that were tested) and a deficiency of immunoglobulins A, G and M. Light and electron microscopy as well as anti-canine immunoglobulin G immunoperoxidase staining studies were performed on one case which had died because of the disease. From these four cases, it appears that P carinii pneumonia in the miniature dachshund may be the result of an immunodeficiency. It does not, however, appear to be a classic primary severe combined immunodeficiency syndrome as the dogs appeared to respond to treatment, did not show growth failure and did not manifest overwhelming commensurate bacterial infections.

Sukura *et al*. (1996) reported a Cavalier Ring Charles Spaniel (1.5 years, male) with respiratory signs unresponsive to therapy. The dog had suffered many problems during its first year, including tibial fracture, a large abscess in the stifle region, gastroenteritis, and erosive inflammation in the mouth and tongue. Erosive lesions were detected also at the beginning of the respiratory disease. Therapy with antibiotics (amoxicillin clavulanic acid, tetracycline, cephalexin) produced no response. Some relief, especially at night, was obtained with low-dose corticosteroids (2.5 mg prednisolone/ day for 1 month). The dog was afebrile and dyspneic with increased abdominal effort in respiration. Cyanosis became evident after slight exercise. Lung auscultation revealed respiratory crackles and wheezes. A soft murmur

was auscultated on the left and right thorax. In thoracic radiographs diffuse interstitial and peribronchial densities were seen throughout the lungs, giving the impression of a reticular structure with micronodule formations. Chronic alveolar densities could be seen in all parts of the lungs. Bronchoalveolar lavage (BAL) specimen showed clusters of cysts of ***P. carinii*** and polymorphonuclear leucocytes; stained with May-Grünwald-Giemsa and by commercial monoclonal antibody kit (CMo). Lung sections stained with HE and specific monoclonal antibody (DMo) with a peroxidase technique showed dark brown *P. carinii* cyst and trophozoite stages filling alveolar spaces.. Transmission electron microscopy. demonstrated SMo-antibody localized on electronlucent layer of cyst (C) pellicle. Diagnosis was confirmed by PCR using specific primers.

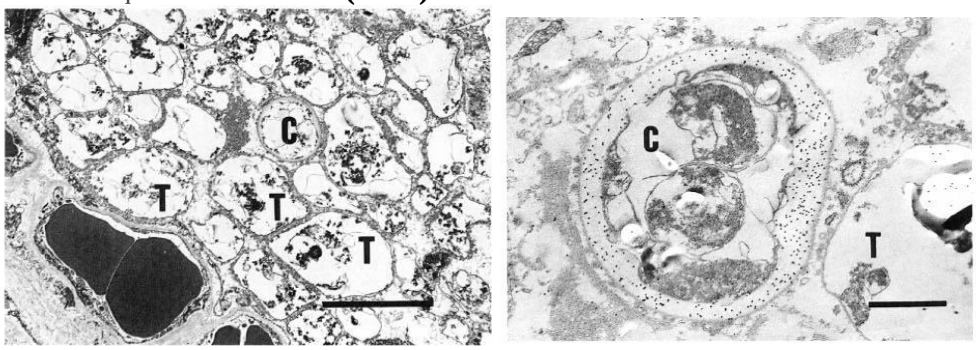


1. (A) Right lateral and **(B)** ventrodorsal thoracic radiographs from the dog. Diffuse symmetrical mixed pulmonary radiodensities and reticulonodular pattern visible. **Sukura *et al*. (1996)**

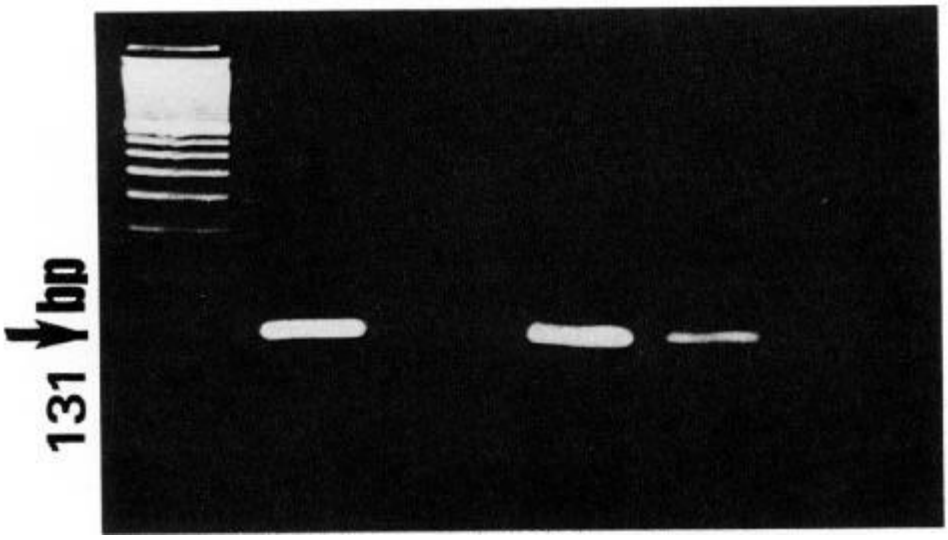


2. Bronchoalveolar lavage (BAL) specimen containing clusters of cysts of *P. carinii* (arrowheads) and polymorphonuclear leucocytes; stained with May-Grünwald-Giemsa. **3.** Clusters of trophozoites and cysts showing a bright apple-green staining pattern; BAL specimen stained by commercial monoclonal antibody kit (CMo).**4.** Lung section containing foamy, eosinophilic exudate in alveolar spaces; paraffin-embedded section stained with HE.**5.** Dark brown *P. carinii* cyst and trophozoite stages filling

alveolar spaces; paraffin-embedded section stained with specific monoclonal antibody (DMo) with a peroxidase technique **Sukura *et al*. (1996)**



Alveolar lumens filled with *P. carinii* organisms. Polymorphic trophozoites (T) seen in close contact with alveolar surface, cysts (C) easy to recognize due to thicker and more rigid pellicle. Transmission electron microscopy. Bar = 5 μm. Ultrastructurally, SMo-antibody localized on electronlucent layer of cyst (C) pellicle. Trophozoites (T) free of gold particles. Immunoelectron microscopy, 15-nm gold particles. Bar = 1 μm. **Sukura *et al*. (1996)**

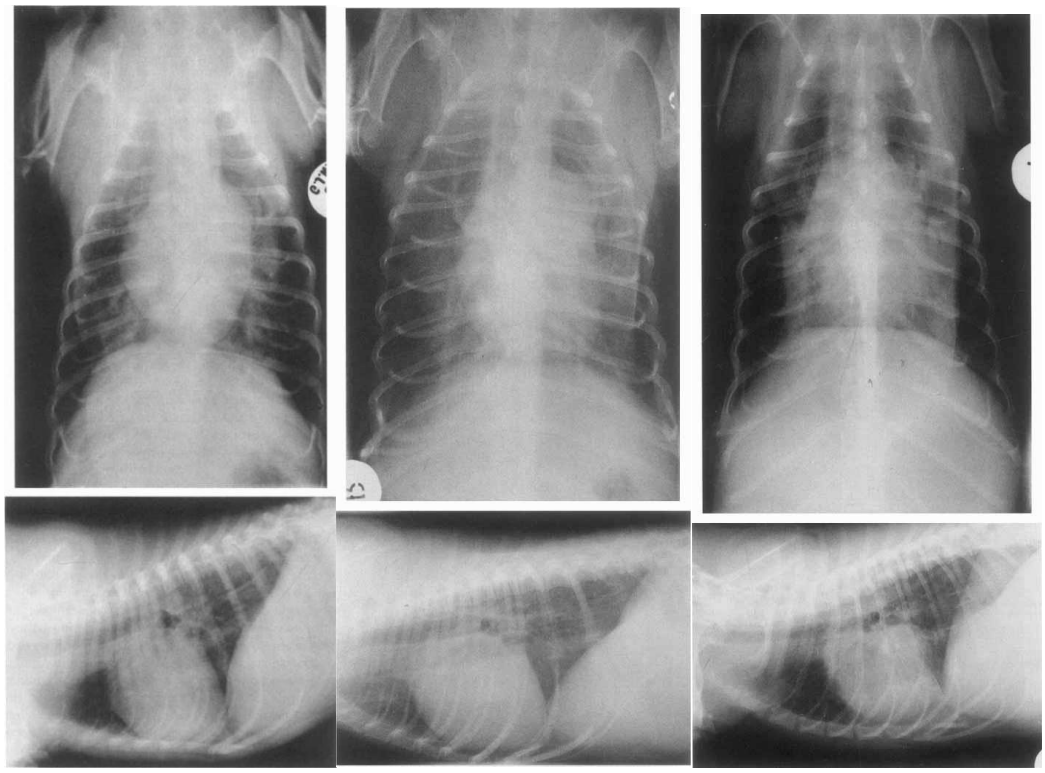


Agarose gel electrophoresis of lung homogenate subjected to PCR. Lane marked M to the left, 100 bp marker; lane 1, positive control (*P. carinii* DNA from human autopsy material); lanes 2-4 amplified DNA from dog lung homogenate (6, 1 and 0.1 μl of the purified DNA, respectively); lane 5, negative control (water). **Sukura *et al*. (1996)**

Yuezhong and Baoping (1996) infected 14 mice trapped in or near houses with ***Pneumocystis carinii*** and the establishment of pneumonia was helped by injecting with cortisone acetate for 6 weeks. Then 16 cats were infected with P. carinii by injection of lung homogenate from the mice which contained from 1.3 x 10(5) to 2.6 x 10(5) P. carinii cysts. The infection resulted in severe cough and tachypnea in Cats 1-8 injected with cortisone acetate, and a subclinical infection in Cats 9-16. In Cats 1-8, the main pathological finding was typical P. carinii pneumonia, but there only was slight swelling of the lungs in Cats 9-16.

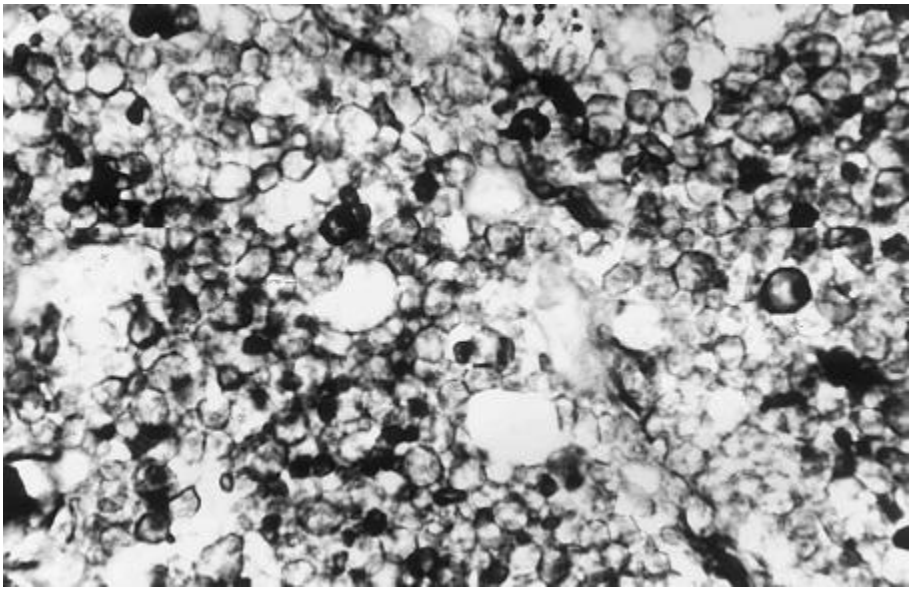
Kirberger and Lobetti (1998) reviewed the thoracic radiographic changes of ***Pneumocystis carinii*** in 7 miniature Dachshunds. The dogs were 7-12 months old and presented with polypnea, exercise intolerance and clinical signs suggestive of immune-incompetence. P. carinii pneumonia was diagnosed in all the dogs using transtracheal aspirate cytology and confirmed at postmortem in 3 dogs that died. Radiographically, diffuse pulmonary changes were present and varied from a mild interstitial and bronchial pattern to an alveolar pattern. Radiographic evidence of cor

pulmonale was present in 1 dog. The most severe radiographic changes were seen in 2 of the dogs that died.

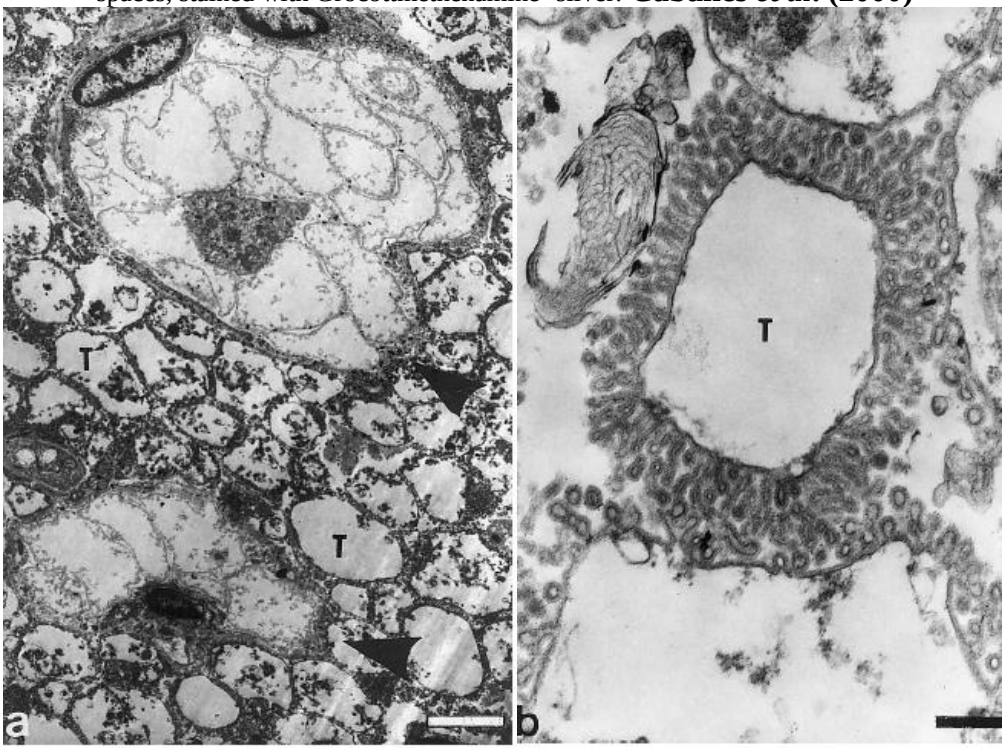


Dorsoventral and right lateral thoracic radiographs of dog 3,which recovered. Moderate diffuse interstitial and peribronchial opacification with less involvement of the cranioventral lung lobes are present. Some faint air bronchogram formation is evident. On the dorsoventral view there is a mildly enlarged pulmonary artery segment and right ventricularenlargement, The vertebral heart size is 10.5. Dorsoventral and right lateral thoracic radiographs of dog 4, which died. Severe diffuse interstitial and mild peribronchial opacification are present. Thin fissure lines are visible on the dorsoventral view. The vertebral heart size is 9.8. Dorsoventral and left lateral thoracic radiographs of dog 7, which died. On the lateral view diffuse interstitial opacification in the caudal lobes with an alveolar pattern in the middle lobes is present. Moderate alveolar pattern results in border effacement of the cardiac silhouette in the dorsoventrdl view. The vertebral heart size is 10.5.

Cabañes *et al*. (2000) presented a 14-month-old male Yorkshire terrier with a history of chronic non-productive cough and acute dyspnea. A follow-up radiograph revealed a diffuse, bilaterally interstitial-alveolar lung disease with presence of air bronchograms. The dog died 5 h after admission with severe dyspnea. Histological sections of the necropsy specimens revealed the presence of characteristic ***Pneumocystis carinii*** cysts within alveolar spaces. A diagnosis of P. carinii pneumonia (PCP) was made on the basis of these results. To our knowledge, PCP has not been described in a Yorkshire terrier dog.



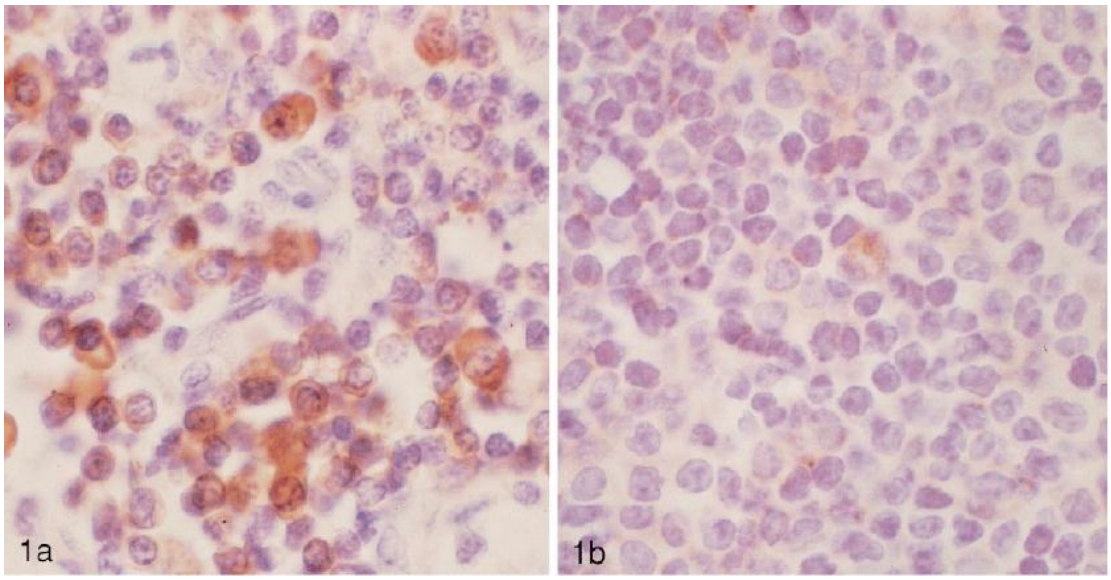
Pneumocystis carinii cyst (dark structures) and trophozoite (light structures) stages . lling alveolar spaces, stained with Grocottmethenamine- silver. **Cabañes *et al*. (2000)**



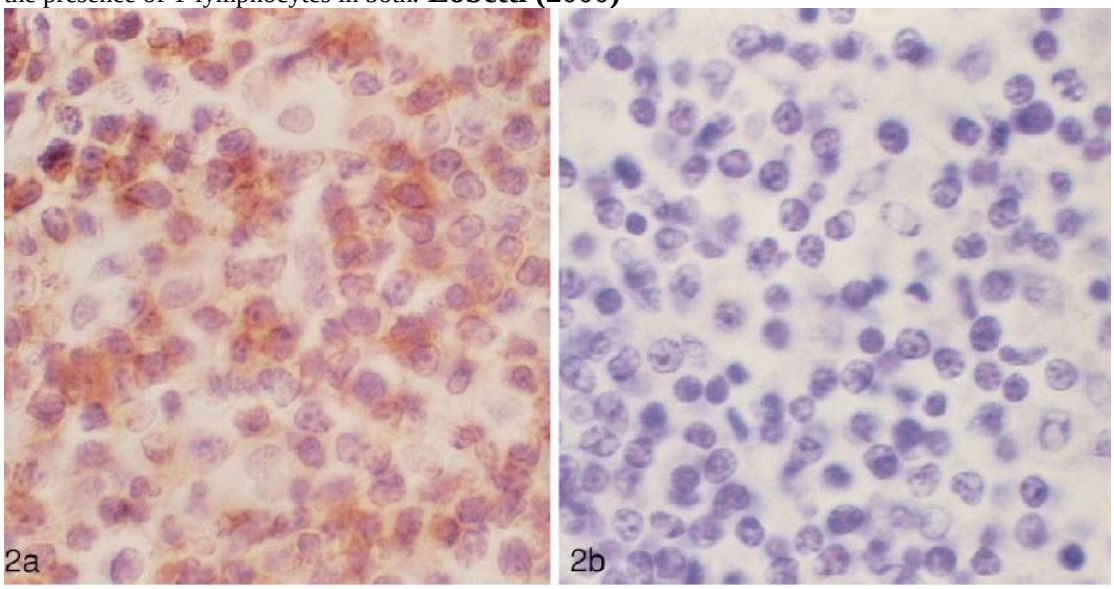
Transmission elect ron micrograph of *Pneumocystis carinii* trophozoites (T) free in the alveolar lumen and inside macrophages (arrowheads) (a) displaying numerous, thin, dendritic . lopodia or tubular extensions (b). Bar¾(a) 3 mm, (b)¾0·3 mm. **Cabañes *et al*. (2000)**

Hagiwara *et a*l. (2001) diagnosed ***Pneumocystis carinii*** pneumonia by postmortem examination of a one-year-old Cavalier King Charles Spaniel with four-week history of dyspnea. Cytologic and histologic examination of lung tissues revealed numerous P. carinii trophozoites and cysts, and P. carinii specific DNA was detected by polymerase chain reaction. The dog showed hypogammagloblinemia and extremely low levels of serum IgG. It was considered that P. carinii pneumonia in this case was associated with an immunodeficient condition which has already been reported in Miniature Dachshunds.

Lobetti (2000) reported 7 miniature dachshunds, all under the age of 1 year, with polypnea, tachypnea, and exercise intolerance as a result of Pneumocystis carinii pneumonia, which was diagnosed on transtracheal aspirate cytology. In all of the dogs, historical and clinical signs were suggestive of immune incompetence. Immunological studies undertaken were leukogram parameters, serum immunoglobulin fraction quantification, lymphocyte transformation assay. CD3 and CD79a lymphocyte markers on lymphoid tissue, and anti-canine immunoglobulin G immunoperoxidase staining. The immunological studies showed hypogammaglobulinemia, deficiency of serum immunoglobulins A, G, and M, decreased lymphocyte transformation response to phytohemagglutinin and pokeweed mitogens and absence of B lymphocytes with presence of T lymphocytes in the lymphoid tissue stained with CD3 and CD79a lymphocyte markers. The preceding findings suggest that P. carinii pneumonia occurring in the miniature dachshund is a result of both a T- and B-cell deficiency. This presentation is not the classic primary severe combined immunodeficiency syndrome but rather combined variable immunodeficiency, which has been well documented in humans but never in the dog.



1. a, A normal and **b**, an affected dog lymph node stained with the CD3 lymphocyte marker showing the presence of T lymphocytes in both. **Lobetti (2000)**



2. a, A normal and **b**, an affected dog lymph node stained with the CD79a lymphocyte marker showing the presence of B lymphocytes in the normal lymph node and absence of B lymphocytes in the affected dog. **Lobetti (2000)**

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11. Lagenidiosis in cats and dogs

Lagenidiosis is life-threatening infections in mammals and birds caused by memebers of the genus *Lagenidium* belonging to the Oomycota. Oomycetes are found in both fresh and salt water as well as in terrestrial environments. They produce flagellated, actively motile spores (zoospores) that are pathogenic to many crop plants and fishes. The majority of species in the genus *Lagenidium* are parasites of algae, fungi, nematodes, crustaceans, and insect larvae.

Two species of this genus have been isolated from dogs with skin lesions. Clinical signs are similar to those associated with cutaneous pythiosis. A number of infected dogs have had frequent exposure to lakes or ponds. Affected dogs have one or multiple skin or tissue lesions on the legs, mammary region, or trunk which look like as firm nodules or as ulcerated, thickened areas with numerous draining tracts.

Lagenidium giganteum forma caninum infection causes severe cutaneous and disseminated disease in dogs..

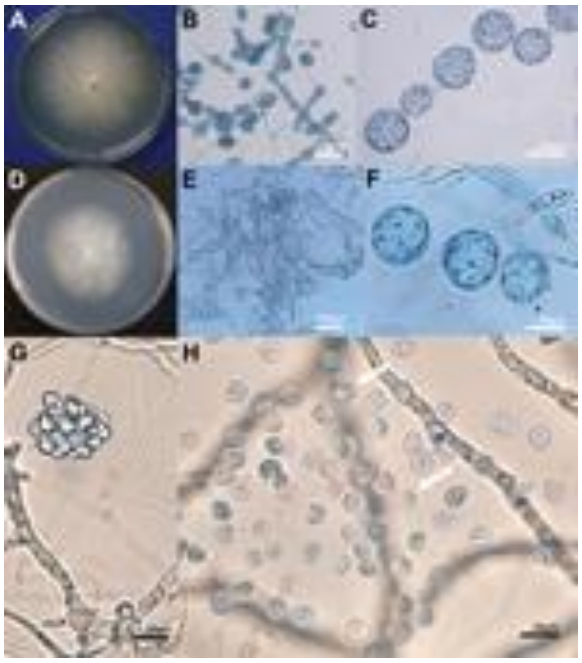
Pythiosis, lagenidiosis, and zygomycosis share similar clinical and histologic characteristics, making them difficult to distinguish from one another; however, distinguishing between these pathogens is important because of differences in epidemiology, choice and duration of therapy, and prognosis.

Aggressive surgical resection of infected tissues is the treatment of choice for lagenidiosis. In animals with lesions limited to a single limb, amputation is recommended. Because response to medical therapy is poor, dogs infected with the more aggressive pathogen have a grave prognosis. In dogs infected with the less aggressive species, surgery may be curative. As with pythiosis, medical therapy for lagenidiosis is usually ineffective.

Lagenidium giganteum forma caninum infection causes severe cutaneous and disseminated disease in dogs. Currently, diagnosis requires culture and rRNA gene sequencing.

Currently, diagnosis requires culture and rRNA gene sequencing.

Lagenidium giganteum Couch, Mycologia 27: 376 (1935)

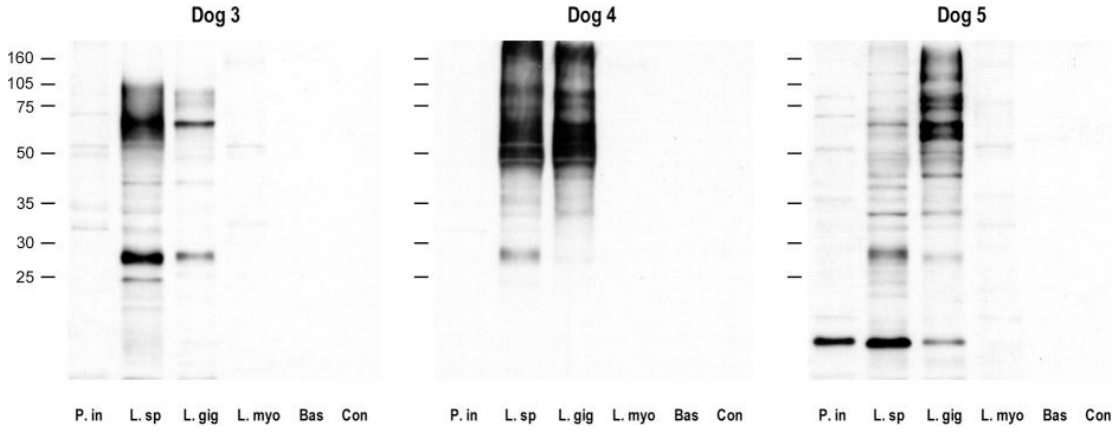


Morphologic features of isolates of *Lagenidium giganteum* mosquito control agent and *L. giganteum* mould from mammals. Panel A shows henotypic features in culture of the mammalian pathogen (ATCCMYA-4933, type strain) wwwnc.cdc.gov

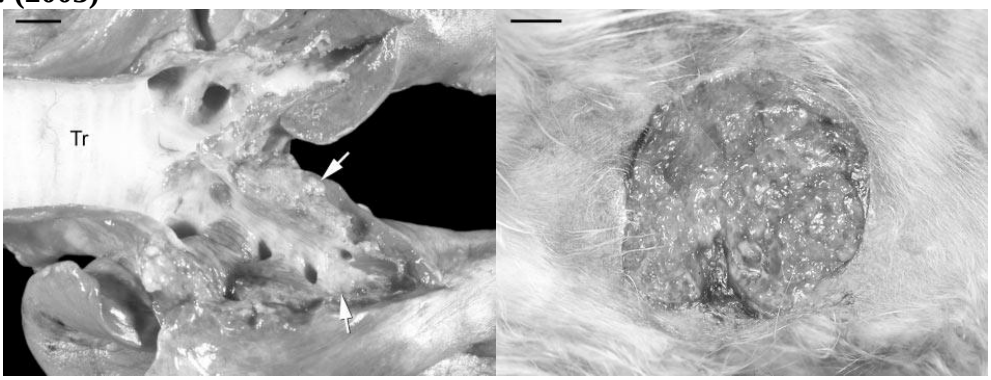
Reports

Grooters *et al.* (2003) isolated an oomycotic pathogen in the genus Lagenidium from tissues obtained from 6 dogs with progressive cutaneous disease. Initial clinical findings in 5 dogs included multifocal cutaneous lesions, subcutaneous lesions, or both associated with regional lymphadenopathy: the 6th dog initially was presented for evaluation of mandibular lymphadenopathy. Cutaneous lesions were ulcerated, exudative regions (often with necrosis and draining tracts) or multiple firm dermal or

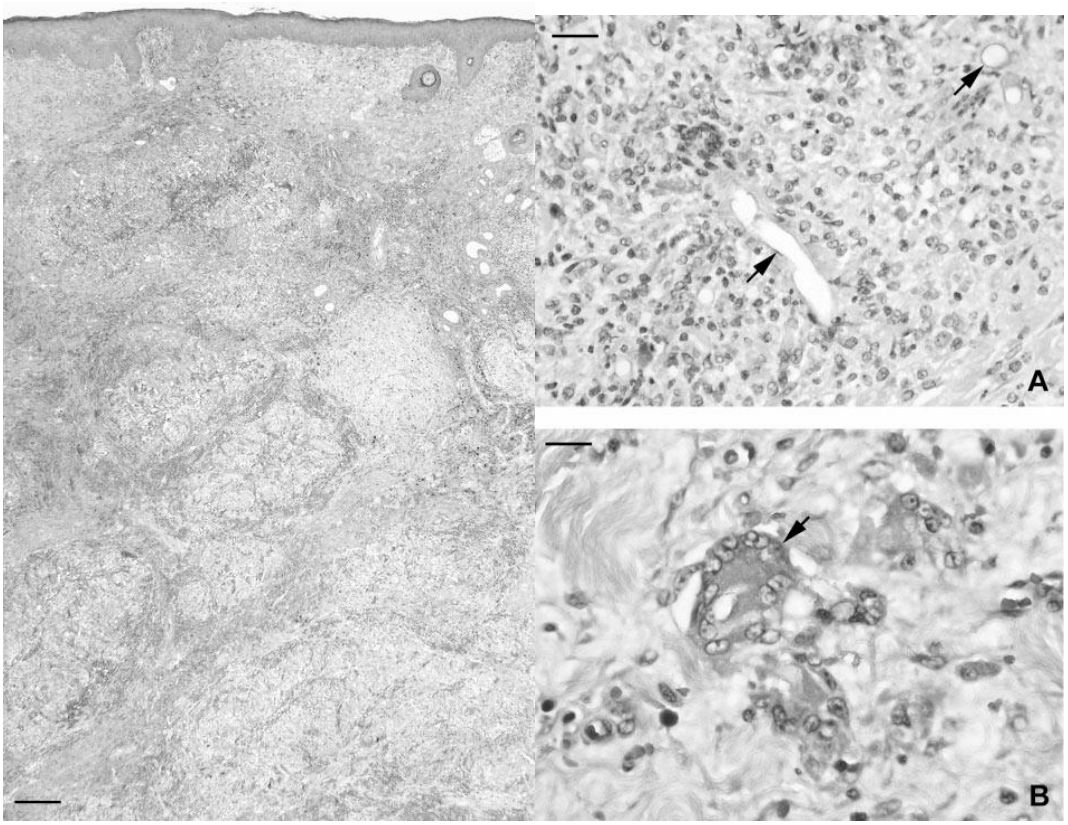
subcutaneous nodules. Two dogs subsequently developed haemoabdomen from great vessel rupture and died acutely. Four dogs were euthanized because of progression of subcutaneous lesions or lymphadenopathy. On postmortem examination, regional granulomatous lymphadenitis was found in all 6 dogs, great vessel invasion in 3 dogs, pulmonary lesions in 2 dogs. ureteral obstruction in 1 dog, mediastinal lymphadenitis in 1 dog, and hilar lymphadenitis with invasion of the distal esophagus and trachea in 1 dog. Histologically, lesions were similar to those associated with pythiosis and zygomycosis and were characterized by severe eosinophilic granulomatous inflammation (often with numerous large multinucleated giant cells) centered around broad (7-25 micro), infrequently septate hyphae. Immunoblot analysis of the serologic response of 4 dogs to a soluble mycelial extract of ***Lagenidium giganteum*** indicated that each dog's serum recognized at least 10 different antigens of L. giganteum. Culture of infected tissues yielded rapid growth of colorless to white submerged colonies. Microscopically, mature hyphae in culture were broad (25-40 micro), segmented, and occasionally branching and produced motile laterally biflagellate zoospores in water culture. This report is the 1st description of infection caused by an oomycete other than Pythium insidiosum in any mammalian species.



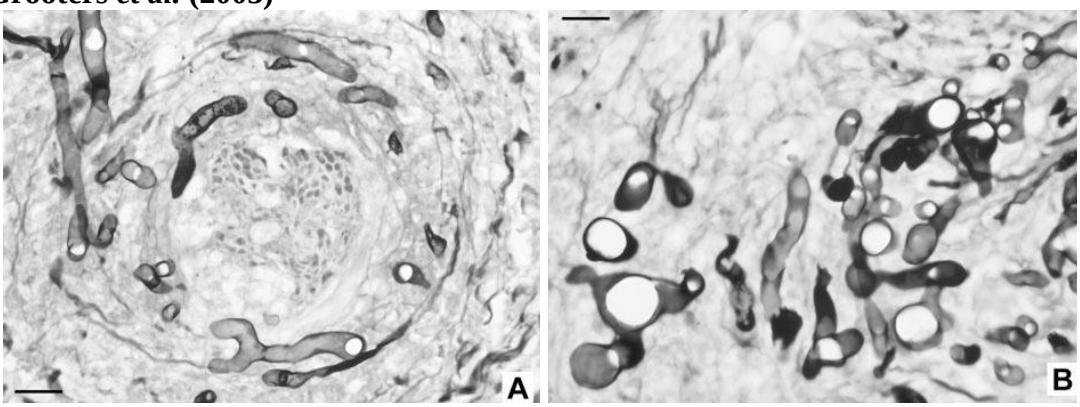
Immunoblot analysis demonstrating the ability of serum from dogs 3, 4, and 5 to recognize antigens of *Pythium insidiosum* (P. in), the canine pathogenic *Lagenidium* species isolated from dog 1 (L. sp), *Lagenidium giganteum* (L. gig), *Lagenidium myophilum* (L. myo), *Basidiobolus ranarum* (Bas), and *Conidiobolus coronatus* (Con). Markers on left indicate molecular weight in kilodaltons, **Grooters *et al.* (2003)**



Gross photograph of the tracheal bifurcation of dog 4. There is a mass (arrows) surrounding the left mainstem bronchus and invading the adjacent pulmonary parenchyma. Histologically, this mass was characterized by eosinophilic granulomatous inflammation with intralesional hyphae. Tr, trachea. Bar 5 2 cm., Gross photograph of a large, raised, ulcerated and exudative cutaneous lesion on the ventral abdomen of dog 5. Histologic examination of this lesion revealed severe ulcerative granulomatous dermatitis with intralesional hyphae. Bar 5 2 cm. **Grooters *et al.* (2003)**



Photomicrograph of a cutaneous lesion on the hind limb of dog 1, showing severe multifocal to coalescing pyogranulomatous inflammation of the dermis and subcutis. Hematoxylin and eosin stain. Bar 5 200 m., Photomicrographs showing *Lagenidium* sp. hyphae in tissue. **(A)** Extracellular hyphae with visible cell wall (arrows) within infected lung tissue from dog 6; **(B)** intracellular hyphae within giant cell (arrow) in infected lymph node from dog 5. Hematoxylin and eosin stain. Bar 5 20 m. **Grooters *et al.* (2003)**



Photomicrographs of tissue sections stained with Gomori's methenamine silver. **(A)** Broad, thick-walled, irregularly septate hyphae associated with granulomatous vasculitis in a lymph node from dog 6; **(B)** numerous hyphae of varying diameter (some of which have a round or bulbous appearance) in an infected lymph node from dog 5. Bar 5 20 m. **Grooters *et al.* (2003)**

Hartfield *et al.* (2014) conducted a study to develop and evaluate an ELISA for quantitation of anti-L. giganteum f. caninum IgG in canine serum. Sera were evaluated from 22 dogs infected with ***L. giganteum f. caninum***, 12 dogs infected with Paralagenidium karlingii, 18 dogs infected with Pythium insidiosum, 26 dogs with nonoomycotic fungal infections or other cutaneous or systemic diseases, and 10 healthy dogs. Antigen was prepared from a soluble mycelial extract of L. giganteum f. caninum. Optimal antigen and antibody concentrations were determined by

checkerboard titration. Results were expressed as percent positivity (PP) relative to a strongly positive control serum. Medians and ranges for PP for each group were: L. giganteum f. caninum (73.9%, 27.9-108.9%), P. karlingii (55.0%, 21.0-90.6%), P. insidiosum (31.3%, 15.8-87.5%), nonoomycotic fungal infection or other cutaneous or systemic diseases (19.2%, 3.2-61.0%), and healthy dogs(9.9%, 7.6-24.6%). Using a PP cutoff value of 40%, sensitivity and specificity (with 95% CI) of the ELISA for detecting L. giganteum f. caninum infection in clinically affected dogs were 90.9% (72.2-97.5%) and 73.2% (60.4-83.0%), respectively. Specificity in dogs infected with P. karlingii was 41.7% (19.3-68.1%) and with P. insidiosum was 66.7% (43.8-83.7%). It was concluded that quantitation of anti-L. giganteum f. caninum antibodies for detection of this infection in dogs has moderately high sensitivity but poor specificity, the latter because of substantial cross-reactivity with anti-P. karlingii and anti-P. insidiosum antibodies.

Vilela *et al.* (2015) conducted a phylogenetic study of 21 mammalian **Lagenidium** isolates; they found that 11 cannot be differentiated from L. giganteum strains that the US Environmental Protection Agency approved for biological control of mosquitoes; these strains were later unregistered and are no longer available. L. giganteum strains pathogenic to mammals formed a strongly supported clade with the biological control isolates, and both types experimentally infected mosquito larvae. However, the strains from mammals grew well at 25°C and 37°C, whereas the biological control strains developed normally at 25°C but poorly at higher temperatures. The emergence of heat-tolerant strains of L. giganteum pathogenic to lower animals and humans is of environmental and public health concern.

Mendoza *et al.* (2016) studied 21 Lagenidium strains isolated from dogs and a human available in their collection. Molecular phylogenetic studies and phenotypic characteristics were used to characterize the strains. They reported the finding of three novel species, herein designated as Lagenidium ajelloi, sp. nov., Lagenidium albertoi sp. nov, and Lagenidium vilelae sp. nov. Their morphological and growth features are also presented.

References

1. Grooters AM, Hodgin EC, Bauer RW, Detrisac CJ, Znajda NR, Thomas RC. Clinicopathologic findings associated with Lagenidium sp. infection in 6 dogs: initial description of an emerging oomycosis. J Vet Intern Med. 2003 Sep-Oct;17(5):637-46.
2. Hartfield JN, Grooters AM, Waite KJ. Development and evaluation of an ELISA for the quantitation of anti-Lagenidium giganteum forma caninum antibodies in dogs. J Vet Intern Med. 2014 Sep-Oct;28(5):1479-84.
3. Mendoza L, Taylor JW, Walker ED, Vilela R. Description of three novel Lagenidium (Oomycota) species causing infection in mammals. Rev Iberoam Micol. 2016 Feb 25.
4. Vilela R, Taylor JW, Walker ED, Mendoza L. Lagenidium giganteum pathogenicity in mammals. Emerg Infect Dis. 2015 Feb;21(2):290-7..

12. *Oxyporosis in cats and dogs*

The filamentous basidiomycetous fungus, Oxyporus corticola, has not previously been reported in the human or veterinary medical literature. Only 2 papers were found concerning dogs. The first was published by Brochus *et al.* (2009) on disseminated

Oxyporus corticola infection in a German shepherd dog and the second byMiller *et al.* (2012), who described the isolation of the fungus Oxyporus corticola from multiple lymphocutaneous tissues of a Beagle dog**.**

Aetiology

Oxyporus corticola (Fr.) Ryvarden, Persoonia 7 (1): 19 (1972

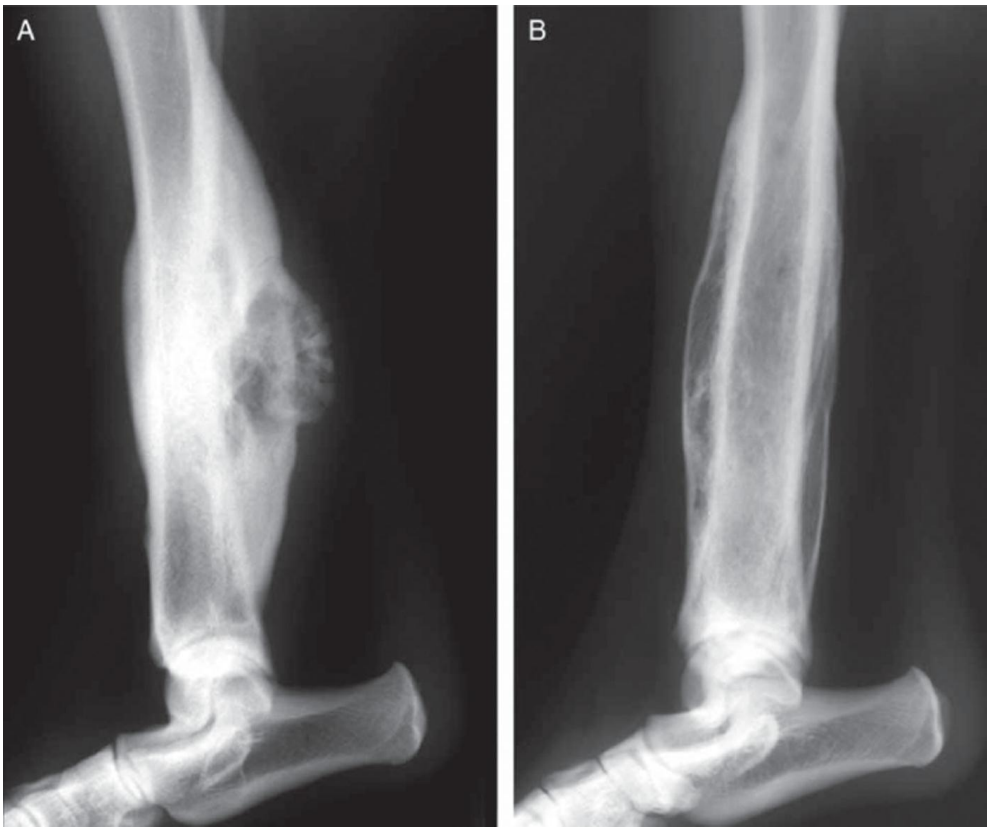
≡Polyporus corticola Fr., Systema Mycologicum 1: 385 (1821)
≡Polyporus polystictus Pers., Mycologia Europaea 2: 111 (1825)
≡Polyporus reticulatus var. corticola (Fr.) P. Kumm., Der Führer in die Pilzkunde: 59 (1871)
≡Physisporus corticola (Fr.) Gillet, Les Hyménomycètes ou Description de tous les Champignons qui Croissent en France: 696 (1878)
≡Poria corticola (Fr.) Cooke, Grevillea 14 (72): 113 (1886)
≡Muciporus corticola (Fr.) Juel, Kungl. Svenska Vetenskapsakad. Handl.: 23 (1897)
≡Coriolus corticola (Fr.) Pat., Essai taxonomique sur les familles et les genres des Hyménomycètes: 94 (1900)
≡Chaetoporus corticola (Fr.) Bondartsev & Singer, Annales Mycologici 39 (1): 51 (1941)
≡Rigidoporus corticola (Fr.) Pouzar, Folia Geobotanica et Phytotaxonomica 1 (4): 368 (1966)
=Polyporus salviae Berk. & M.A. Curtis, Grevillea 1 (4): 54 (1872)
=Polyporus rostafinskii P. Karst., Bidrag till Kännedom av Finlands Natur och Folk 25: 274 (1876)
=Physisporus tener Har. & P. Karst., Revue Mycologique Toulouse 12: 128 (1890)
=Poria separans Murrill, Mycologia 12 (6): 305 (1920)
=Polyporus separans Murrill, Mycologia 12 (6): 305 (1920)
=Poria vicina Bres., Mycologia 17 (2): 76 (1925)
=Poria pearsonii Pilát, Transactions of the British Mycological Society 19 (3): 195 (1935)

Oyxporus corticola is a white-rot decay fungus of woody angiosperms and gymnosperms and is widely distributed in North America and Europe. It is characterized by resupinate or effuse-reflexed, soft and leathery fruiting bodies with a cream to light brown pore surface. The hyphae are simple, septate with thin to thickened walls, bearing short, clavate basidia on each of which are formed four, ovoid to broadly ellipsoid spores, 5–9×3.5–4.5 µm. Two kinds of cylindrical cystidia are present, i.e., one is apically encrusted with hyaline crystals and the other contains a refractive substance.

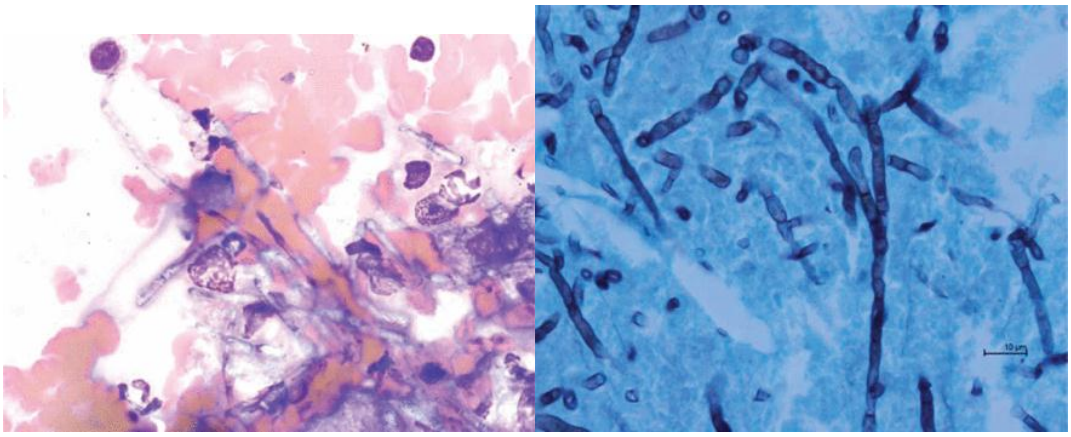
Reports:

Brockus *et al*. (2009) reported a 6-year-old female spayed German shepherd with a painful boney mass on the right distal tibia that had caused the animal to limp for 4 weeks. On physical examination, right hind limb lameness was present with a palpable painful right tibia mass. Mild generalized lymphadenopathy also was noted. Medications and supplements that had been used included carprofen, (Rimadyl, Pfizer, Inc., Exton, PA), glucosamine, chondroitin, brewer's yeast, cod liver oil, and multivitamins. A proliferative mass was observed on the distal right tibia on radiographs but thoracic radiographs were within healthy parameters. Chorioretinal lesions of unknown cause were observed on retinal examination. A fine needle aspirate (FNA) and a biopsy specimen of the right tibial lesion were submitted for study. Macrophages with phagocytosed branching, septate hyphae with nearly parallel sides were observed in the FNA and fungal hyphae were also noted in the biopsy specimen. Initially, this was interpreted as probable aspergillosis. Infrequent fungal hyphae were also present on a prescapular lymph node FNA indicating a disseminated infection. Rapid growth of a white, filamentous, unidentified fungus occurred in the

aerobic bacterial cultures at 35°C and on the fungal cultures at both 25 and 35°C, but no bacterial growth was detected. The isolate was initially identified as a basidiomycetous fungus, and subsequently confirmed as ***Oxyporus corticola*** by sequencing .



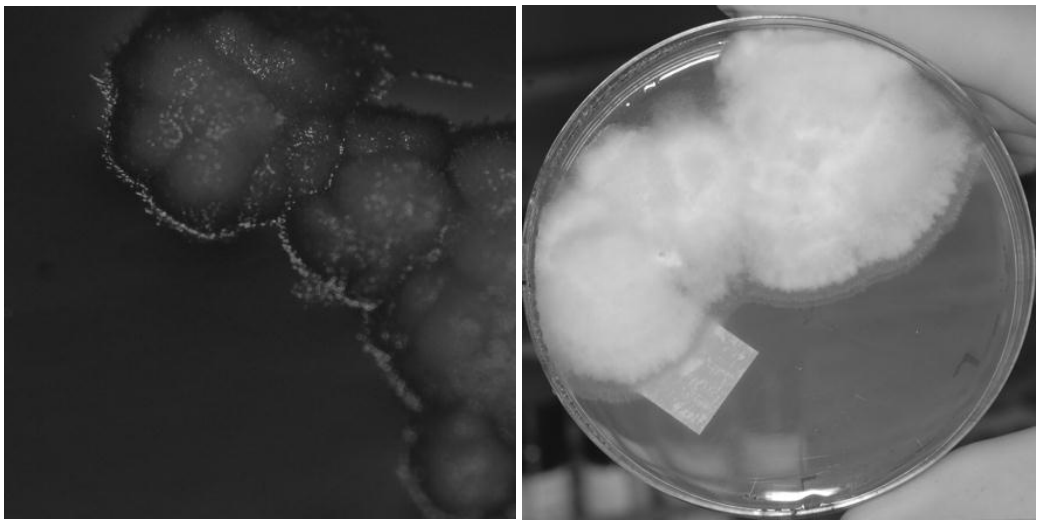
An aggressive mixed productive and proliferative lesion is present in the mid/distal-tibia region (A). A smoother appearance but proliferation remained after 6 months of treatment. Productive changes have progressed distally (B). **Brockus *et al*. (2009)**



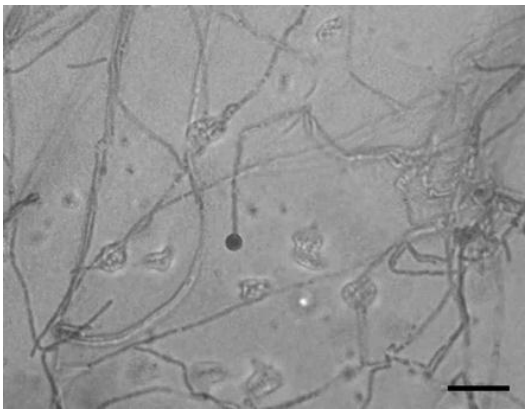
Cytological photomicrograph of a cytocentrifuge fine needle aspirate specimen of the tibial lesion from the dog. Branching, septate, fungal hyphae with parallel walls were initially thought to be compatible with an Aspergillus species (Wright's stain)., Photomicrograph of the adrenal gland illustrating fungal hyphae disseminated throughout the gland (Gomori methenamine silver staining). **Brockus *et al.* (2009)**

Miller *et al.* (2012) described the isolation of the fungus ***Oxyporus corticola*** from multiple lymphocutaneous tissues of a Beagle dog. Until recently, this fungus had not been reported in the human or veterinary medical literature as a cause of animal disease. A single previous report also involved infection in a German Shepherd Dog,

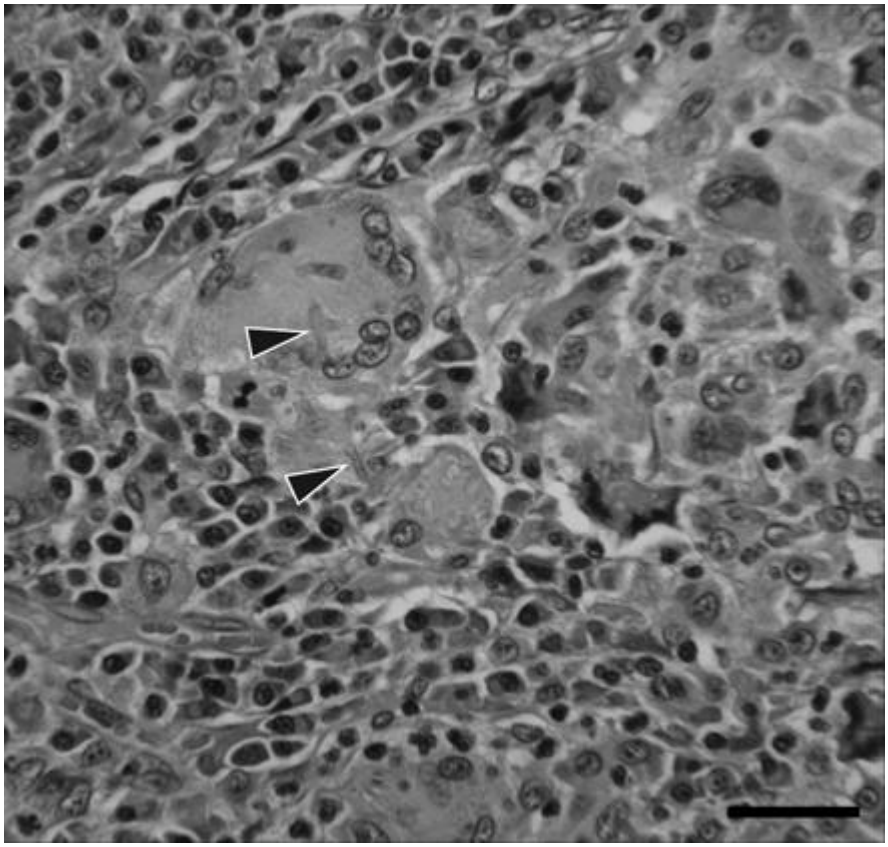
a breed with reported increased susceptibility to disseminated fungal infection and dysfunctional immune response. Isolates were non-sporulating and required molecular identification methods for prompt differentiation from other fungal pathogens. Risk factors for infection with O. corticola are unknown.



Oxyporus corticola isolate lacking aerial mycelia when grown on blood agar incubated at 35°C (7 days). *Oxyporus corticola* isolate on inhibitory mold agar incubated at 30°C (31 days) **Miller *et al.* (2012)**



Microscopic appearance of *Oxyporus corticola* isolate from inhibitory mold agar incubated at 30°C (31 days) showing a rare spore-like structure. Lactophenol cotton blue. Bar = 37 µm **Miller *et al.* (2012)**



Hematoxylin and eosin–stained histopathology slide of lymph node biopsy showing macrophage with associated hyphal elements (arrows). Bar = 112 µm **Miller *et al.* (2012)**

References:

1. Brockus CW, Myers RK, Crandell JM, Sutton DA, Wickes BL, Nakasone KK. Disseminated Oxyporus corticola infection in a German shepherd dog. Med Mycol. 2009 Dec;47(8):862-8.
2. Miller SA, Roth-Johnson L, Kania SA, Bemis DA. Isolation and sequence-based identification of Oxyporus corticola from a dog with generalized lymphadenopathy. J Vet Diagn Invest. 2012 Jan;24(1):178-81.

13. Acremoniosis

Simpson *et al.* (1993) examined a 4-year-old female German Shepherd dog to determine the cause of ataxia, progressive head tilt, anorexia, lethargy, and weight loss of 3 weeks' duration. A vestibular syndrome, generalized lymphadenopathy, bilateral uveitis, and chorioretinitis with complete detachment of the left retina were detected. Abnormal clinicopathologic findings were isosthenuria and hyperglobulinemia. The non-functional left eye was enucleated and fungal organisms resembling Aspergillus spp were identified on histologic examination. Microbial culture of a urine sample yielded **Acremonium sp,** which was initially considered a contaminant. The dog was considered to have systemic aspergillosis and was treated

with itraconazole for 7 months, until it was euthanatized because of persistent vomiting and anorexia. Postmortem examination revealed multisystemic pyogranulomatous and necrotizing inflammation of the myocardium, pericardium, liver, and kidneys; and granulomatous splenitis, lymphadenitis, retinitis, endometritis, and meningoencephalitis. Fungal culture of affected organs yielded Acremonium sp. These findings indicated that Acremonium spp can be pathogenic and should not be ignored when cultured.

Reference:

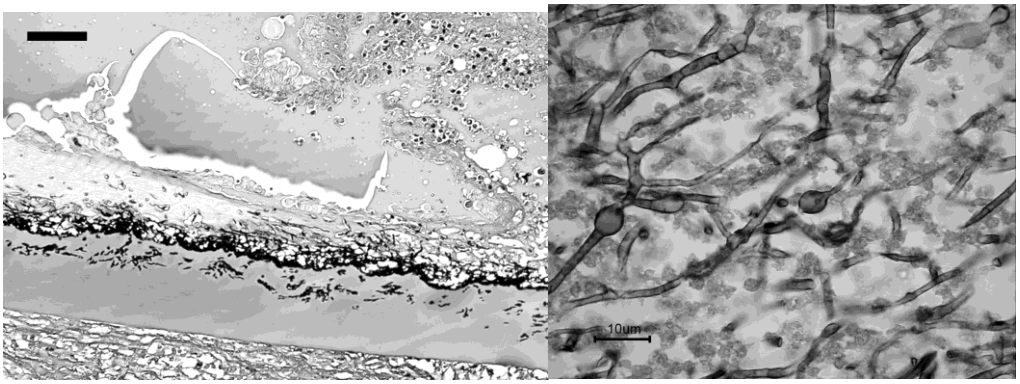
1. Simpson KW, Khan KN, Podell M, Johnson SE, Wilkie DA. **Systemic mycosis caused by Acremonium sp in a dog.** J Am Vet Med Assoc. 1993 Nov 1;203(9):1296-9.

14. *Geosmithiosis* in cats and dogs

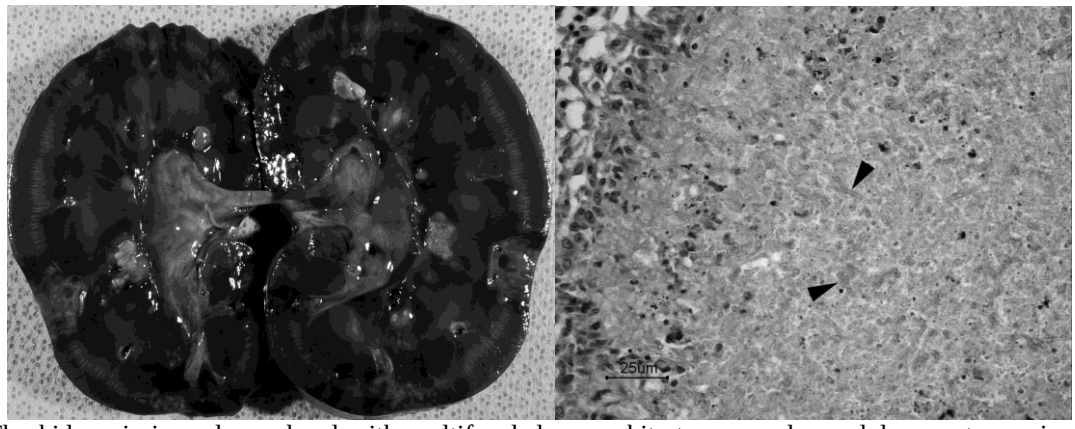
The genus *Geosmithia* currently contains numerous species formerly classified as *Penicillium*. *Geosmithia argillacea* (Stolk, H.C. Evans & T. Nilsson) , was originally described as a new thermotolerant *Penicillium* species by Stolk *et al*. who isolated the type strain from a high-temperature mine waste tip in 1969 21. In 1979 Pitt erected the genus *Geosmithia* to distinguish isolates previously known as *Penicillium* spp. but which formed conidia borne as cylinders from cylindrical, rough-walled phialides lacking narrow necks, as in *Penicillium* and *Paecilomyces*, and that produced conidia that were not typically some shade of green.

Report:

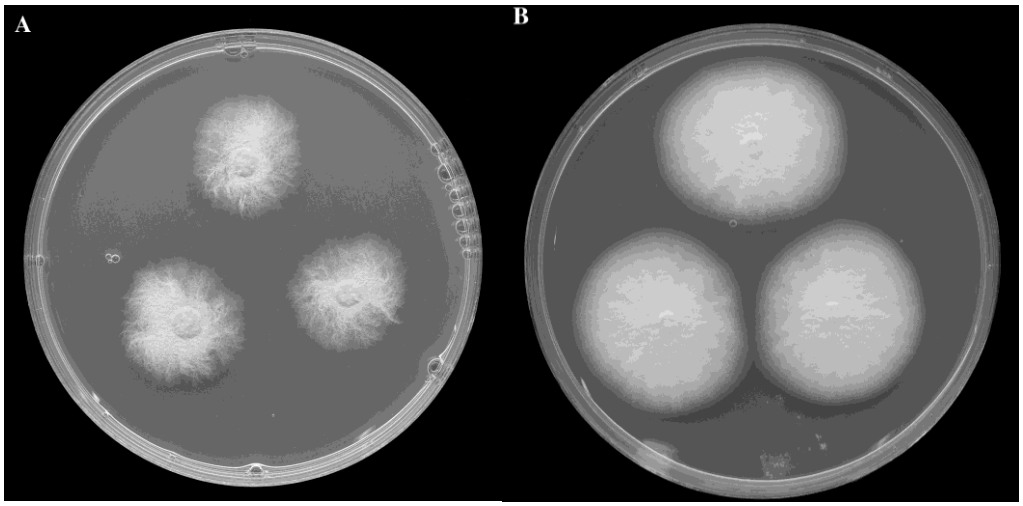
Grant *et al.* (2009) reported a systemic mycosis in a German Shepherd dog caused by ***Geosmithia argillacea***. Although this etiologic agent microscopically resembles a Penicillium species, and is histopathologically compatible with members of the genus Aspergillus, morphologic features and molecular characterization clearly separate it from these genera. This appears to be the first report of disseminated disease by this species in humans or animals. In vitro antifungal susceptibility testing suggests resistance to amphotericin B and voriconazole and susceptibility to caspofungin, itraconazole, and posaconazole.



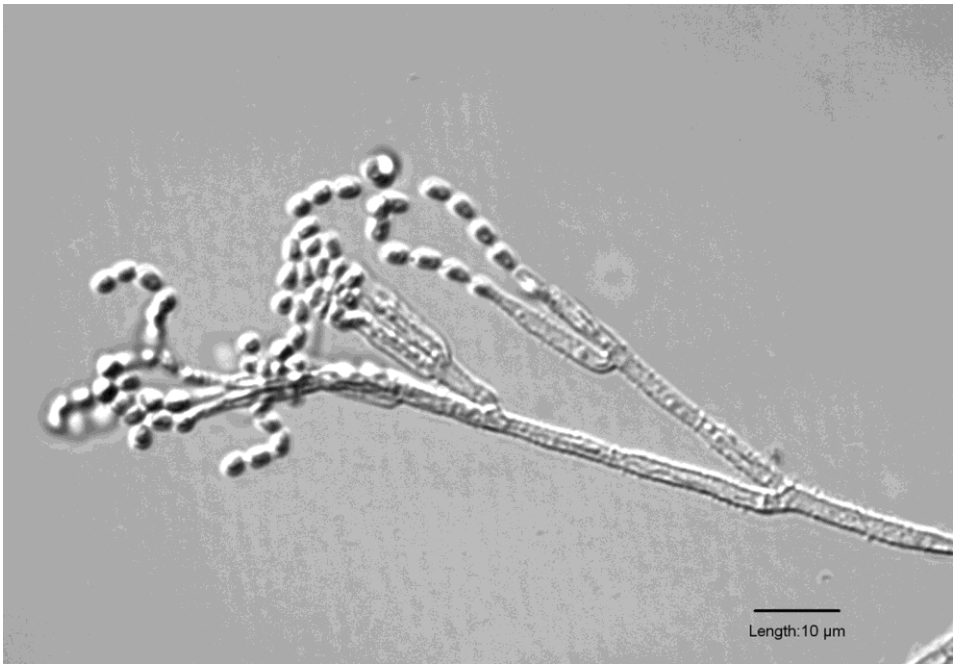
GMS stain, eye, (bar equals 50 microns). Multiple septate hyphae invading the anterior lens capsule and lens cortical material. GMS stain, sternebra, (bar equals 10 microns). Multiple septate hyphae with bulbous endings are dispersed throughout. **Grant *et al.* (2009)**



The kidney is irregular and red with multifocal, large, white-tan, granular nodules most prominent along the renal pelvis. There is a wedge shaped pale area extending from the cortex to the medulla consistent with an infarct. H&E, kidney (bar equals 25 microns). The centers of granulomas are necrotic and contain poorly staining septate, dichotomous branching fungal hyphae (arrowheads) with bulbous endings. **Grant *et al.* (2009)**



Macroscopic morphology of *Geosmithia argillacea* on malt extract agar. (A) 16 days at 23°C. (B) 8 days at 35°C.



Microscopic morphology of *Geosmithia argillacea* demonstrating branching stipes, monoverticillate and asymmetric biverticillate penicilli, cylindrical and appressed phialides, and smooth, hyaline, cuniform to ellipsoidal conidia borne in long, columnar chains. Roughened stipes, metulae, and phialides are a distinctive microscopic feature of this species (bar equals 10 microns), **Grant *et al.* (2009)**

Reference

1. Grant DC, Sutton DA, Sandberg CA, Tyler RD Jr, Thompson EH, Romanelli AM, Wickes BL. Disseminated Geosmithia argillacea infection in a German shepherd dog. Med. Mycol. 47:221–226

D. Diseases in cats and dogs caused by diphasic fungi

1. Blastomycosis in cats and dogs

1.1. Introduction

Blastomycosis is a systemic fungal infection caused by the dimorphic fungus *Blastomyces dermatitidis*, which has a relatively wide distribution in North America, including the Mississippi, Missouri, and Ohio river valleys; the Middle Atlantic states; southern Saskatchewan; Manitoba; Quebec; and Ontario. Blastomycosis is most commonly diagnosed in 2- to 4-year-old intact male large-breed dogs living in endemic regions, as they have a greater tendency to roam and to sniff and dig in the soil, resulting in greater exposure to the organism. Sporting dogs and hound breeds are predisposed, most likely because of increased exposure to high-risk areas during hunting. Residence near a river or lake and access to recently excavated sites have been demonstrated to increase the risk of infection. Most cases of

canine blastomycosis are diagnosed in late summer or early fall (**Harasen and Randall 1986; Rudmann et al.,1992;. Arceneaux et al., 1998;.Kerl 2003; Baumgardner et al., 1995; Baumgardner et al., 2005;Brömel and Sykes, 2005;Legendre, 2006**).

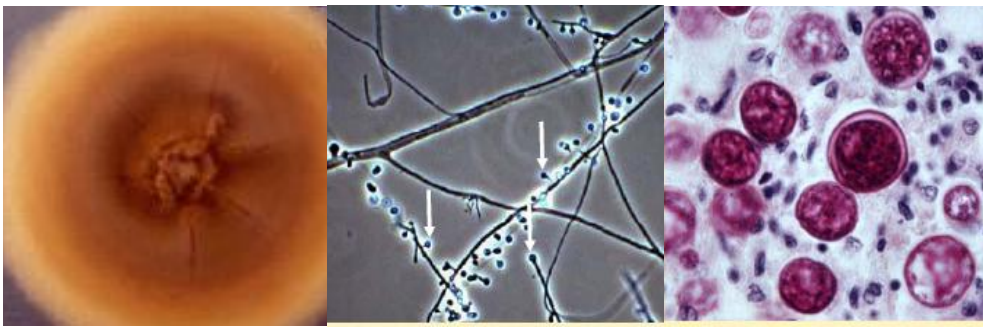
Infection most commonly occurs after inhaling spores from contaminated soil. At normal canine body temperature, the organism transforms to a yeast that can infect the lungs and spread systemically. Although infection almost always begins in the lungs before being disseminated through hematogenous or lymphatic routes to other body tissues, lung lesions occasionally resolve by the time infection in other sites becomes apparent. The most common sites of clinically apparent infection in dogs include the lungs, lymph nodes, eyes, skin, and bone. Subclinical or spontaneously resolving infection is uncommon.

1.2. Aetiology:

Blastomyces dermatitidis GILCHRIST et STOCKES 1898

Synonyms: *Oidium dermatitidis* RICKETTS 1901-
Cryptococcus gilchrisii VUILLEMIN 1902-
Zymonema gilchrisii BEUREMANN et GOUGEGOT 1901
Glenospora gammeli POLACCI et NANNIZZI 1927-
Blastomycoides tulanensis CASTELLANI 1928-
Monosporium tulanensis AGOSTIN 1932
Perfect stage: *Ajellomyces dermatitidis* McDONOUGH et LEWIS 1968

B. dermatitidis is a thermically dimorphic fungus which is assumed to be a soil saprophyte in nature. It grows at room temperature as mould, developing glabrous, tan, non-conidiating colonies, or colonies with fluffy white mycelium. The colonies mature in 2 weeks and may attain dark brown colour on age. Microscopically, the mycelium consists of hyaline and septate hyphae which bear delicate conidiophores that carry on their tips round, oval or pear-shaped conidia. The fungus readily converts to the yeast phase when plated on blood agar and incubated at 37 C. The yeast colonies are wrinkled and folded, glabrous and tan or creamy in colour. Microscopically, the yeast cells are characterized by broad-based buds.



B. dermatitidis colony at 25^{o}C oval or pear-shaped conidia broad-based buds intissues

1.3. Diagnosis

1.3.1. Clinical signs

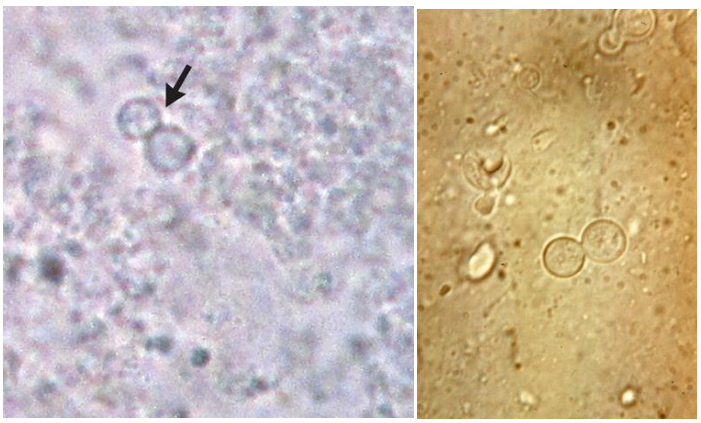
- Nonspecific signs of illness, including anorexia, weight loss, and lethargy, are common, and fever
- Involvement of the Lung pathology results in exercise intolerance, cough, tachypnea, cyanosis, or respiratory distress.
- Enlargement of one or more peripheral lymph nodes
- endophthalmitis, chorioretinitis, optic neuritis, serous or granulomatous retinal detachment, hyalitis and Panophthalmitis may also develop
- Dermatologic manifestations such as granulomatous proliferative mass-like lesions and ulcerated skin lesions draining serosanguineous or purulent fluid are most common, involving the nasal planum, face, and nail beds.
- Lameness may occur as a result of bone infections of the distal limbs.
- Symptoms resulting from involvement of internal organs like prostate, kidneys, testes, joints, nasal passages, and brain. straightforward.

1.3.2. Thoracic radiography

Diffuse miliary to nodular interstitial and bronchointerstitial pulmonary changes are most common Less often, lung lobe consolidation or a solitary mass within the lung parenchyma is identified

1.3.3. Direct microscopic examination

The diagnosis of blastomycosis can usually be confirmed by demonstration of the characteristic broad based budding organisms in exudates or aspirates from dermal lesions and fine-needle aspirates from enlarged, infected lymph nodes, transtracheal aspiration, bronchoalveolar lavage, and transthoracic lung aspiration, cerebrospinal fluid and vitreal aspirates and subretinal aspirates from infected eyes etc by KOH prep, cytology, or histology. Thick-walled spherical yeast cells, 8-30 microns may be detected in sputum,aspirates from cutaneous lesions or biopsy material, when examined directly by the microscope. The yeast cells have broad-based buds and the cytoplasm usually shrinks away from the wall.



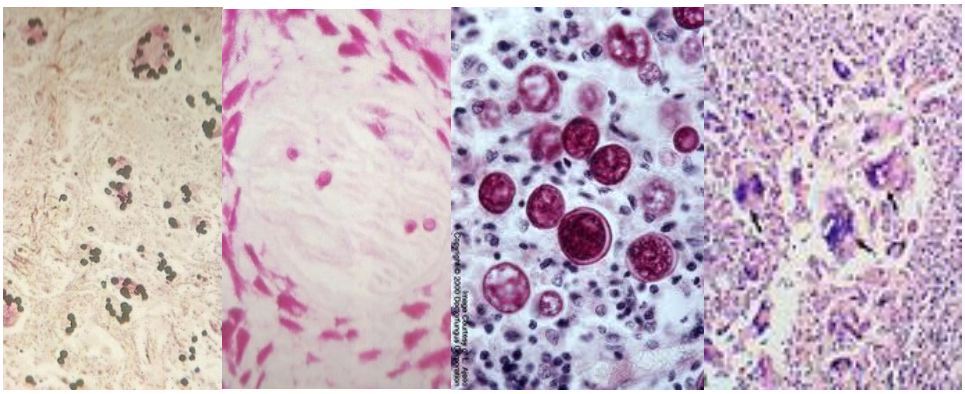
Cutaneous scrapings pus

1.3.4. Serological tests

- The agar gel immunodiffusion test for antibodies against the *Blastomyces* A antigen is the most commonly used serologic test
- Radioimmunoassay tests to detect serum antibodies against the WI-01 antigen
- Enzyme immunoassay test to detect *B. dermatitidis* antigen in urine
- ELISA
- PCR tests are used as supportive evidence to complete a clinical picture rather than as a sole method of diagnosis.

1.3.5. *Histopathology*

B. dermatitidis in tissue appears as yeasts, 8 to 15 µm in diameter, have thick refractile cell walls, and may show a single, broad-based bud. The thick refractile cell wall of this organism gives the appearance of a space between the fungal cell contents and the surrounding tissue when hematoxylin and eosin (H&E) stain is used. Inside the cell wall, the multiple nuclei of the yeast stain with hematoxylin. The contour of the yeast is best highlighted by staining the cell wall with fungal silver stains such as GMS or periodic acid-Schiff (PAS) stain. The inflammatory reaction accompanying the yeasts is primarily granulomatous with various degrees of neutrophilic infiltrate.



Lung biopsy,Gomory stain skin biopsy, PAS stain Broad-based budding cells Granuloma with suppuration

1.3.6. Isolation of the organism

Suspected material is plated on Sabouraud agar and blood agar and incubated at 25 and 37 C , respectively. Growth is quite slow and may need 2 months or more.

1.4. Treatment

1.4.1. Amphotericin B, The desoxycholate form of amphotericin B is most commonly administered as an intravenous infusion in doses of dose (0.5 mg/kg) diluted in 5% dextrose in water solution or 2.5% dextrose in 0.45% saline solution (500 ml for dogs < 20 kg; 1,000 ml for dogs >

20 kg).**It** may be administered during the initial treatment of dogs with severe or rapidly progressive blastomycosis to improve the rate of recovery.

1.4.2. Itraconazole is the azole most often recommended for treating dogs with blastomycosis since it is as effective as amphotericin B but is associated with fewer adverse effects and can be administered orally at a dose of 5 mg/kg orally twice a day for five days, followed by 5 mg/kg once daily, or divided twice daily, for the remainder of the treatment period.

1.4.3. Fluconazole (2.5 to 5 mg/kg orally or intravenously twice a day)

1.4.4. Voriconazole (5 to 10 mg/kg orally or intravenously twice a day)

1.5. PROGNOSIS

- Most dogs with brain involvement will die
- Dogs with severe diffuse pulmonary blastomycosis often deteriorate during the first two or three days of treatment

1.6. Reports

1.6.1. Reports on blastomycosis in dogs

Clinical

1. **Pulmonary blastomycosis (McGuire *et al,* 2002, Crews *et al.*, 2008a, Crews *et al.*, 2008b, McMillan and Taylor, 2008, Reed *et al.*, 2010))**
2. **Nasal blastomycosis (Wehner *et al.*, 2008, Parker *et al.*, 2013)**
3. **Ocular blastomycosis (Hendrix *et al.*, 2004, Baron *et al,* 2011))**
4. **Cardiovascular lesions (Schmiedt *et al.*, 2006**
5. **Central nervous system blastomycosis (Lipitz *et al.*, 2010. Hecht *et al,*. 2011, Bentley *et al.*, 2013)**
6. **Blastomycotic osteomyelitis/arthritis (Harasen, 2007, Whelen, 2008, Oshin *et al.*, 2009, Woods *et al.*,2013)**
7. **Blastomycotic prostatitis (Totten *et al,* 2011)**
8. **Blastomycotic mastitis (Ditmyer and** Craig, 2011)
9. **Systemic blastomycosis** with neurologic involvement **(Gaunt *et al.*, 2009**

Diagnosis

1. **Radiographic examination (Crews *et al.*, 2008a, Oshin *et al.*, 2009, Hecht *et al,*. 2011)**
2. **Enzyme-linked immunosorbent assay** (**Bono *et al.*, 1995, Fisher *et al.*, 1995, Fisher *et al.*, 1997, Wakamoto *et al.*, 1997a, Wakamoto *et al.*, 1997b, Shurley *et al.*, 2005, Sestero and Scalarone, 2006, Gaunt *et al.*, 2009, Boyd *et al.*, 2013, Mondada *et al.*, 2014, Mourning *et al.*, 2015)**
3. **Enzyme immunoassay** (EIA) **(Spector *et al.*, 2008, Foy *et al.*, 2014))**
4. **Agar gel immunodiffusion (AGID (Spector *et al.*, 2008, Mourning *et al.*, 2015)**
5. **Histological examination (Crews *et al.*, 2008b, Gaunt *et al.*, 2009,**
6. **PCR** assay (**Bialek *et al.*, 2003**
7. **Molecular typing (Anderson *et al.*, 2013**)

Treatment

1. **Itraconazole (Hendrix *et al.*, 2004, Finn *et al.*, 2007, Crews *et al.*, 2008b, Spector *et al.*, 2008, Wehner *et al.*, 2008, Whelen, 2008, Mazepa *et al.*, 2011, Totten *et al*, 2011, Parker *et al.*, 2013)**
2. **Fluconazole (Mazepa *et al.*, 2011, Totten *et al*, 2011)**
3. **Amphotericin B (Finn *et al.*, 2007, Oshin *et al.*, 2009)**
4. **Vaccine (Wüthrich *et al.*, 2011)**

Zoonotic aspects (MacDonald *et al.*, 2006, Herrmann *et al.*, 2011, Anderson *et al.*, 2013
Risk factors of canine blastomycosis (Chen *et al.*, 2008

Bono *et al.* (1995) prepared a ***Blastomyces dermatitidis*** (dog isolate T-58) yeast phase lysate **antigen**, which was then concentrated and separated by Rotofor preparative isoelectric focusing cell (Bio-Rad). The pH values of the fractions were determined and equilibrated to pH 7.2 and then analysed by **enzyme-linked immunosorbent assay** using horseradish peroxidase enzyme system against serum specimens from dogs with blastomycosis, histoplasmosis, aspergillosis, and coccidioidomycosis. The results showed a peak absorbance at pH 3.89-4.31 (fractions 4 and 5) with the blastomycosis serum specimens. This was a single sharp peak while the rest of the fractions were lower. In contrast the sera from dogs with histoplasmosis showed a peak absorbance at pH 5.54-5.97 (fractions 9 and 10), while the other mycoses showed patterns that did not resemble the blastomycosis or histoplasmosis specimens. Serum specimens from dogs with blastomycosis being treated with itraconazole were also assayed (pre-treatment and 1, 2, 3, and 12 months post-treatment sera). The characteristic peak for blastomycosis was observed and a decrease in the peak was seen as the treatment progressed. Fractions 3-12 were also used to detect delayed dermal hypersensitivity in hyperimmunized hairless guinea-pigs. Fraction 5 (pH 4.31) elicited the optimal response in B. dermatitidis-immunized animals, while no cross-reactivity was observed in guinea-pigs sensitized with Histoplasma capsulatum killed cells.

Fisher *et al.* (1995) prepared ***Blastomyces dermatitidis*** yeast lysate **antigen** (T-58, dog isolate) fractions using the Rotofor preparative isoelectric focusing (IEF) cell (Bio-Rad) and compared them with B. dermatitidis yeast lysate and filtrate reagents with respect to the detection of antibodies in sera from dogs with blastomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis and aspergillosis. A horseradish **peroxidase enzyme immunoassay** with Turbo TMB substrate was used in the study. One particular IEF fraction (pH 4.3) was optimal in the assay, and it exhibited greater sensitivity (100%) and specificity (93%) than the lysate or filtrate preparations. The highest degree of cross-reactivity was encountered with the histoplasmosis and coccidioidomycosis specimens and considerably less with the cryptococcosis and aspergillosis sera. Studies are in progress to purify further the optimal IEF fraction.

Fisher *et al.* (1997) analyzed fractions of a ***Blastomyces dermatitidis*** yeast lysate **antigen** for the presence of glycoproteins that may lead to cross-reactivity in immunoassays for the diagnosis of blastomycosis. Five major glycoproteins were apparent, two of which showed cross-reactivity when used in Western blots with sera obtained from dogs with histoplasmosis and coccidioidomycosis. These five glycoproteins were characterized for linkage to the proteins using N-glycosidase F (NGF) and for their lectin binding properties. The cross-reactive 235- and 160-kDa

glycoproteins were found to possess mainly O-linked, high-mannose-type carbohydrates, and periodate-mediated oxidation of these molecules eliminated cross-reactivity observed with heterologous sera. Thus, the periodate-treated IEF antigens described here may be useful in solid-phase enzyme immunoassays for the diagnosis of blastomycosis.

Wakamoto *et al.* (1997a) prepared yeast-phase lysate antigens from 10 different isolates of ***Blastomyces dermatitidis***. Comparative studies were performed using the lysate antigens in an enzyme-linked immunosorbent assay **(ELISA)** for the detection of antibodies in sera from dogs with blastomycosis andhistoplasmosis. In order to evaluate the ability of the lysate reagents to elicit delayed dermal hypersensitivity (DTH) responses, the lysates were compared as **skin-testing antigens** in hairless guinea pigs that were previously sensitized with B. dermatitidis or Histoplasma capsulatum killed whole yeast cells. All ten of the lysate reagents were able to detect antibody with the ELISA in the serum specimens from dogs with blastomycosis (absorbance values ranged from 0.184 to 0.272; mean value 0.235). In contrast, when the lysates were assayed against sera from dogs withhistoplasmosis, the absorbance values ranged from 0.053 to 0.151, with a mean value of 0.092. All ten lysate antigens were able to elicit a DTH response in the B. dermatitidis-immunized animals (mean axes of induration values ranged from 7.0 to 14.4 mm; mean value 8.6 mm). On the other hand, only minimal reactivity was evidenced in the guinea pigs immunized with H. capsulatum (mean axes of induration values ranged from 0.8 to 2.9 mm; mean value 1.8 mm).

Wakamoto *et al.* (1997b) performed comparative evaluations to assess the stability, sensitivity and specificity of eight lots of yeast lysate antigen prepared from a ***Blastomyces dermatitidis*** dog isolate (T-58). These antigens were prepared during the period from 1989 to 1995. The lysates were used in an **ELISA** for the detection of antibodies in serum specimens from dogs with blastomycosis and histoplasmosis. In order to evaluate the ability of the lysates to elicit delayed dermal hypersensitivity (DTH) responses, they were compared as **skin-testing antigens** in guinea pigs that were previously sensitized with B. dermatitidis or Histoplasma capsulatum killed whole yeast cells. All 8 of the lots of antigen detected antibody in the sera from dogs with blastomycosis (absorbance values ranged from 0.432 to 0.543; mean value of 0.508). The absorbance values ranged from 0.283 to 0.439 (mean value of 0.326) when the lysates were assayed against sera from dogs with histoplasmosis. All of the antigens were able to elicit a DTH response in B. dermatitidis immunized animals (mean axes of induration values ranged from 10.5 mm to 12.5 mm; mean value of 11.6 mm). In contrast, only minimal cross-reactivity was evidenced in the guinea pigs immunized with H. capsulatum (mean axes of induration values ranged from 0 to 4.5 mm; mean value of induration of 1.7 mm).

Bateman (2002) diagnosed systemic blastomycosis in a 5-year-old German shepherd histologically at necropsy. Diagnosis and treatment were difficult due to unusual neurological symptoms, the absence of abnormalities on diagnostic tests, and the advanced stage of the disease at presentation.

McGuire *et al.* (2002) presented an 8-year-old, male castrated golden retriever with cough and increased respiratory effort. Radiographs revealed an alveolar pattern in the right caudal lung lobe and an opacity at the carina suspected to be enlarged tracheobronchial lymph nodes. The disease progressed to involve the right middle lung lobe. Cytopathology of a fine-needle aspirate and bronchoalveolar lavage fluid

were non-diagnostic. Surgical removal of the right caudal lung lobe and biopsy of the perihilar lymph nodes revealed **pulmonary thromboembolism** and reactive lymph nodes. The dog died several days postoperatively, and necropsy revealed diffuse pulmonary thromboembolism. Additionally, ***Blastomyces dermatitis*** organisms were identified in a pyogranulomatous mass surrounding the trachea near the carina.

Bialek *et al.* (2003) developed a ***Blastomyces dermatitidis*** nested **PCR** assay targeting the gene encoding the Wisconsin 1 (WI-1) adhesin and compared it with a nested PCR targeting the 18S rRNA gene (rDNA) of members of the family ONYGENACEAE. They examined 73 paraffin-embedded tissue samples obtained from nine dogs which died of blastomycosis and nine dogs which succumbed to lymphosarcoma according to autopsy findings; amplifiable canine DNA was extracted from 25 and 33 specimens from the two groups, respectively. The B. dermatitidis PCR amplified DNA from 8 of 13 tissue samples in which yeast cells were detected by microscopy. Sequencing revealed that all PCR products were homologous to the B. dermatitidis WI-1 adhesin gene. No PCR product was amplified from 12 microscopically negative biopsy specimens from dogs with blastomycosis or from 33 biopsy specimens from dogs with lymphosarcoma. The 18S rDNA PCR amplified DNA from 10 and 9 tissue samples taken from dogs which died of blastomycosis and lymphosarcoma, respectively. Only six products were identified as being identical to B. dermatitidis 18S rDNA; they were exclusively obtained from specimens positive by the B. dermatitidis nested PCR. For specificity testing, 20 human biopsy specimens proven to have histoplasmosis were examined, and a specific H. capsulatum product was amplified by the 18S rDNA PCR from all specimens, whereas no product was obtained from any of the 20 samples by the B. dermatitidis PCR assay. In conclusion, the PCR targeting a gene encoding the unique WI-1 adhesin was proved to be as sensitive as but more specific than the PCR targeting the 18S rDNA for detection of B. dermatitidis in canine tissue.

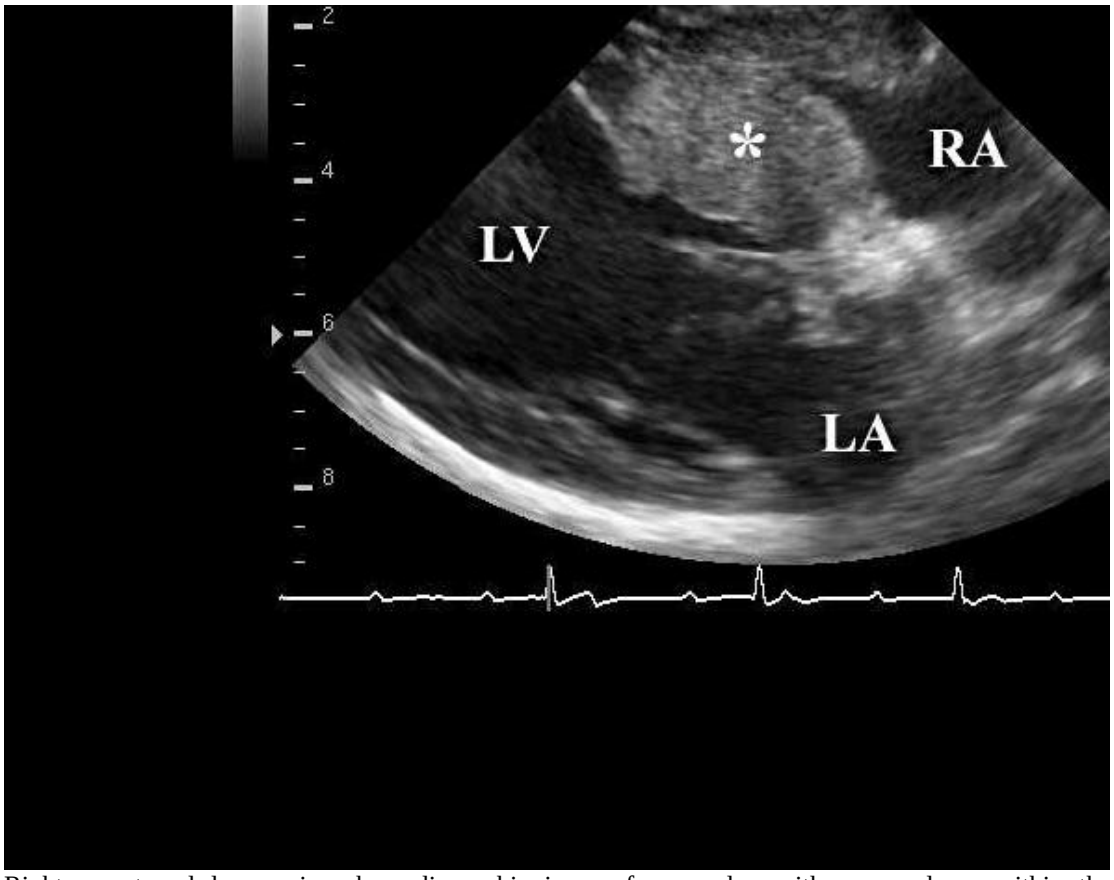
Hendrix *et al.* (2004) performed a retrospective study to compare prevalence of organisms and histologic changes in eyes from 36 dogs with **endophthalmitis** associated with blastomycosis that were either untreated or undergoing treatment with **itraconazol**e. Signalment, results of ophthalmic examination, and duration of treatment with itraconazole were extracted from medical records. Histologic sections from eyes were examined for prevalence and viability (ie, budding) of fungal organisms. A scoring system was devised to assess the degree of inflammation. Clinically, all eyes were blind and had signs of severe endophthalmitis. Histologically, the type and degree of inflammation and prevalence of ***Blastomyces dermatitidis*** were not significantly different between dogs treated with itraconazole and untreated dogs or among groups of dogs treated for different time periods (4 to 14, 15 to 28, or 29 to 72 days). Replication of the organisms in vascular tissues as well as avascular spaces in the eyes was similar in treated and untreated dogs. Lens rupture was seen in 12 of 29 (41%) eyes.

Shurley *et al.* (2005) used a competitive binding inhibition enzyme linked immunosorbent assay (**ELISA**) to detect ***Blastomyces dermatitidis*** antigens in urine specimens from dogs with blastomycosis. Sera from rabbits immunized with B. dermatitidis killed whole yeast cells were used as the primary antibody in the competitive ELISA. This initial study was performed to determine if B. dermatitidis antigen detection was possible and to test the efficacy of the rabbit sera as a primary antibody. An indirect ELISA was also performed to compare antigen detection in urine to antibody detection in the sera of the infected dogs. The results indicate 100% (36/36 specimens) detection of both antigen and antibody. Cross reactivity with

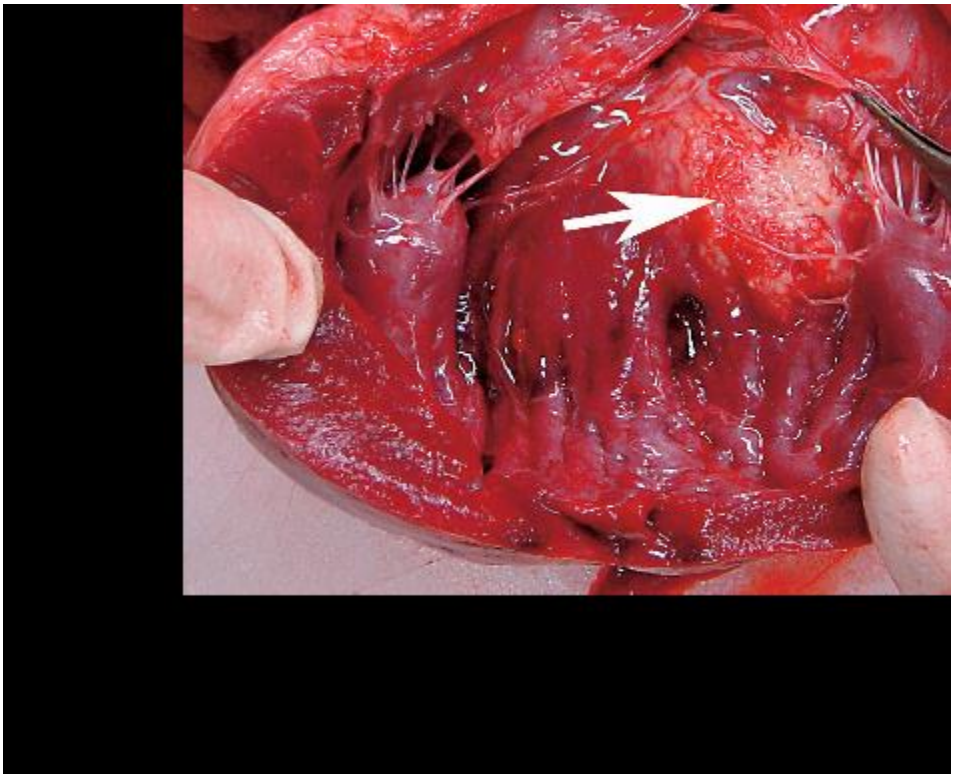
Histoplasma capsulatum, as well as non-specific binding with the normal urine specimens, was observed with the competitive binding inhibition ELISA.

MacDonald *et al.* (2006) investigated a cluster of blastomycosis in 8 humans and 4 dogs in a rural North Carolina community. Delayed diagnosis, difficulty isolating Blastomyces dermatitidis in nature, and lack of a sensitive and specific test to assess exposure make outbreaks of this disease difficult to study

Schmiedt *et al.* (2006) identified dogs with cardiovascular lesions caused by blastomycosis from retrospective evaluation of medical records. Five dogs had de novo infections and 3 had recurrences of previously treated infections. Harsh labored breathing, lethargy, and anorexia were the most common historic complaints. Three dogs had syncope. Physical examination and clinicopathologic data were typical of blastomycosis and included dyspnea, increased lung sounds, and lethargy. In addition, 3 dogs had heart murmurs and 1 had a third-degree atrioventricular block. Four dogs had myocarditis and 2 had pericarditis or epicarditis. Two dogs had cardiac signs attributed to extracardiac compression by fungal granulomas and clinical signs were relieved by treatment. Half of the remaining 6 dogs were euthanized; 2 of these were not treated. Of the remaining 3 dogs, 1 dog died acutely while sleeping; the second died intraoperatively during an attempt to place an epicardial pacemaker; and the third had **Blastomyces-induced endocarditis** and died of heart failure.



Right parasternal long axis echocardiographic image from a dog with a granuloma within the interventricular septum due to Blastomyces dermatitidis infection. Asterisk indicates the focal thickening of the interventricular septum at the level of the lesion. LA, left atrium; LV, left ventricle; RA, right atrium. **Schmiedt *et al.* (2006)**



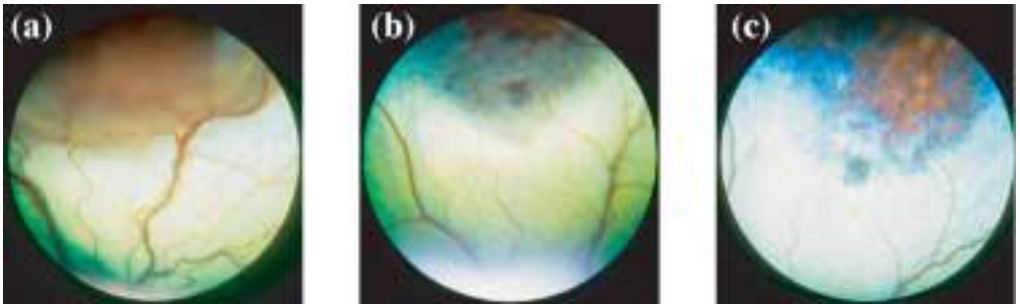
Postmortem specimen from the same dog. The left ventricle is opened and the interventricular septal granuloma is indicated by the arrow **Schmiedt *et al.* (2006)**

Sestero and Scalarone (2006) carrrried out a study to compare the efficacy of eight ***Blastomyces dermatitidis*** yeast phase lysate **antigens** (T-58: dog, Tennessee; T-27: polar bear, Tennessee; ERC-2: dog, Wisconsin; B5894: human, Minnesota; SOIL: soil, Canada; B5896: human, Minnesota; 48089: human, Zaire; 48938: bat, India) in the detection of the immunoglobulins IgG and IgM in serum specimens from canines with blastomycosis. An indirect enzyme-linked immunosorbent assay (**ELISA**, peroxidase system) was used to analyze sera collected during four different intervals post-infection. The yeast lysate antigen 48938 was a reactive antigen for the detection of both IgG (mean absorbance value range: 1.198-2.934) and IgM (mean absorbance value range: 0.505-0.845). For the same sera, antigen T-27 was also effective in the detection of IgG (mean absorbance value range: 0.904-3.356) and antigen 48089 was useful for the detection of IgM (mean absorbance value range: 0.377-0.554). The yeast lysate antigen B5894 proved to be a poor antigen for the detection of both IgG and IgM (mean absorbance value ranges: 0.310-0.744 for IgG, 0.025-0.069 for IgM). Inherent variations in yeast lysate antigens such as these may be utilized to develop improved immunoassay procedures for the specific detection of IgG or IgM in cases of blastomycosis.

Crews *et al.* (2007) performed a retrospective study to determine blood ionized calcium (iCa) and serum total calcium (tCa) concentrations in dogs with blastomycosis and to evaluate whether serum tCa concentration, albumin-adjusted serum calcium concentration (AdjCa-Alb), and total protein-adjusted serum calcium concentration (AdjCa-TP) accurately predict iCa status. The study covered 38 client-owned dogs with a cytologic diagnosis of blastomycosis. Dogs were classified as hypocalcemic, normocalcemic, or hypercalcemic on the basis of blood iCa concentration, serum tCa concentration, AdjCa-Alb, and AdjCa-TP; classification on the basis of serum tCa concentration, AdjCa-Alb, and AdjCa-TP was

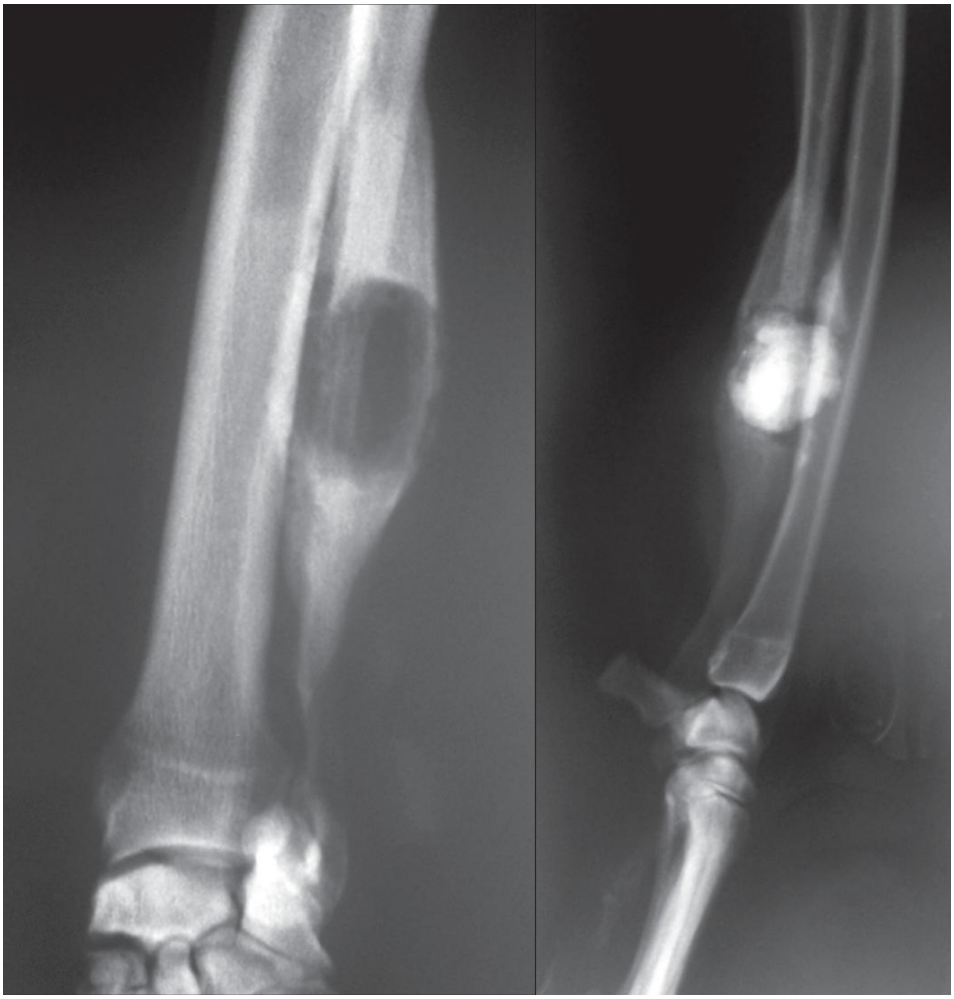
compared with blood iCa concentration. Except for 2 hypercalcemic dogs, all dogs had blood iCa concentrations within the reference interval. Use of serum tCa concentration overestimated hypocalcemia in 57.9% (22/38) of dogs and underestimated hypercalcemia in 1 dog. Use of AdjCa-Alb correctly reclassified all dogsas normocalcemic that were classified as hypocalcemic on the basis of serum tCa concentration, but failed to predict hypercalcemia in 1 dog. Use of AdjCa-TP correctly reclassified all but 2 dogs as normocalcemic that were classified as hypocalcemic on the basis of serum tCa concentration, and failed to predict hypercalcemia in 1 dog. No correlation was found between blood iCa concentration and serum concentrations of tCa, total protein, and albumin; AdjCa-Alb; or AdjCa-TP. It was concluded that high blood iCa concentration was uncommon in dogs with blastomycosis. Hypoalbuminemia contributed to a low serum tCa concentration despite a blood iCa concentration within reference limits. The use of serum tCa concentration, AdjCa-Alb, and AdjCa-TP may fail to identify a small number of dogs with high blood iCa concentrations.

Finn *et al.* (2007) carried out a retrospective study to evaluate the success of the use of systemic corticosteroids and antifungal medications in the treatment of dogs with ocular lesions associated with systemic blastomycosis. Medical records of 25 dogs diagnosed with blastomycosis, via either cytology or histopathology, at the Purdue University Veterinary Teaching Hospital between 1 January 2000 and 1 January 2005, were reviewed. Data collected from the medical records included signalment, presence and progression of ocular lesions, antifungal drugs administered, oral and topical corticosteroid administration, length of follow-up, response to treatment, and visual outcome. Of the 25 cases reviewed, 12 dogs (19 eyes) with follow-up information were found to have lesions consistent with **ocular blastomycosis**. Length of follow-up in the 12 cases ranged from 1 month to 31 months with a mean of 9 months. Antifungal therapy for all cases consisted of oral **itraconazole** (5 mg/kg every 24 h) initially. In seven cases, the antifungal drug administered was changed from itraconazole to oral fluconazole. Two of these also received intravenous **amphotericin B**, and two received additional treatment with itraconazole. All 12 dogs also received oral prednisone. The dose of oral prednisone utilized ranged from 0.2 mg/kg/day to 1.4 mg/kg/day with a mean of 0.7 mg/kg/day; the duration of oral prednisone administration ranged from 2 weeks to 8.5 months with a mean of 3 months. Topical prednisolone was a component of the treatment of 16 of the 19 eyes. Duration of topical prednisolone treatment ranged from 1 month to 30 months with a mean of 5 months. Lesions not located in the eyes exhibited a positive response to treatment in 11 (92%) of the 12 dogs. Overall, 14/19 (74%) affected eyes were visual at the time of their final recheck. All eyes with mild or moderate lesions and 5/10 (50%) severely affected eyes were visual at their last recorded recheck examination. The administration of systemic corticosteroids did not appear to adversely affect the survival rate and might have played a role in preservation of vision in a majority of dogs in this group with ocular blastomycosis.



Fundus photograph from a 5-year-old, neutered male Boxer diagnosed with blastomycosis. Lung, bone and skin lesions were present in addition to the fundic lesion depicted here. (a) At presentation a subretinal granuloma and associated serous retinal detachment affecting approximately 30% of the fundus. (b) After 2 weeks of treatment with systemic itraconazole and systemic prednisone the retinal detachment has flattened and the granuloma has decreased in size. (c) After an additional 6 weeks of therapy the granuloma has further decreased in size and the lesion is becoming a chorioretinal scar. **Finn *et al.* (2007)**

Harasen (2007) described a lytic lesion in the distal region of the ulna of a 2-year-old spayed female boxer boxer presented with a 3-week history of right front lameness. The owner had noticed a firm swelling on the lateral aspect of the distal part of the ante-brachium, just proximal to the radiocarpal joint. Radiographs of the limb showed an oval area of bone lysis in the distal part of the ulna approximately 5 cm long by 2 cm wide. The lesion was surgically curetted and samples from it submitted for histopathologic analysis. The remaining defect was filled with a combination of autogenous cancellous bone from the proximal part of the ipsilateral humerus and a silica-based synthetic particulate bone substitute. The histopathologic diagnosis on the curetted material was **pyogranulomatous osteomyelitis** due to ***Blastomyces dermatitidis***. The owner declined treatment options and the dog was euthanized.



A lytic lesion in the distal region of the ulna of a 2-year-old boxer caused by *Blastomycosis osteomyelitis.* Postoperative view of the lesion after surgical curettage and packing with autogenous cancellous and synthetic graft material. **Harasen (2007)**

Chen *et al.* (2008) investigated environmental and host risk factors of canine blastomycosis in Knox County, Tennessee, USA. Data on 78 cases and 146 randomly selected controls were extracted from the medical database at the University of Tennessee Veterinary Teaching Hospital for the period 1977-1999. Home addresses of cases and controls were geocoded and linked to environmental risk factor data using a Geographic Information System (GIS) software. Multiple logistic regression analysis was performed to identify environmental and host factors associated with risk of canine blastomycosis. Important risk factors in the study area were sex, breed, age, and proximity to water whereas, soil type, pH, and organic matter content had no significant associations with blastomycosis risk in this study area. Males were 2.7 times (OR =2.7; 95% CI =1.3, 5.3) more likely to have blastomycosis than females. Blastomycosis risk was also higher in working (OR =4.6; 95% CI =1.5, 14.0) and sporting dogs (OR =6.2; 95% CI =2.4, 16.0) than other breeds. Disease risk was highest in 2-4-year-old dogs (OR =11.6; 95% CI =4.6, 29.1) and increased among dogs living near water bodies. Blastomycosis control strategies need to be designed with knowledge of the important risk factors in effect at the geographic location of interest.

Crews *et al.* (2008a) carried out a retrospective to identify radiographic patterns in 125 dogs with pulmonary blastomycosis and radiographic factors associated with

outcome. Medical records were reviewed, and for each lung lobe, the primary radiographic pattern and percentage of lobar involvement at the time of initial examination were recorded. 79 dogs survived, 38 died, and 8 were euthanized without treatment. The initial radiographic pattern was variable and not significantly associated with outcome. Mean half-time for radiographic resolution of pulmonary infiltrates was 41.4 days for all patterns except masses, for which mean half-time to resolution was 90.8 days. Transient radiographic worsening was seen in 20 of 87 (23%) dogs but was not associated with a poor prognosis. Pulmonary bullae were seen in 20 (16%) dogs, most often in association with an alveolar pattern. Accuracy of using percentage of right caudal lung lobe involvement (<or= 20% vs > 20%) to predict outcome was 74.4%; accuracy of using number of affected lobes (< 4 vs >or= 4) to predict outcome was 65.8%. Results suggested that a non-uniform distribution of pulmonary infiltrates was equally as likely as a diffuse nodular interstitial pattern in dogs with pulmonary blastomycosis. On the basis of half-time for resolution of pulmonary infiltrates, follow-up radiography should be performed no more often than every 4 to 6 weeks in clinically stable patients. Transient radiographic worsening that occurred during the initial weeks of treatment was not associated with a poorer prognosis.

Crews *et al.* (2008b) carried out a retrospective study to compare results of the most common diagnostic tests for **pulmonary blastomycosis** in dogs, identify factors associated with outcome, and determine response to various antifungal treatment protocols on125 dogs with pulmonary blastomycosis. Medical records were reviewed, and information was obtained regarding diagnostic methods, results of routine laboratory testing, and radiographic response to antifungal treatment. 79 dogs survived, 38 died, and 8 were euthanized. Transthoracic fine-needle aspiration and transtracheal lavage were the most common diagnostic methods. Results of an agar gel immunodiffusion test for antibodies against Blastomyces dermatitidis were negative in 12 of 24 (50%) dogs. Only 3 of 94 (3.2%) dogs in which cytologic or histologic examination or bacterial culture of pulmonary samples were performed had any evidence of concurrent bacterial infection. The half-time for radiographic resolution of pulmonary infiltrates did not vary significantly with antifungal treatment, and use of a loading dosage of **itraconazole** was not associated with significant improvements in outcome or time to disease resolution.Dogs that died had a higher number of band neutrophils at initial examination, compared with those that survived.

McMillan and Taylor (2008) performed a retrospective study which identified B. dermatitidis organisms in 76% of samples when transtracheal aspiration was performed in 17 non-sedated dogs with pulmonary blastomycosis.

Spector *et al.* (2008) used an **enzyme immunoassay** (EIA) for detection of ***Blastomyces dermatitidis*** galactomannan antigen in body fluids has been used for rapid diagnosis of blastomycosis in dogs. Serum and urine samples from 46 dogs with confirmed blastomycosis were tested for Blastomyces antigen and serum was tested for anti-Blastomyces antibodies. The sensitivity for the detection of antigen in urine was 93.5% and it was 87.0% in serum. The sensitivity of antibody detection by agar gel immunodiffusion (AGID) was 17.4% and it was 76.1% by EIA. Antigen and antibody decreased during itraconazole treatment.

Wehner *et al.* (2008) examined a 2-year-old 38.9-kg (85.58-lb) sexually intact male German Shepherd Dog because of a 4-month history of severe nasal swelling and

nasal mucosa congestion. The signs were slowly progressive. Physical examination revealed that the dorsal aspect of the dog's nose was swollen and hard. Mucous membranes in both nostrils were hyperemic and edematous. Diagnostic investigation revealed severe **nasal osteolysis and pyogranulomatous rhinitis and nasopharyngitis** attributable to blastomycosis. Oral administration of **itraconazole** was initiated (5 mg/kg [2.27 mg/lb], q 12 h for 5 days and then q 24 h). After a treatment period of 3 months, the nose had regained its normal appearance. After 5 months of treatment, the Blastomyces infection was eliminated as confirmed by results of rhinoscopy and biopsy specimen examination. No relapse was evident within 1 year after discontinuation of treatment.

Whelen (2008) suggested a protocol for treatment of canine blastomycotic osteomyelitis as follows: itraconazole 5 mg/kg BW, PO, q12h for at least 1–2 wk (or until the animal shows signs of feeling better), then a maintenance dose of 5 mg/kg BW, PO, q24h for a minimum of another 90 d. Some animals will be fine with a shorter course of treatment, but some need a significantly longer period on the medication. He found that osteomyelitis responded very well to the treatment.

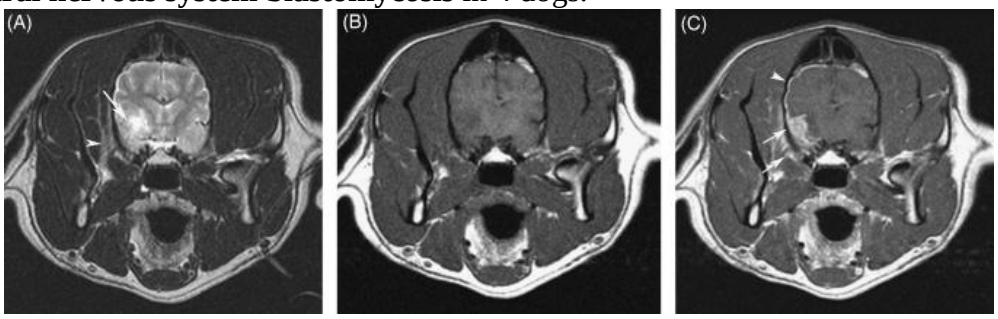
Gaunt *et al.* (2009) presented a 6-year-old spayed female, golden retriever from Kenora, Ontario for progressive neurologic dysfunction. Clinical examination suggested brainstem disease. Blastomycosis was diagnosed based on fine-needle aspiration cytology of a normal sized lymph node of the swollen mandibular region and a positive blastomycosis urine antigen test. On gross examination, a suppurative exudate was present in and around the pituitary gland and an approximately 1.0 cm in diameter focus of caseous necrosis was noted in the dorsolateral aspect of the left caudal lung lobe. Histological examination of the left caudal lung lobe revealed multiple, variably sized (0.1 to 0.5 mm), coalescing nodules comprised of an inflammatory infiltrate and intralesional yeast organisms, as described in the cerebrum. Inflammation and identical yeast organisms were identified on histopathological examination of the pituitary gland and retropharyngeal lymph node. The histopathological diagnosis was systemic blastomycosis. The previously submitted urine blastomyces antigen enzyme-linked immunosorbent assay (**ELISA**) test result was available 1 wk later, with a strong positive result. ***Blastomyces dermatitidis*** was isolated from material cultured from the pituitary gland. The case was diagnosed as **systemic blastomycosis** with neurologic involvement.

Oshin *et al.* (2009) presented a 4-year-old, spayed female, mixed-breed dog suffering from chronic left **hind-limb lameness**. Lytic lesions were observed in the left patella on radiographs of the stifle. A biopsy of the patella led to a histopathological diagnosis of blastomycosis. Surgical debridement followed by a 90-day course of itraconazole and physical rehabilitation resolved the clinical signs and stopped the progression of radiographic lesions. Blastomycosis should be considered as a differential diagnosis for stifle joint lameness with lytic lesions in the patella.

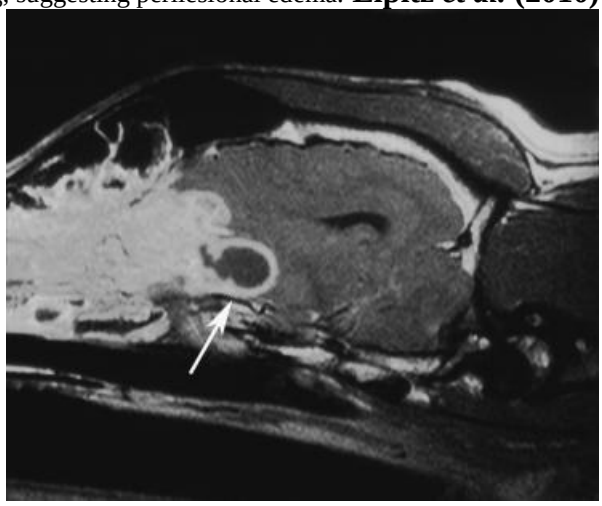
Varani *et al.* (2009) obtained two swabs each from the nares of 110 asymptomatic, physically normal dogs from a veterinary practice in Eagle River, WI, USA, an area highly endemic for blastomycosis. Four of the tested dogs had past histories of blastomycosis. Samples were placed on yeast extract phosphate (Smith's) media at 20 degrees C but growth of Blastomyces dermatitidis was not observed on any of the 220 cultures. One dog developed cytologically confirmed B. dermatitidis one month following culture of its samples, 6 died of other illnesses, while 91/103 dogs completing follow-up have remained asymptomatic for three years. They

did not observe nasal colonization by B. dermatitidis in this population of dogs with potential for sniffing and digging in an environment highly endemic for this fungus.

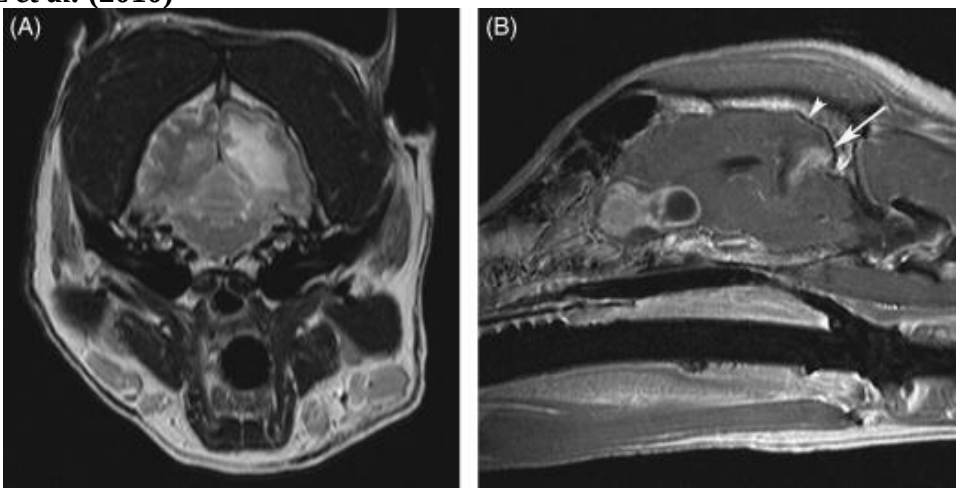
Lipitz *et al.* (2010) reported the clinical and magnetic resonance imaging features of **central nervous system blastomycosis** in 4 dogs.



(A) T2-weighted transverse magnetic resonance image from dog 1. There were hyperintense lesions in the right temporal-piriform lobes (arrow) and the right temporalis muscle (arrowhead). (B) Transverse T1-weighted image. The cerebral lesion is hypointense to grey matter and the muscle lesion is isointense to normal muscle. (C) Transverse T1-weighted postcontrast image. Note the strong, homogenous contrast enhancement in the right cerebral and temporal muscle lesions (arrows) and meningeal enhancement (arrowhead). The hyperintensity on T2- weighted images (A) is larger than the area contrast enhancing, suggesting perilesional edema. **Lipitz *et al.* (2010)**

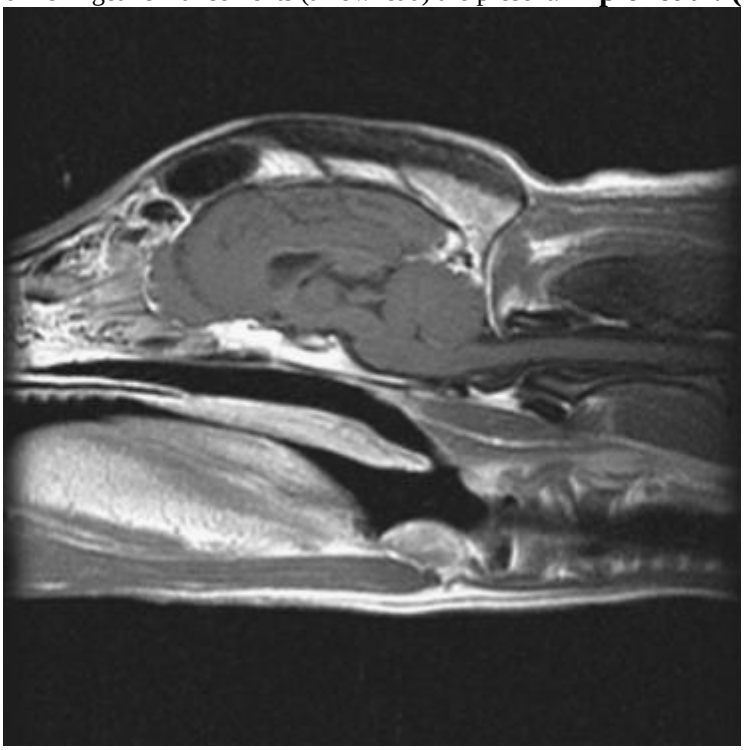


T1-weighted postcontrast sagittal magnetic resonance image from dog 2. Lesions in the nasal cavity and frontal cortex enhance strongly and homogenously with an area of ring enhancement (arrow). **Lipitz *et al.* (2010)**

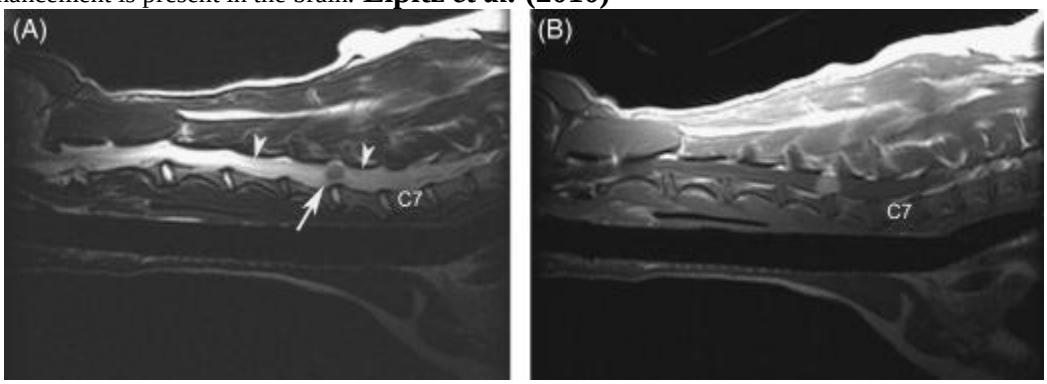


(A) T2-weighted transverse magnetic resonance image from dog 3 at the level of the occipital cortex. Marked edema is present in the white matter of the left occipital cortex; the edema obscures the occipital mass lesion. (B) T1-weighted postcontrast sagittal image. Note the uniform enhancement of

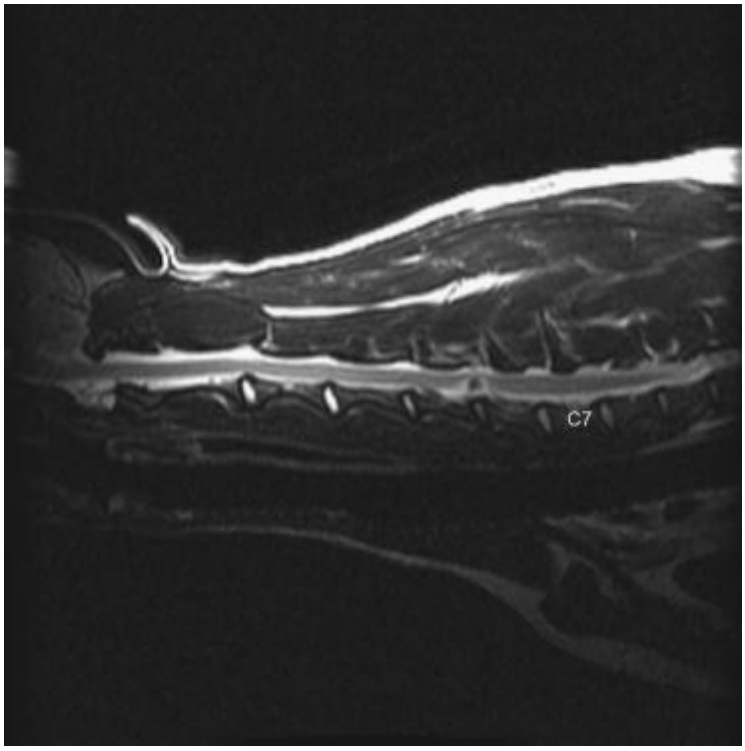
the lesion in the frontal cortex with an area of ring enhancement. Diffuse enhancement in the occipital cortex (arrow) and meningeal enhancements (arrowhead) are present. **Lipitz *et al.* (2010)**



T1-weighted sagittal postcontrast image from dog 3 acquired 1 year after original magnetic resonance imaging examination The previously described frontal and occipital lobe lesions have resolved and no enhancement is present in the brain. **Lipitz *et al.* (2010)**



(A) T2-weighted fat saturation sagittal image from dog 4. The mass in the spinal cord over the C5-6 intervertebral disc space is hypointense centrally with a hyperintense rim (arrow). Note the perilesional edema cranial and caudal to the mass (arrowheads). (B) Sagittal T1-weighted postcontrast image. There is strong, uniform contrast enhancement of the mass. **Lipitz *et al.* (2010)**



Sagittal T2-weighted image from dog 4 acquired 20 months after original magnetic resonance imaging examination. The mass in the spinal cord over the C5-6 intervertebral disc space is reduced in size and there is resolution of perilesional edema. **Lipitz *et al.* (2010)**

Reed *et al.* (2010) reported a case of granulomatous pneumonia, prostatitis and uveitis with intralesional yeasts consistent with Blastomyces in a 7-year-old sexually intact male German Short-haired Pointer.

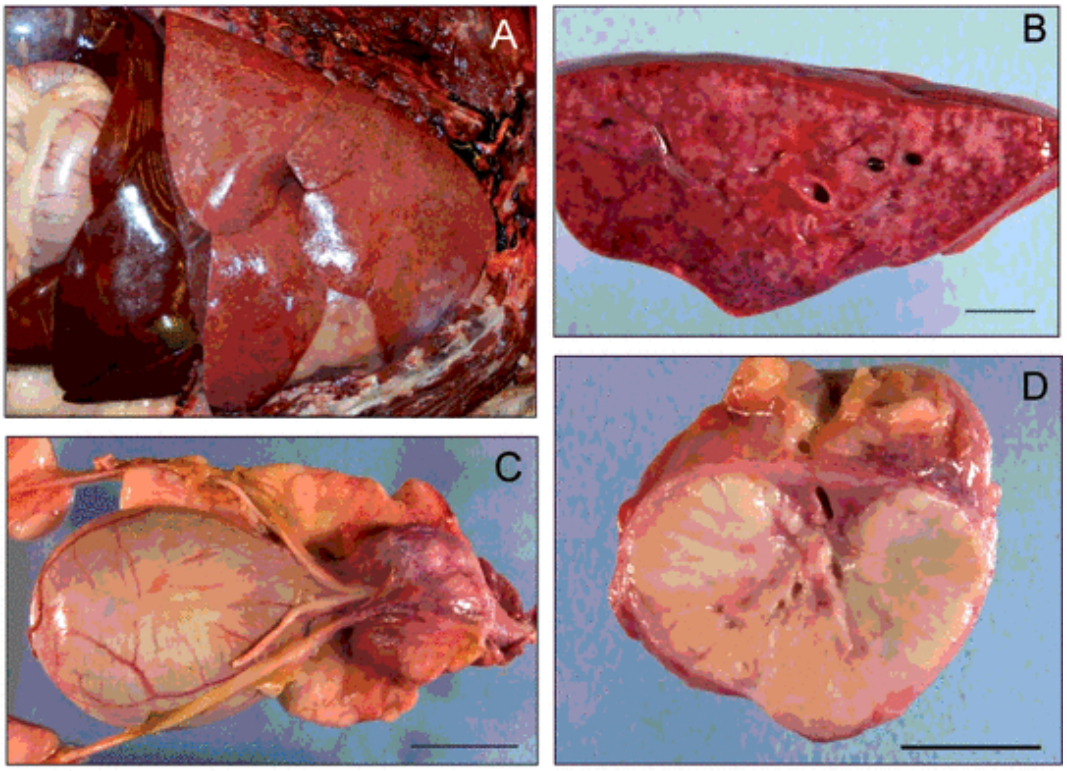
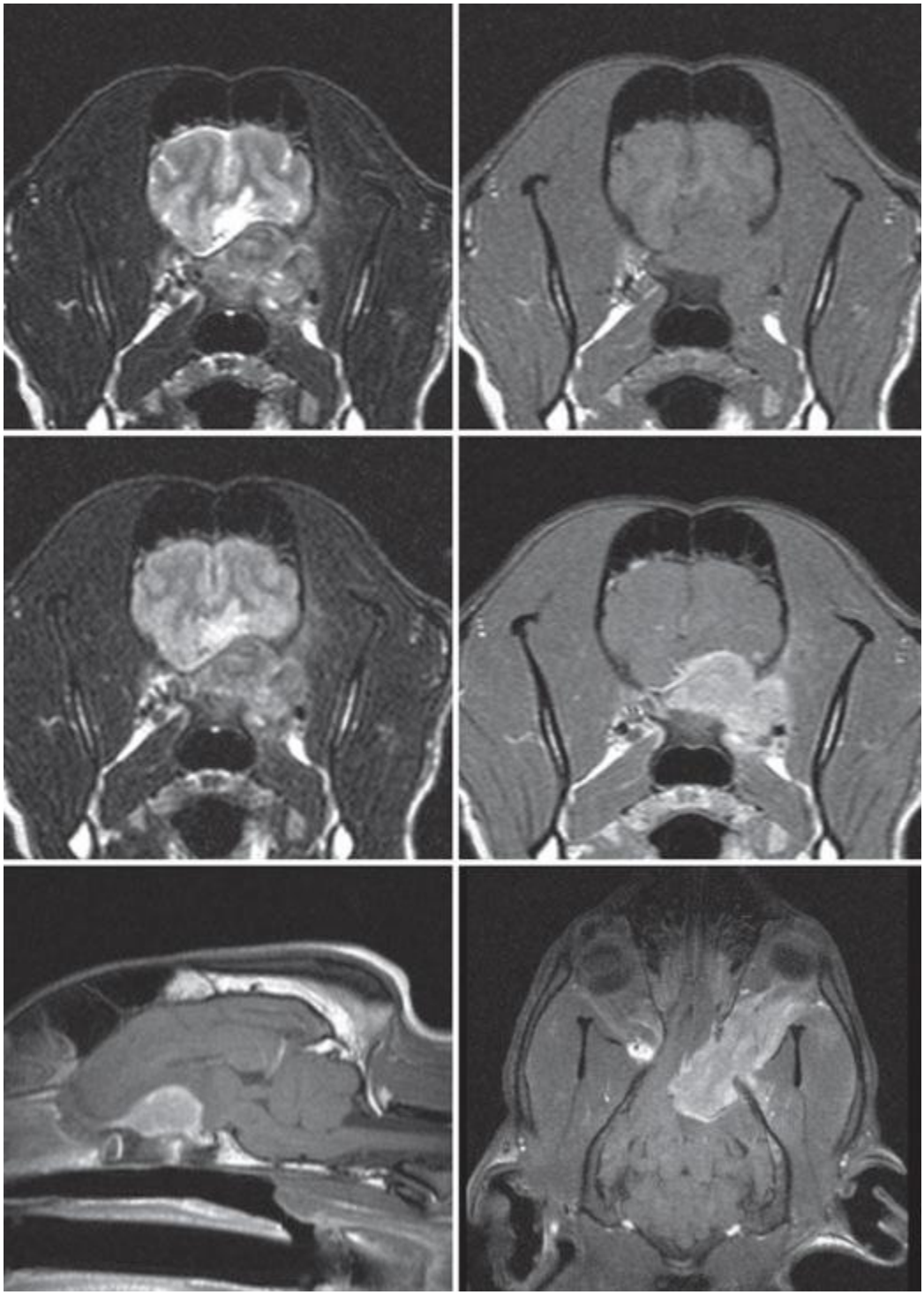


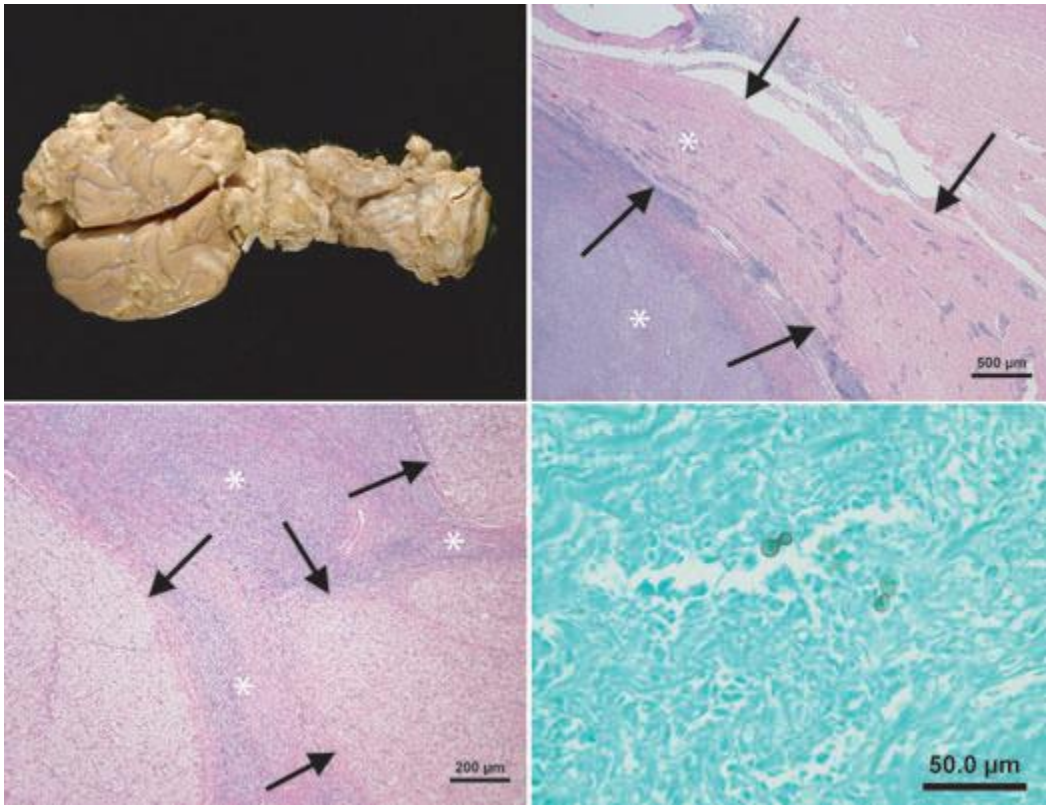
Figure 1—Photographs of the thoracic cavity (A), cut surface of lung tissue (B), urinary tract and prostate gland (C), and cut surface of the cross-sectioned prostate gland (D) of a 7-year-old sexually intact male German Short-haired Pointer that was evaluated because of sudden onset of respiratory distress and a 3-day history of prostatomegaly, tenesmus, and anorexia. In panel B, bar = 2 cm; in panels C and D, bar = 4 cm.

Reed *et al.* (2010)

Baron *et al.* (2011) reported a case that illustrated the clinical, MRI and histopathologic findings in a dog with invasion of a **retrobulbar blastomycotic** lesion into the calvarium. A 5-year-old intact female Weimaraner was referred for a 2-month history of change in behavior and recent onset of visual deficits. Magnetic resonance imaging (MRI) examination revealed a large (5.8 × 2.0 × 2.5 cm) mass extending from the left orbit through a circular defect in the left cranioventral aspect of the calvarium caudally to the level of the pituitary fossa and interthalamic adhesion. The mass was heterogeneously iso- to hypointense on T2-W images, slightly hypointense on T1-W images, did not attenuate on fluid attenuated inversion recovery (FLAIR) images, and did not show evidence of susceptibility artifact on T2*-W gradient recalled echo (GRE) images. Vasogenic edema and associated mass effect were noted. The mass showed strong homogeneous contrast enhancement with well-defined margins and had thickening of the adjacent meninges (dural tail sign). Based on MRI findings a malignant neoplastic process was considered most likely and the patient was placed on oral prednisone to decrease peri-tumoral inflammation. The dog initially improved but was euthanized 3 weeks later for worsening clinical signs. Histopathologic assessment of the mass revealed **marked pyogranulomatous optic neuritis** with intralesional fungal yeasts consistent with blastomycosis (***Blastomyces dermatitidis***).



Transverse pre- (a–c) and postcontrast (d–f) images of the brain and orbit. The retro-bulbar mass with intracranial extension is heterogeneously iso- to hypointense to brain parenchyma on T2-W images (a: TR = 5230 ms, TE 98 ms) and slightly hypointense on T1-W images (b: TR = 387 ms, TE = 15 ms). There is no evidence of attenuation of any portion of the mass on fluid attenuated inversion recovery (FLAIR) images (c: TR = 8000 ms, TE = 87 ms, TI = 2300 ms). Rightward shift of the falx cerebri and T2 hyperintensity to brain parenchyma bordering the mass is consistent with mass effect and edema. On post contrast transverse T1-W images (a: TR = 746; TE = 15), sagittal T1-W images (b: TR = 635, TE = 15) and dorsal T1-W images with fat saturation (a: TR = 500, TE = 15) there is strong homogeneous contrast enhancement of the mass with well-defined margins. **Baron *et al.* (2011)**



Photograph and photomicrographs of necropsy specimen. (a) Photograph of dissected left eye, optic nerve and brain (oriented with globe to the right) demonstrating mass effect of inflammatory infiltrate along the optic nerve. (b) Photomicrograph of inflammatory infiltrate (asterisks) compressing and infiltrating meninges (arrows) and frontal portion of cerebrum. Hematoxylin and eosin stain. (c) Photomicrograph of retro-bulbar tissue demonstrating separation and entrapment of nerves (arrows) by inflammatory infiltrates and necrosis (asterisks). Hematoxylin and eosin stain. (d) Photomicrograph of retro-bulbar tissue with multiple Blastomyces dermatitidis organisms (yeast) exhibiting narrow-based budding. Gomori methenamine silver stain. **Baron *et al.* (2011)**

Baumgardner *et al.* (2011) studied over 18 years 219 dogs with blastomycosis from a single veterinary practice in Northern Wisconsin. The 202 Vilas County dog addresses were compared to 200 random-number selected addresses from the practice registry. Street addresses were geocoded and mapped using ArcGIS, including ratio of cases/random addresses to construct a control chart. Stepwise and linear regression was used to model case counts by season and by 6 month warm (April-September) and cold periods, using lagged local weather data. The geographic distribution of cases was found to be similar regardless of season and time period, and no season exceeded control chart limits. Seasonal distribution of cases was; winter (n = 53, 24%), spring (39, 18%), summer (79, 36%), fall (48, 22%), p = 0.002. When cases were considered by 6-month warm/cold periods, 67% of variation is explained by the total precipitation which occurred two periods prior, and lower average temperature, but higher maximum temperature one period prior (p = 0.000). Weather parameters along with fixed and variable environmental factors likely determine the occurrence of B. dermatitidis, perhaps as part of a 'grow and tolerate change' model.

Ditmyer and Craig (2011) described three cases in which mammary tissue samples were submitted to the Department of Pathobiology, University of Tennessee, College of Veterinary Medicine with clinical suspicion of neoplasia or postpartum bacterial mastitis. **Pyogranulomatous to granulomatous mastitis** and **dermatitis** with intralesional yeast consistent with ***Blastomyces dermatitidis*** were diagnosed. Two of

the three dogs also had lymph node and pulmonary involvement. Mycotic mastitis due to Blastomyces dermatitidis is rarely reported and blastomycosis should be considered in the differential diagnosis of dogs with mammary lesions from endemic areas.

Hecht *et al.* (2011) carried out a study to describe clinical and imaging findings in dogs with **intracranial blastomycosis** (***Blastomyces dermatiditis***). The radiology database was searched retrospectively for patients with a diagnosis of intracranial blastomycosis which had computed tomography performed as part of their diagnostic work-up. Medical records and imaging studies were reviewed. Five dogs met the inclusion criteria. Major presenting complaints were stertor/nasal discharge (n=2), exophthalmos (n=1), and seizures (n=2). Clinical and laboratory findings were variable. Computed tomographic examination revealed a single contrast-enhancing intra-axial mass (n=1), a nasal mass disrupting the cribriform plate (n=3), and an intracranial mass extending into the orbit and nasal cavity (n=1). Findings in intracranial blastomycosis in dogs were variable, and the disease may mimic other inflammatory disorders or neoplasia.

Herrmann *et al.* (2011) carried out a retrospective cross-sectional survey to compare the temporal and spatial distribution of cases of blastomycosis among **humans and dogs** in Illinois from 2001 through 2007. For each year, human population data were obtained from the US Census Bureau, and the total number of dogs was estimated by use of a human population-based formula. Data regarding infections with ***Blastomyces dermatitidis*** in humans were accessed from the Illinois Department of Public Health. Data regarding B dermatitidis infections in dogs were acquired through a survey of a random sample of the 747 veterinary medical practices in Illinois. Statistical analyses of human and canine data were performed by use of t tests, ANOVA, odds ratio assessment, and regression modeling. Estimated annual incidence of human cases of blastomycosis in Illinois increased from 3.8 to 10.7 cases/1 million persons/y from 2001 through 2007. Analysis of data from 221 veterinary practices revealed that the mean estimated annual incidence of canine cases of blastomycosis was 8.3 times the mean estimated annual incidence of human cases, with a similar pattern of change and regional distributions. Thirty-eight counties reported either human or canine cases but not both. In conclusion, the estimated annual incidence of blastomycosis in humans and dogs in Illinois increased during the period of interest. Veterinarians, physicians, and public health agencies should be encouraged to communicate with each other regarding diagnoses of blastomycosis in either species to facilitate early diagnosis and treatment.

Mazepa *et al.* (2011) performed a study to compare incidence of clinical remission and death; treatment duration; total drug cost; incidence of relapse; and incidence of increased ALT activities in dogs with blastomycosis treated with fluconazole or itraconazole. One hundred and forty-four dogs with systemic blastomycosis treated with **itraconazole or fluconazole** from 1998 to 2008 were included in the study. Retrospective case review. Information obtained included signalment, body weight, clinical signs, drug regimen, treatment duration, time to clinical remission, and laboratory results. Neither treatment efficacy between fluconazole (75% remission) and itraconazole (90% remission) nor relapse rate (18% for itraconazole, 22% for fluconazole) was significantly different (P = .13, .75, respectively). Treatment duration was significantly longer for fluconazole (median 183 days) than for itraconazole (138 days; P = .001). Costs for fluconazole (median $1,223) were significantly less than for itraconazole ($3,717; P < .001). Incidence of increased ALT activities was not significantly different between groups (17% [3/18] for fluconazole,

26% [6/23] for itraconazole; P = .71). It was concluded that fluconazole was associated with survival to clinical remission in 75% of dogs with blastomycosis. Although dogs receiving fluconazole were treated longer, drug costs were one-third those of itraconazole. Hepatotoxicosis, as estimated by increases in serum ALT activity, could be observed with similar incidence for both drugs.

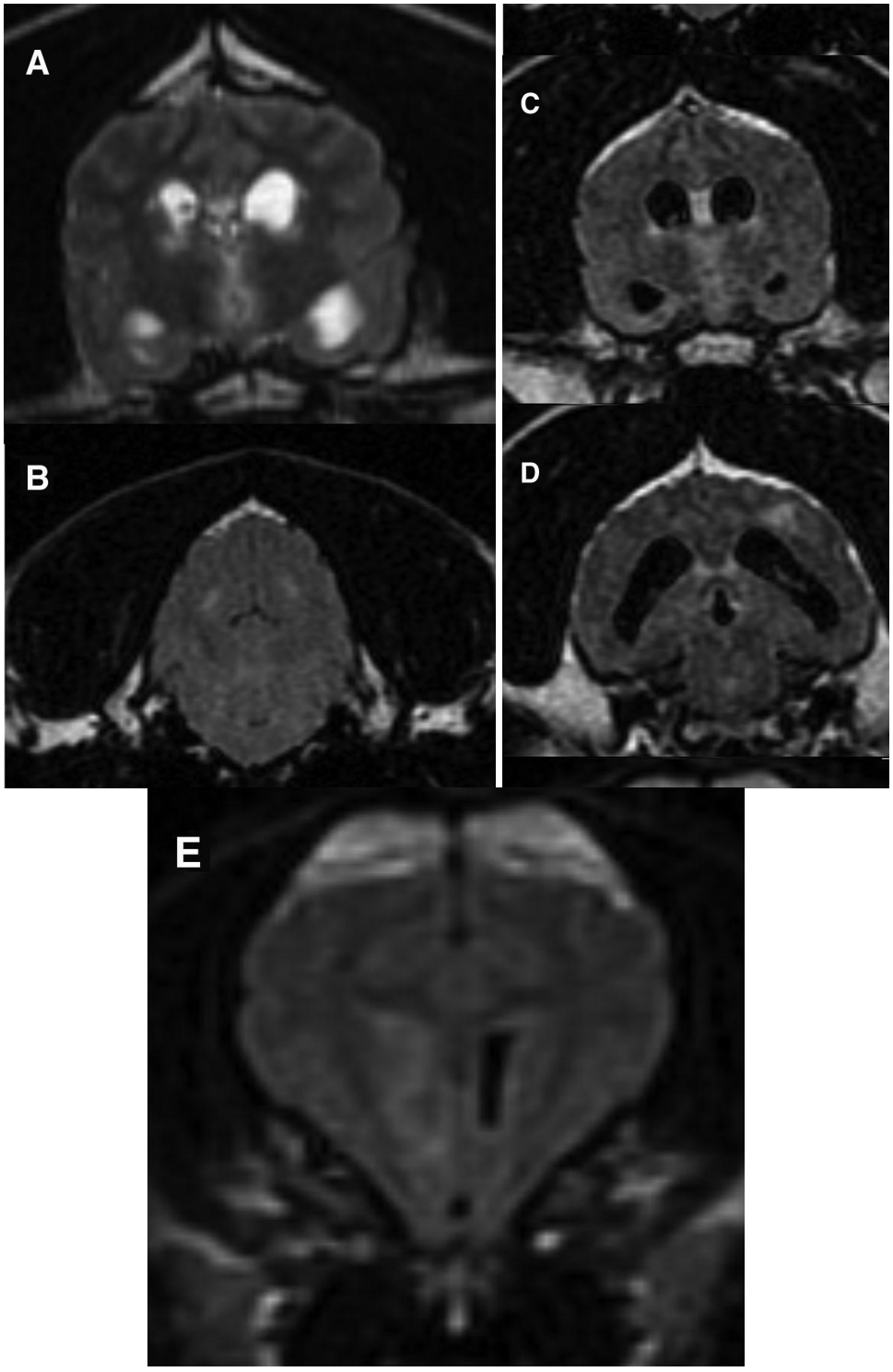
Totten *et al.* (2011) performed a retrospective case study of eight dogs diagnosed with **prostatic or testicular B. dermatitidis infection.** Signalment, clinical presentation, diagnostic procedures, and treatment options were evaluated. Review of medical records of dogs diagnosed with blastomycosis at the University of Illinois Veterinary Teaching Hospital from 1992 to 2005 yielded four dogs with prostatic blastomycosis (PB) and four dogs with testicular blastomycosis (TB). Three of the four dogs with PB and all four dogs with TB had evidence of urogenital disease. Three dogs with PB had an elevated body temperature and all had systemic disease. All dogs with TB had a normal body temperature, and three had systemic disease and one had clinical signs limited to testicular disease. Cytology or histopathology was used to diagnose PB or TB. Treatment included **itraconazole or fluconazole** with or without nonsteroidal anti-inflammatory drugs. PB and TB are infrequently recognized and may be under diagnosed due to failure to specifically evaluate these tissues. PB or TB should be considered in the evaluation and staging of male dogs with blastomycosis. Male dogs with urogenital signs should be evaluated via prostatic or testicular cytology or histopathology since proper identification and management of PB or TB may improve overall treatment success.

Wüthrich *et al.* (2011) studied the safety, toxicity, and immunogenicity of a **genetically engineered live-attenuated strain of *B. dermatitidis*** lacking the major virulence factor BAD-1, which successfully vaccinated against lethal experimental infection in mice, in dogs, using 25 beagles at a teaching laboratory and 78 foxhounds in a field trial. In the beagles, escalating doses of live vaccine ranging from 2×10^4 to 2×10^7 yeast cells given subcutaneously were safe and did not disseminate to the lung or induce systemic illness, but a dose of $< 2 \times 10^6$ yeast cells induced less fever and local inflammation. A vaccine dose of 10^5 yeast cells was also well tolerated in vaccinated foxhounds who had never had blastomycosis; however, vaccinated dogs with prior infection had more local reactions at the vaccine site. The draining lymph node cells and peripheral blood lymphocytes from vaccinated dogs demonstrated gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF) specifically in response to stimulation with Blastomyces antigens. Thus, the live-attenuated vaccine against blastomycosis studied here proved safe, well tolerated, and immunogenic in dogs and merits further studies of vaccine efficacy.

Anderson *et al.* (2013) used veterinary and human isolates matched with epidemiological case data from the same geographic area and time period to determine: (i) if differences in genetic diversity and structure exist between clinical veterinary and human isolates of B. dermatitidis and (ii) if comparable epidemiologic features differ among veterinary and human blastomycosiscases. **Genetic typing** of 301 clinical ***B. dermatitidis*** isolates produced 196 haplotypes (59 unique to veterinary

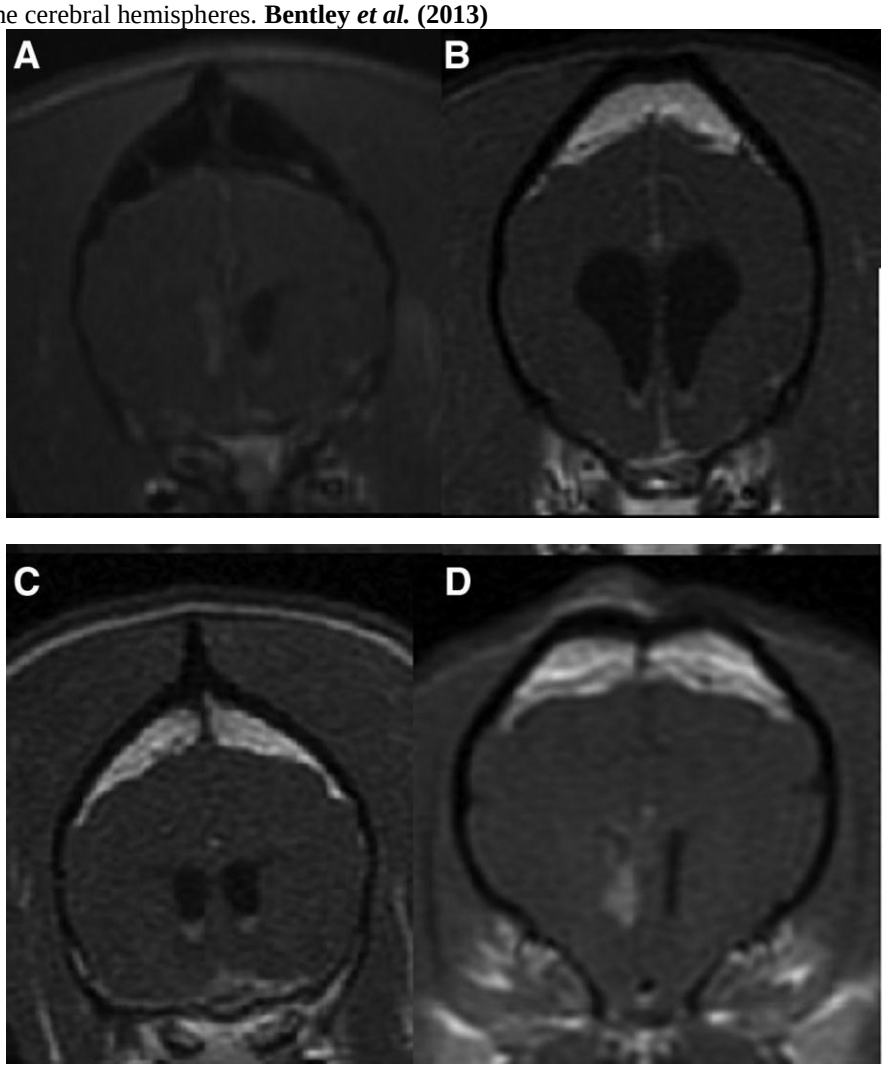
isolates, 134 unique to human isolates, and 3 shared between canine and human isolates). Private allelic richness was higher in veterinary (median 2.27) compared to human isolates (median 1.14) ($p = 0.005$). Concordant with previous studies, two distinct genetic groups were identified among all isolates. Genetic group assignment was different between human and veterinary isolates ($p < 0.001$), with more veterinary isolates assigned to Group 2. The mean age of dogs diagnosed with blastomycosis was 6 years. Thirty cases were in male dogs (52%) and 24 were females (41%). The breed of dog was able to be retrieved in 38 of 58 cases with 19 (50%) being sporting breeds. Three of four felines infected with blastomycosis were domestic shorthair males between ages 6-12, and presented with disseminated disease. The other was a lynx with pulmonary disease. The equine isolate was from an 11-year-old male Halflinger with disseminated disease. Disseminated disease was reported more often in veterinary (62%) than human cases (19%) ($p < 0.001$). It was concluded that isolates from all hosts clustered largely into previously identified genetic groups, with 3 haplotypes being shared between human and canine isolates confirming that B. dermatitidis isolates capable of infecting both species occur in nature. Allelic diversity measures trended higher in veterinary samples, with a higher number of total alleles and private alleles.

Bentley *et al.* (2013) performed a retrospective study to determine whether other MRI characteristics of CNS blastomycosis may also occur. Medical records of the Purdue University Veterinary Teaching Hospital were searched and four dogs met inclusion criteria. Magnetic resonance imaging characteristics included periventricular edema, periventricular and meningeal contrast enhancement, and ventriculomegaly. Periventricular lesions most commonly involved the rostral horn of the lateral ventricles and the third ventricle. Increased meningeal contrast enhancement involved the cerebrum, thalamus, sella turcica, and brainstem. Findings indicated that, in addition to mass lesions, MRI characteristics of periventricular hyperintensity, contrast enhancement, and ventriculomegaly may also occur in dogs with CNS blastomycosis.

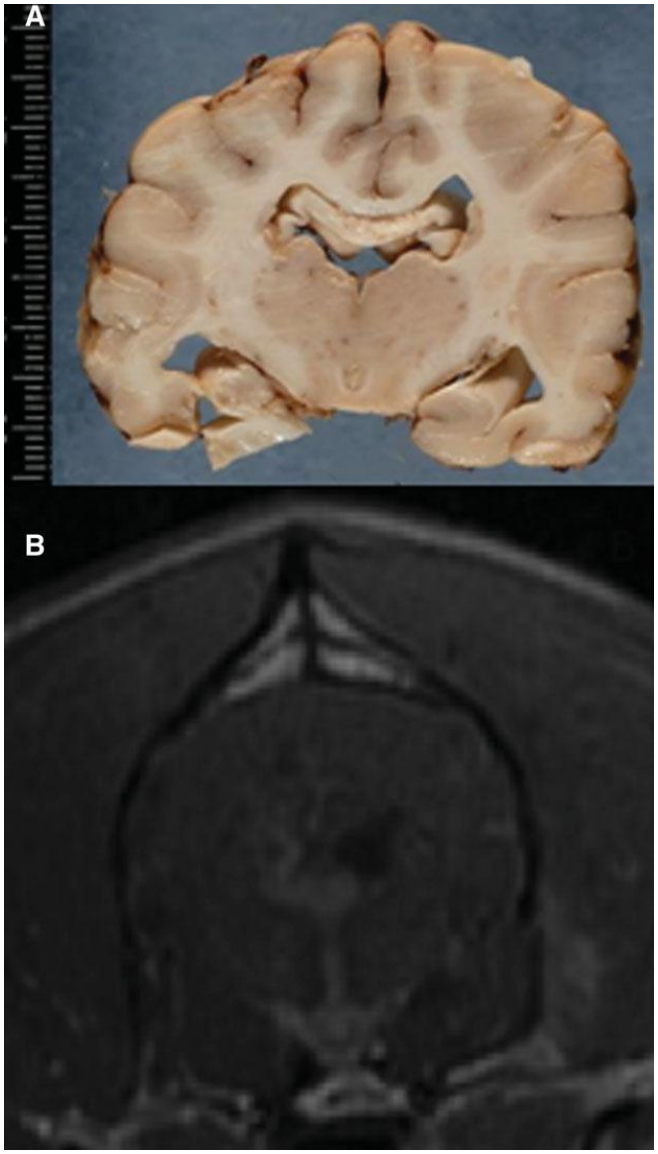


Transverse T2-weighted (A) and fluid attenuation inversion recovery (B–E) magnetic resonance images of four dogs with central nervous system blastomycosis. Images are at the level of the diencephalon (Case 1;A), the occipital lobes (Case 2; B), the level of the diencephalon and the rostral midbrain (Case 3; C and D, respectively) and the frontal lobe (Case 4; E). With respect to normal cerebrospinal fluid, the signal from the lumen of the third ventricle is T2-hypointense; a T2-hypointense structure is also seen in the region of the choroid plexus of the right lateral ventricle (A). With respect to normal periventricular gray and white matter as appropriate, periventricular hyperintensity is present surrounding the lateral ventricles (A, C) and in both occipital lobes immediately caudal to the lateral ventricles (B), the third ventricle (A), the caudal part of the caudate nuclei and the diencephalon immediately rostral to the third ventricle (C), the corpus callosum (A, C, and D), the rostral occipital lobe, and the midbrain ventral to the mesencephalic aqueduct (D), and encircling the rostral horns of the lateral ventricles (E). Note that the 4th ventricle (B), mesencephalic

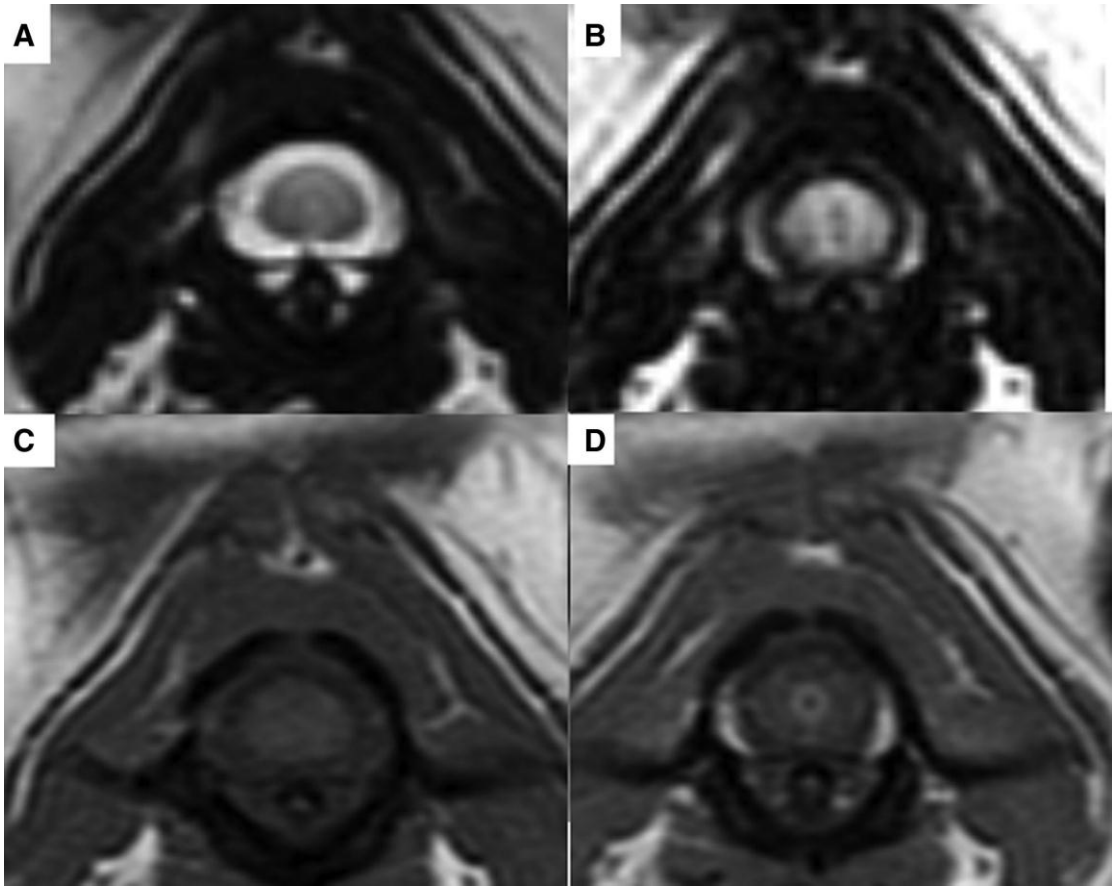
aqueduct (D), and one right lateral ventricle (E) cannot be well distinguished due to collapse, or displacement of cerebrospinal fluid by abnormal fluid or tissue. There is relative sparing of parenchyma besides the periventricular lesions. The subarachnoid space is difficult to visualize around much of the cerebral hemispheres. **Bentley *et al.* (2013)**



Transverse postcontrast T1-weighted magnetic resonance images at the level of the rostral horn of the lateral ventricle in four dogs with central nervous system blastomycosis (A, B, C, D; Cases 1–4, respectively). Ependymal or periventricular parenchymal contrast enhancement is present in the ventral portion of the rostral horn in seven of the eight lateral ventricles. A lateral ventricle is completely (A) or mostly (D) effaced by contrast enhancing material in two dogs. Segmental enhancement of the meninges is also variably present across the four cases; note the lack of parenchymal enhancement. **Bentley *et al.* (2013)**



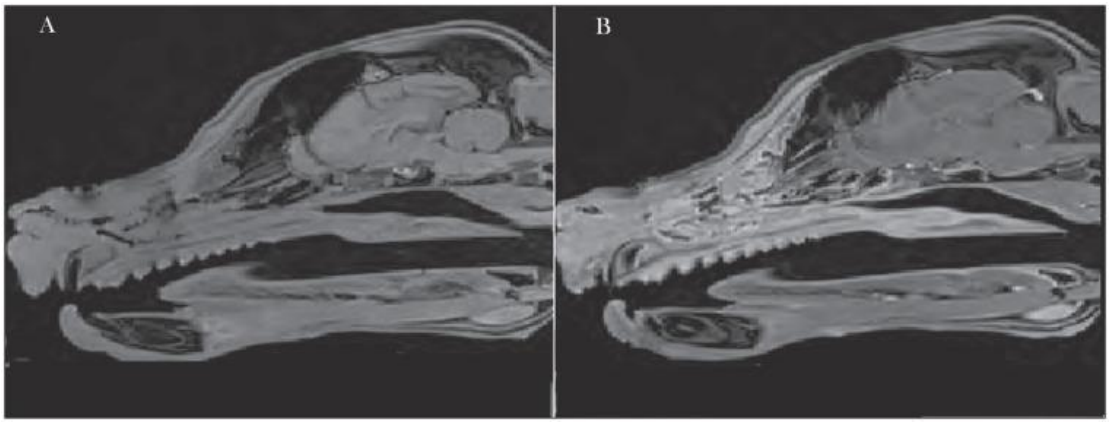
A section of the brain (A) and matching, slightly more rostral postcontrast T1-weighted transverse magnetic resonance image (B) from a 3-year-old male neutered Golden Retriever dog with central nervous system blastomycosis (Case 1) at the level of the diencephalon. The ventral portion of the third ventricle is occluded (A, B). The choroid plexus of the right lateral ventricle, and its extension into the dorsal third ventricle, is irregularly thickened (A, B). The ventral portion of the third ventricle is filled with contrast enhancing material and there is also contrast enhancement in the region of the sella turcica, the ventral meninges of the thalamus and segmental enhancement of the meninges of the cerebral cortex (B). **Bentley *et al.* (2013)**



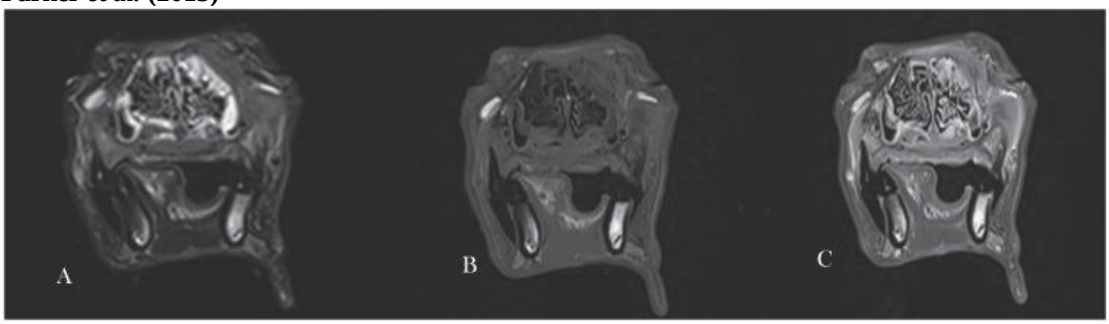
Transverse magnetic resonance images at the level of the C1 vertebra of a dog with central nervous system blastomycosis (Case 4). T2-weighted (A), fluid attenuation inversion recovery (B), T1-weighted (C), and T1-weighted postcontrast (D) images. Compared to normal gray and white matter respectively, the gray matter and the dorsal funiculus are indistinctly T2-hyperintense (A, B). The outline of the central canal is difficult to visualize (B, C). There is severe, circumferential contrast enhancement of the ependyma or adjacent parenchyma but not the meninges (D). **Bentley *et al.* (2013)**

Boyd *et al.* (2013) performed a study to evaluate the diagnostic sensitivity of ***Blastomyces dermatitidis*** yeast lysate **antigens** with respect to antibody detection in dogs with blastomycosis. Lysate antigens were prepared from B. dermatitidis isolates T-58 and T-66 (dogs, Tennessee) and WI-R and WI-J (dogs, Wisconsin). Based on results obtained from a preliminary comparative study, five combinations of these isolates and one individual isolate were tested against 92 serum specimens from dogs with culture-proven or histologically-confirmed blastomycosis, using the indirect enzyme-linked immunosorbent assay (**ELISA**). Mean absorbance values obtained from the sera ranged from 0.905 with the individual T-58 antigen to 1.760 using an antigen combination (T-58 + T-66 + WI-R). All of the 6 antigenic preparations were able to detect antibody in the serum specimens, but the antigen combinations detected antibody to a higher degree than the individual antigen. The study provided evidence that combinations of the yeast lysate reagents were more efficacious for antibody detection in dog sera.

Parker *et al.* (2013) described a case of localized **oronasal** blastomycosis mimicking oral neoplasia. Long-term therapy with **itraconazole** resulted in clinical cure.

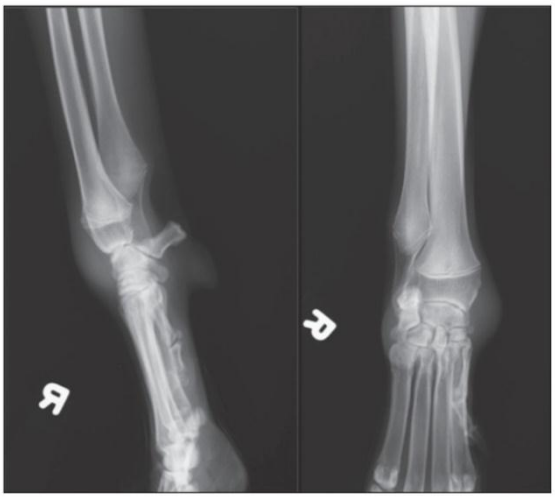


Sagittal MRI images of the lesions affecting the hard and soft palates, nasal passages, and dorsal nose using an ultrashort T1, Fat Saturated, MPR, VIBE sequence in pre (A) and post (B) IV contrast for maximal spatial resolution. Notice the extent of the infiltration as depicted by the hyperintense contrast enhancement throughout the oral, pharyngeal, dorsal nasal, nasal, and mandibular lymph node tissues. **Parker *et al.* (2013)**



Transverse MRI images at the level of the maxillary carnassial (108 and 208) teeth. On T2W (A) the nasal and palate tissues are hyperintense to normal muscle and thickened. There is hyperintense thickened nasal mucosa. In the precontrast T1W image (B), the thickened tissues are isointense to normal soft tissues and the nasal mucosa is hypointense. All the affected nasal and palate tissues contrast enhance (C: Post Contrast T1W) demonstrating the extensive infiltrative process. On all images the dorsal left nasal bone is disrupted, along with a portion of the hard palate **Parker *et al.* (2013)**

Woods *et al.* (2013) presented a 6-month-old male castrated Labrador retriever for coughing and forelimb **lameness**. ***Blastomyces dermatitidis*** was identified in cytology of sputum and synovial fluid. Repeat arthrocentesis 7 months later revealed resolution of septic **arthritis.**



Lateral (left) and dorsopalmar (right) radiographs of the right carpus, which identify increased soft tissue opacity confined to the carpal joint capsule. Bony structures are unremarkable.Carpal intra-articular blastomycosis in a Labrador retriever. **Woods *et al.* (2013)**

Foy *et al.* (2014) monitored 21 dogs with newly diagnosed blastomycosis until clinical remission (Treatment Phase), and 27 dogs over 1 year from the time of antifungal discontinuation or until clinical relapse (After Treatment Phase) monthly, with a complete history, physical exam, chest radiographs, and ocular exam. Urine and serum Blastomyces **antigen** concentrations were measured at each visit using a quantitative **enzyme immunoassay.** At enrollment in the Treatment Phase, Blastomyces antigen was positive in all 21 urine samples (100% sensitivity; 95% CI 85-100%), and in 18 of 20 serum samples (90% sensitivity; 95% CI 70-97%). At 2-4 months of treatment, urine antigen was more sensitive for clinically detectable disease (82%; CI 60-94%) than serum antigen (18%; CI 6-41%). The sensitivity of the urine test for clinical relapse was 71% (CI 36-92%), with close to 100% specificity (CI 84-100%) during after treatment surveillance in this population. It was concluded that urine Blastomyces antigen testing had high sensitivity for active disease at the time of diagnosis and during treatment, and moderate sensitivity but high specificity for clinical relapse. Urine testing should be useful at the time of diagnosis, when treatment discontinuation is being considered, and anytime there is poor clinical response or suspicion of relapse.

Mondada *et al.* (2014) conducted a study to compare the reactivity of two ***B. dermatitidis*** yeast lysate **antigens** prepared from dog isolates (ERC-2, Wisconsin; T-58, Tennessee) and two lysate antigens prepared from human isolates (B5931 and B5896, Minnesota) against 48 serum specimens from dogs with confirmed blastomycosis using the indirect enzyme-linked immunosorbent assay (ELISA). Secondarily, we used three different **ELISA** substrates (Ultra TMB: A, SureBlue: B, and SureBlue Reserve: C) to compare the effectiveness of each substrate. Mean absorbance values ranged from 0.446 (B) to 0.651 (C) for the B5931 antigen and from 0.393 (B) to 0.540 (C) for the ERC-2 antigen in Trial 1. In Trial 2, the absorbance values ranged from 0.628 (B) to 0.909 (A) for the B5896 antigen and from 0.828 (B) to 1.375 (C) for the T-58 antigen. In Trial 1, the lysate antigen prepared from the human isolate B5931 exhibited the highest absorbance value and in Trial 2 the lysate prepared from the dog isolate T-58 was the most reactive. The overall results thus indicated that the T-58 lysate was the optimal reagent when used to detect antibody with the Sure-Blue Reserve substrate. Our laboratory is continuing to study B. dermatitidis antigen and substrate combinations for the reliable immunodiagnosis of blastomycosis in humans and animals.

Mourning *et al.* (2015) performed a study to evaluate the sensitivity and specificity of an **enzyme immunoassay (EIA)** for antibodies to a recombinant **Blastomyces adhesin-1** repeat **antigen** (rBAD-1) to aid in the diagnosis of blastomycosis in dogs and compare the findings with results from other tests used for this purpose using serum and urine samples from 70 dogs with and without blastomycosis. Samples were collected from dogs with blastomycosis (n = 21), histoplasmosis (8), or non-fungal pulmonary disease (21) and from healthy control dogs living in a blastomycosis-endemic area (20). Serum was tested for antibodies against Blastomyces dermatitidis with the rBAD-1 antibody EIA and an A-antigen antibody agar gel immunodiffusion (AGID) assay. Serum and urine were

tested for B dermatitidis antigen with a quantitative EIA. Sensitivity of the quantitative antigen EIA was 100% in serum and urine samples from dogs with blastomycosis, with specificity of 95% in urine samples from dogs with nonfungal pulmonary disease and 100% in urine samples from healthy dogs. Sensitivity of the rBAD-1 antibody EIA (95%) was significantly greater than that of the A-antigen antibody AGID assay (65%). Specificity of the antibody EIA was 88% in dogs with histoplasmosis, 95% in healthy dogs, and 100% in dogs with nonfungal pulmonary disease.

1.6.2. Reports on blastomycosis in cats

Nasisse *et al.* (1985) examined a domestic shorthair cat because of dyspnea. It was found to have ophthalmoscopic and radiographic changes suggestive of systemic mycosis. The cat died despite antifungal therapy. Histologic examination revealed Blastomyces dermatitidis in the eyes, brain, lungs, stomach, liver, kidneys, spleen, pancreas, and adrenal glands. The pathologic changes were similar, but more widespread than those typically seen with canineblastomycosis.

Breider *et al.* (1988) reviewed medical records of 5 cats with blastomycosis at the University of Tennessee Veterinary Teaching Hospital from 1979 to 1986. Clinical signs of blastomycosis varied depending on the organ(s) affected, but respiratory tract disease was most common, followed by CNS signs and ocular problems. A definitive diagnosis was made by identification of characteristic fungal organisms in biopsy or necropsy specimens. Two cats treated with amphotericin B did not respond to treatment and died or were euthanatized. The lungs, brain, eyes, and lymph nodes commonly were affected, but one cat had only cutaneous and regional lymph node involvement. The respiratory tract appeared to be a common primary site of infection, with dissemination to other organ systems. The typical host response was a pyogranulomatous cellular infiltrate with numerous fungal organisms evident.

Meschter and Heiber (1989) diagnosed blastomycosis in a cat from lower New York State by cytologic examination of aspirates from lymph nodes. This represents a novel geographic distribution of this disease

Gilor *et al.* (2006) conducted a retrospective study to evaluate clinical and laboratory findings, treatment, and clinical outcome in 8 cats with blastomycosis. Medical records of the University of Illinois Veterinary Teaching Hospital were searched for cases of blastomycosis in cats diagnosed via cytologic or histopathologic findings. Clinical and laboratory findings, treatment, and clinical outcome were determined. Radiographs were reviewed for the 8 cases. All cats were systemically ill. Respiratory tract signs and dermal lesions were most commonly observed. All cats had radiographic evidence of respiratory tract disease. Seven of the 8 cats had ill-defined soft-tissue opacities (nodules or masses) or alveolar consolidation of the lungs. Antemortem diagnosis was achieved cytologically in 6 of the 8 cats, and 3 were successfully treated and survived.

Blondin *et al.* (2007) investigated an outbreak of blastomycosis among five urban, indoor cats diagnosed at three veterinary clinics March 3-July 13, 2005, in suburban Chicago, Illinois, by owner interviews, site visits, environmental cultures for B. dermatitidis, GIS analysis, and analysis of local weather data. There were no environmental exposures common to the five cats that lived a median of 300 m from nearest body of water, in homes on a loam soil. Closest and farthest case home sites were 3.4 and 26.1 km, respectively. All cats were confined indoors except one cat that

averaged 15 min/week in his backyard and was exposed to excavation. B. dermatitidis was not isolated from any of 60 environmental samples. The annualized incidence rate March through July 2005 among 6,761 cats in these practices was 178/100,000, compared to none in the previous 4 years, and 0.14/100,000 cat visits from a nationwide animal hospital registry. Precipitation January through June 2005 was 9.30 versus period mean of 14.05 +/- 1.69 inches the previous 4 years (P = 0.01). Circumstantial evidence suggests acquisition of B. dermatitidis from the home site environment in five cats. Relative drought may have contributed to an apparent outbreak of blastomycosis in this urban locale.

Smith *et al.* (2007) examined an 8-year-old domestic shorthair cat because of signs of depression, circling, and visual deficits. The cat had no cutaneous lesions, and results of an ophthalmologic examination and thoracic radiography were within reference limits. Computed tomography of the brain revealed a mass lesion involving the right parietal, temporal, and occipital lobes; the mass was in broad-based contact with the skull and smoothly marginated and had strong homogenous enhancement after contrast agent administration. During craniectomy, samples of the mass were collected for cytologic and histopathologic evaluations and microbial culture. A diagnosis of ***Blastomyces dermatitidis***-associated **meningoencephalitis** with secondary pyogranulomatous inflammation was made. **Amphotericin B** (0.25 mg/kg [0.11 mg/lb], IV) was administered on alternate days (cumulative dose, 1.75 mg/kg [0.8 mg/lb]). To minimize the risk of nephrotoxicosis, assessments of serum biochemical variables (urea nitrogen and creatinine concentrations) and urinalyses were performed at intervals. The third dose of amphotericin B was postponed 48 hours because the cat became azotemic. The cat subsequently received **fluconazole** (10 mg/kg [4.5 mg/lb], PO, q 12 h) for 5.5 months. Six months after discontinuation of that treatment, the cat appeared healthy and had no signs of relapse.

Stern *et al.* (2011) reported a 9 yr old domestic shorthair cat with cutaneous and pulmonic blastomycosis. Severe persistent ionized hypercalcemia and excess circulating concentration of calcitriol were documented in association with blastomycosis. Ionized hypercalcemia resolved when the granulomatous lesions of blastomycosis resolved and the calcitriol levels decreased.

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2. Coccidioidomycosis in cats and dogs

2.1. Introduction

- Coccidioidomycosis is a respiratory fungal infection with occasional systemic dissemination. The disseminated coccidioidomycosis is considered a multifaceted disease. In medicine, disseminated coccidioidomycosis is included within a group of infectious diseases that have been referred as the great imitators. In many cases,

malignancies are included in the presumptive diagnosis. In veterinary medicine, disseminated coccidioidomycosis is common in dogs. Nonetheless, despite of being a diagnostic dilemma, disseminated coccidioidomycosis is underestimated and frequently not included into differentials, even in endemic zones **(Ramírez-Romero *et al.*, 2016)**.

- Coccidioidomycosis or Valley Fever (VF) is an emerging soil-borne fungal zoonosis affecting humans and animals. Most non-human cases of VF are found in dogs, which we hypothesize may serve as sentinels for estimating the human exposure risk **(Gautam *et al.*, 2013).**
- The dimorphic fungi Coccidioides immitis and Coccidioides posadasii are the causative agents of coccidioidomycosis. Dogs and cats residing in and visiting endemic areas are at risk of exposure to infectious arthrospores. The primary infection is pulmonary and frequently results in chronic cough. Disseminated disease is common and causes cutaneous, osseous, cardiac, ocular, nervous system, or other organ disease. Radiographic changes include a variable degree of interstitial pulmonary infiltration, hilar lymphadenopathy, and osseous lesions. Serological titers support the diagnosis, but definitive diagnosis relies on identification of Coccidioides in cytological or tissue samples. Coccidioidomycosis should be considered in any dog or cat that has been potentially exposed during the previous 3 years and is presented with chronic illness, respiratory signs, lameness, lymphadenopathy, nonhealing cutaneous lesions, or neurological, ocular, or cardiac abnormalities **(Graupmann-Kuzma *et al.*, 2008)**.

- Coccidioides spp. appear capable of infecting all mammals and at least some reptiles. Development of disease as a result of infection is species-dependent. Dogs seem to have a susceptibility similar to that of humans, with subclinical infections, mild-to-severe primary pulmonary disease, and disseminated disease. Whereas central nervous system disease in humans is typically meningitis, brain disease in dogs and cats takes the form of granulomatous parenchymal masses. Osteomyelitis is the most common form of disseminated disease in the dog, while skin lesions predominate in the cat. Orally administered azole antifungal agents are the backbone of therapy in animals as they are in humans **(Shubitz, 2007).**

- Clinical coccidioidomycosis is quite common in the dog; though less frequently recognized in the cat, disease is often severe at the time of diagnosis. Diagnosis can be a challenge because serology, while specific, is not very sensitive and quantitative titration of antibodies does not correlate entirely with clinical disease in dogs. Radiographs, serum biochemistry tests and complete blood counts are beneficial additions to the database when establishing a diagnosis; cytology, histopathology, and culture are definitive when available. Advanced imaging can detect central nervous system and subtle skeletal lesions. Disease can occur in most organs of the body and may prove a diagnostic challenge requiring several modalities. **(Shubitz and Dial, 2005).**

- **Greyhounds** seem particularly susceptible to VF, perhaps due to their normally low white blood cell count. Natural immunity plays a part in determining which dogs contract VF (a new arrival to the area is more susceptible than a dog that grew up there). Valley Fever is a disease that can be obscure and may progress before the owner sees sufficient reason to visit a

veterinarian. Some dogs display no specific signs, especially early on, appearing to be not as well, eating inconsistently, or losing weight. Despite the name, half of dogs with VF have normal temperatures at presentation. They may, however, run fluctuating fevers at home and have times of appearing not as well, interspersed with times of lethargy, and inevitably go on to develop more specific signs, if their condition is undiagnosed and untreated. The most common signs are poor appetite, weight loss, lameness, bone pain, spinal pain, and coughing. In the early (primary) form, the fungus infects the lungs, then moves on to infect the bones (secondary form). Lungs and bones are affected in most cases; other systems that can be affected are the central nervous system (CNS), eyes, and, less commonly, the heart or skin. The coughing stage is seldom seen in greyhounds; most cases present with bone involvement or nonspecific illness and weight loss. Other dogs tend to present with equal proportions of the lung versus the bone form. Of particular concern with greyhounds is that, on radiographs, VF bone lesions resemble osteosarcoma. Lesions can be either osteoproliferative or osteolytic, so it is important to obtain an antibody titer to *C. immitis.* In fact, a titer should be obtained for any greyhound from Arizona that is sick for any reason. Ketoconazole is the first line of treatment. It is used at a dose of 5 mg/kg BW, q12h, with food. Minimum treatment time is 1 y, unless there is only lung involvement, in which case it is 6 mo. Treatment is continued until titers are negative and radiographs are clear, if bone has been involved **(Rubensohn and Stack, 2003)**.

- Little published information is available to guide therapy for canine and feline patients with Coccidioides infections involving the central nervous system (CNS).

2.2. Aetiology

Coccidioides immitis RIXFORD et GILCHRIST 1896

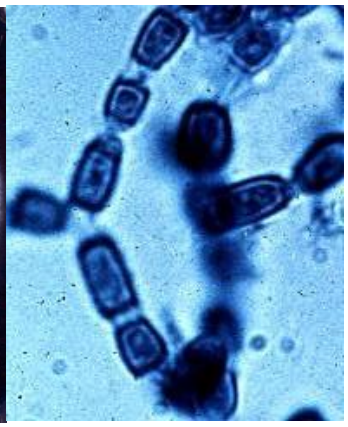
Synonyms: *Posadasia esferiformis* CANTON 1898
Blastomycoides immitis CASTELLANI 1928
Pseudococcidioides mazzai DA FONSECA 1928
Geotrichum immite AGOSTINI 1932
Coccidioides esferiformis MOORE 1932
Glenospora metaeuropea CASTELLANI 1933
Glenospora louisianoideum CASTELLANI 1933
Trichosporon proteolyticum NEGRONI et DE VILLAFANE 1938

Perfect stage: unknown

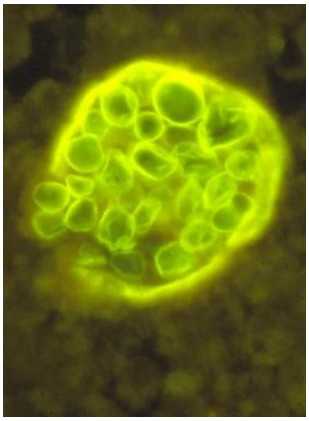
C. immitis is a thermically dimorphic fungus that grows in nature, soil, or in the laboratory at room temperature as a mould and in tissues or in the laboratory at 37C as a yeast. The mould phase grows at first as moist, glabrous and grayish colonies that rapidly develop abundant, floccose, aerial mycelium. The mycelium is initially white, but usually becomes tan to brown with age. Microscopically, the fungus develops thin and septate hyphae that produce side branches that are much more thicker and have numerous septations. Thick-walled arthroconidia are produced in these side-branches. The arthroconidia alternate with thin-walled empty cells.

The arthroconida are barrel-shaped, 2.5-4 by 3-6 um and are released by fragmentation of the mycelium. The arthroconidia are highly resistant to desiccation, temperature extremes and deprivation of nutrients and may remain viable for years. In the tissues the arthroconidium develops into spherules within a few hours or days. They become more rounded as they transform and enlarge. At maturity the spherules are 30-60 um in diameter, with a thick and prominent cell wall. Endospores are formed inside the spherules which are 2-5 um in diameter and may reach to hundreds in one spherule. At maturation the spherule ruptures and the endospores are released, which in turn develop into spherules.

C. immitis at 25^{o}C



arthroconidia

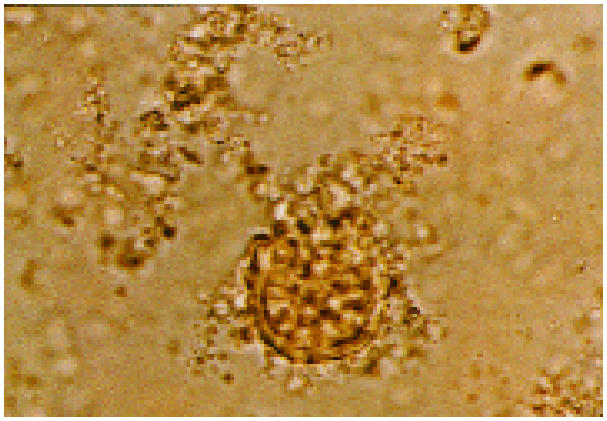


spherule

2.3. Diagnosis

2.3.1. Direct microscopic examination:

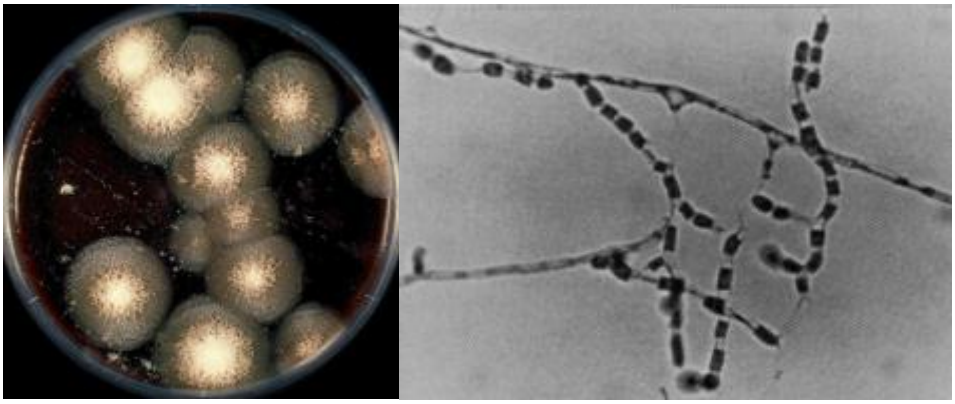
Spherules can be detected in sputum, pus, exudate and biopsy material, either in fresh or stained preparation by methods of Papanicolaou or Gomori's methenamine silver staining. These stains can demonstrate spherules and surrounding inflammation.



A spherule in a direct film

2.3.2. Isolation and identification:

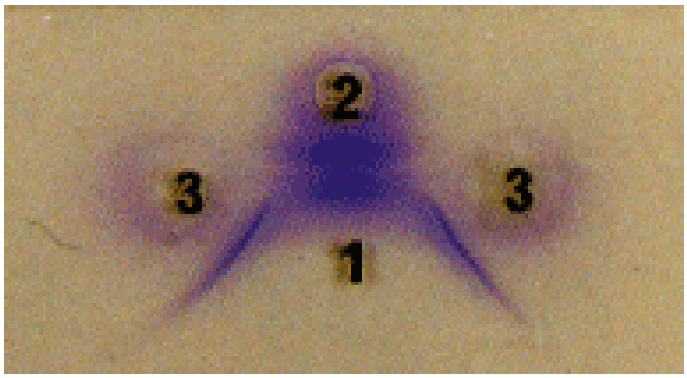
Material to be examined is plated on Sabouraud's dextrose agar and incubated at room temperature and on brain-heart dextrose agar and incubated at high carbon dioxide concentration at 37 C. Growth of the mould phase is rather rapid and the colony matures within 2 weeks. Confirmation requires the conversion into yeast phase by subculturing the mould on brain-heart or blood agar and incubating at 37 C.



Colonies of *C. immitis* and arthroconidia

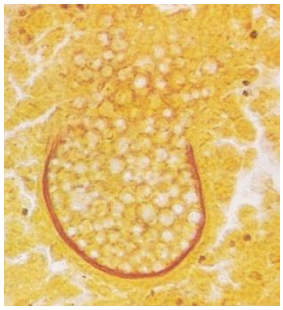
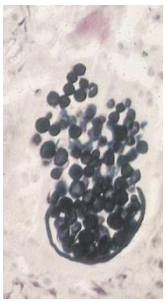
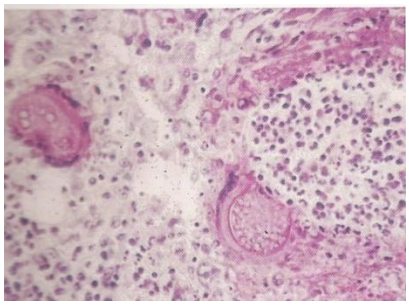
2.3.3. Serology :

Complement-Fixation is excellent for coccidioidomycosis because it is quantitative. However, these antibodies cross-react with some other fungi (Blastomyces and Histoplasma). The C-F test is also a PROGNOSTIC test. If the titer keeps rising, then the patient is responding poorly and the course may be fatal. If the C-F titer is dropping then the prognosis for that patient is favorable. A titer of greater than 1:128 usually indicates extensive dissemination. Life-long immunity usually follows infection with C. immitis. Detection of antibodies to *Coccidioides* can be an important diagnostic tool. Today, IgM and IgG are generally measured using EIA and/or immunodiffusion; however, some laboratories continue to use tube precipitin to measure IgM and complement fixation to measure IgG antibodies. False-negative serology has been seen in up to 38% of patients with hematogenous infection and 46% of fatal cases. Detection of antigens in the urine using EIA has been shown in 71% of patients with coccidioidomycosis but shows cross-reaction in 10% of patients with other endemic mycoses



2.3.4. Histopathology

Spherules of various sizes (10 to 100 μm) with multiple endospores (2 to 5 μm) are characteristic of coccidioidomycosis and can be seen with routine H&E staining.The walls of some of the spherules may appear to be ruptured, and the endospores spill into surrounding tissues. The inflammatory reaction to endospores is predominantly neutrophilic, while reaction to spherules is granulomatous.


Skin biopsy Lung biopsy, Gomory st., , H&E st. granulomatous reaction FA stain

2.3.5. Skin test:

The intradermal injection of 0.1 ml of coccidioidin, which is a 6-10 weeks culture filtrate, evokes in infected individual a positive delayed hypersensitivity reaction larger than 5 mm in diameter after 24-96 hours. The test may be negative at the onset of infection or in case of dissemination.

2.4. Treatment

Disease is often self-limiting, but if chronic respiratory signs or multisystemic disease are present, longterm antifungal therapy is needed; with disseminated infection, treatment of at least 6–12 mo is typical. Fluconazole (2.5–10 mg/kg/day) is the most commonly used drug to treat disseminated or chronic respiratory infections. Ketoconazole (10–30 mg/kg/day) and itraconazole (10 mg/kg/day) are also commonly used to treat dogs with coccidioidomycosis but are more expensive and have a higher incidence of adverse effects. Amphotericin B may be the most effective antifungal drug, but it is highly nephrotoxic. It may be indicated in animals that either do not improve or are unable to tolerate the azole antifungals.

2.5. Reports on coccidioidomycosis

2.5.1. Reports on coccidioidomycosis in dogs

Wolf (1979) diagnosed primary cutaneous coccidioidomycosis in a dog and a cat examined because of lymphangitis and lymphadenitis associated with skin wounds. This benign and self-limiting form of disease was distinguished from the skin lesions associated with systemic coccidioidomycosis by means of historic, physical, and serologic criteria established in human medicine.

Angell *et al.* (1987) examined 33 dogs with ocular lesions. Serologic and/or histologic evaluation confirmed the diagnoses of coccidioidomycosis. Histologic evaluation of the eye revealed a primary posterior segment disease, such as chorioretinitis or retinal separation, with an extension into the anterior segment of the eye.

Hawkins and DeNicola (1990) performed analysis of tracheal wash and bronchoalveolar lavage fluid in 9 dogs that had mycotic infections with pulmonary involvement. Characteristic organisms were identified in tracheal wash fluid in 3 of 7 dogs with blastomycosis. Organisms were identified in bronchoalveolar lavage fluid in 5 of 7 dogs with blastomycosis and in one dog with histoplasmosis. Organisms were not found in either fluid in one dog withcoccidioidomycosis. These procedures should be considered

for dogs with suspected mycotic infections that involve the lungs and that cannot be diagnosed by less invasive means

Plotnick et al. (1997) reported a 13-month-old, female Labrador retriever which developed draining tracts in the right hind limb, 3 weeks after traveling to Arizona,. Primary cutaneous coccidioidomycosis was diagnosed. Initial treatment with itraconazole resulted in exacerbation of clinical signs. Histopathology was suggestive of a cutaneous drug eruption. Discontinuation of the itraconazole caused resolution of the drug eruption. Successful treatment of the fungal infection was achieved using ketoconazole.

Burtch *et al*. (1998) diagnosed granulomatous meningitis attributable to Coccidioides immitis on postmortem examination in a 4-year-old Border Collie. Clinical signs included CNS disease, aspiration pneumonia secondary to a megaesophagus, and otitis externa. Central nervous system signs included central vestibular and cranial nerve dysfunction. Cerebellar and medullary infiltrates seen on histologic examination affected cranial nerves VIII, IX, and X. Despite extensive diagnostics, diagnosis was not made antemortem. Analysis of CSF suggested suppurative meningitis, but bacteriologic culture results were negative. Coccidioides endospores were identified on reexamination of brain tissue. The clinical course of disease and rate of Coccidioides immitis infection is variable. Causative agents of granulomatous or inflammatory CNS disease may include fungal infection more often than is currently suspected.

Wanke *et al*. (1999) described an outbreak of coccidioidomycosis that involved three individuals and eight of their dogs, who had engaged in a successful hunt for nine-banded armadillos (Dasypus novemcinctus) in the environs of Oeiras, a community in Brazil's north eastern state of Piauí. Diagnosis was based on clinical, serological and cultural findings. Four of 24 soil samples collected in and around the burrow of an armadillo yielded cultures of Coccidioides immitis, thus establishing the endemicity of that mould in the state of Piauí. A literature review revealed that C. immitis, aside from that state, is endemic in three other Brazilian states--Bahia, Ceará and Maranhão. These four contiguous states have semi-arid regions where climatic conditions and their flora are similar to those that exist in C. immitis's endemic regions in North, Central and South America.

Shubitz *et al.* (2001) reported a 4-year-old castrated male mixed-breed dog with a history of coccidioidomycosis with abdominal and pleural effusion. Results of radiography, ultrasonography, cytologic evaluation of thoracic fluid, and serologic testing supported a diagnosis of constrictive pericarditis secondary to infection with Coccidioides immitis. Aggressive treatment for presumptive coccidioidomycosis was begun, but the dog's condition continued to deteriorate, and the dog was euthanatized. At necropsy, the pericardium was thicker than normal and fibrotic and adhered to the epicardium. Microscopically, the pericardium and 1 section of epicardium contained lymphoplasmacytic infiltrates with a few macrophages and neutrophils. Coccidioides immitis was cultured from pericardial fluid. A search of records from the Arizona Veterinary Diagnostic Laboratory for 1988 through 1998 revealed that of 46 dogs in which a diagnosis of coccidioidomycosis was confirmed at necropsy, 13 had involvement of the heart or pericardium.

Jeroski (2003) evaluated an 11-year-old, spayed female Alaskan malamute with a history of coccidioidal osteomyelitis for inappetance and lethargy. Findings included generalized lymphadenopathy, pale mucous membranes, tachycardia, and labored breathing. Laboratory findings and radiographic imaging were consistent with

generalized lymphoma and disseminated coccidioidomycosis. Treatment consisted of antibiotics, chemotherapeutic agents, and antifungals

Johnson *et al.* (2003) conducted a etrospective case series study to determine clinical, clinicopathologic, and radiographic abnormalities in dogs with coccidioidomycosis. Clinical information and results of clinicopathologic testing were obtained from medical records. Thoracic radiographs were reviewed to characterize abnormalities. Dogs ranged from 1 to 10 years old at the time of diagnosis, with 12 dogs being between 1 and 3 years old. Historical complaints included cough, lameness, signs of head or neck pain, and difficulty breathing. Mild anemia, neutrophilia, and monocytosis were common. All dogs had hypoalbuminemia, and 8 of 15 had hyperglobulinemia. Thoracic radiographs of 19 dogs were reviewed. Pulmonary infiltrates were seen in 13dogs, with an interstitial pattern of infiltration being most common. Hilar lymphadenopathy was seen radiographically in 10 dogs. Serum from 20 dogswas tested for antibodies against Coccidioides immitis. One dog was positive for IgM antibodies, 5 were positive for IgM and IgG antibodies, and 14 were positive for IgG antibodies. Quantitative IgG titers measured in 14 dogs ranged from 1:2 to 1:128 (median and mode, 1:32). In 6 dogs, histologic examination of biopsy samples revealed fungal spherules ranging from 8 to 70 microm in diameter. Results suggested that in dogs, coccidioidomycosis may be associated with a wide spectrum of nonspecific respiratory and musculoskeletal abnormalities.

Rubensohn and Stack (2003) described a 2-year-old, spayed, female greyhound named Cabby for a postadoption examination on March 9, 2002. She originated in Arizona and had been adopted via the Greyhound Rescue Society. On presentation, Cabby was bright, alert, and responsive. The right submandibular lymph node was enlarged (diameter 2 cm), the right external ear was inflamed, and the nail bed of the left front 2nd digit was infected. She was placed on cephalexin (Novo-Lexin, Novopharm, Toronto, Ontario), 250 mg, PO, q12h for 2 wk and then reexamined; the lymph node had reduced in size (diameter 1.25 cm) and the nail bed infection and ear were healed. On July 13, 2002, Cabby was presented limping on the left hind leg and with a fever of 39.5°C. The clinical examination was unremarkable and she was administered meloxicam (Metacam, Boehringer Ingelheim (Canada), Burlington, Ontario), 0.1 mg/kg bodyweight (BW), PO, q24h. On August 6, 2002, Cabby was checked again. Her left hind limb lameness was worse and she was again febrile (40.5°C), with persistent drainage from the peri-anal fistula. The pyogranulomatous discharge from the fistula contained spherules of *Coccidiodes immitis.* Results from the CBC count showed a monocytosis (1.730×10^9 cells/L; reference range, 0.000 to 0.980×10^9/L) and a basophilia (0.111×10^9 cells/L; reference range, 0 to 0.100×10^9/L). Results of the blood biochemical analysis revealed a severe hyperproteinemia (86 g/L; reference range, 54 to 71 g/L) due to an exaggerated hyperglobinemia (62 g/L; reference range, 20 to 40 g/L). The systemic fungal panel was positive for antibodies to *Coccidiodes* (+1:16). The pelvic radiographs showed granulomatous osteomyelitis. Cabby was administered ketoconozole, 5 mg/kg BW, PO, q12h. She was also placed on a diet of canned puppy food (Medi-Cal Development, Veterinary Medical Diets, Guelph, Ontario) to augment her diet, as ketoconozole can act as an appetite suppressant. Cabby responded well to treatment; her temperature was lower (39.2°C), and she was bright and eating well.

Butkiewicz *et al.* (2005) performed community-based longitudinal and cross-sectional studies to evaluate potential risk factors for Coccidioides infection

among 104 healthy 4- to 6-month-old puppies (longitudinal study) and 381 4- to 18-month-old dogs with unknown serostatus (cross-sectional study) living in a region in which the organism is endemic (Pima and Maricopa counties, Arizona). Dogs in the longitudinal study were tested 3 times at 6-month intervals for anticoccidioidal antibodies; dogs in the cross-sectional study were tested only once. Owners of all dogs completed a questionnaire on potential environmental exposures. In the longitudinal study, the relative risk of infection for dogs that were outdoors during the day was 4.9 times the risk for dogs that were kept indoors. Seropositive dogs in the cross-sectional study were 6.2 times as likely to have access to > 1 acre to roam as were seronegative dogs. Logistic regression analysis indicated that the odds of infection increased with age (odds ratio [OR], 1.1), amount of roaming space (OR, 2.4), and walking in the desert (OR, 2.2). Walking on sidewalks had a protective effect (OR, 0.4). Results suggested that in regions in which the organism is endemic, dogs that spend more time outdoors or have more land in which to roam are at greater risk of infection with Coccidioides spp.

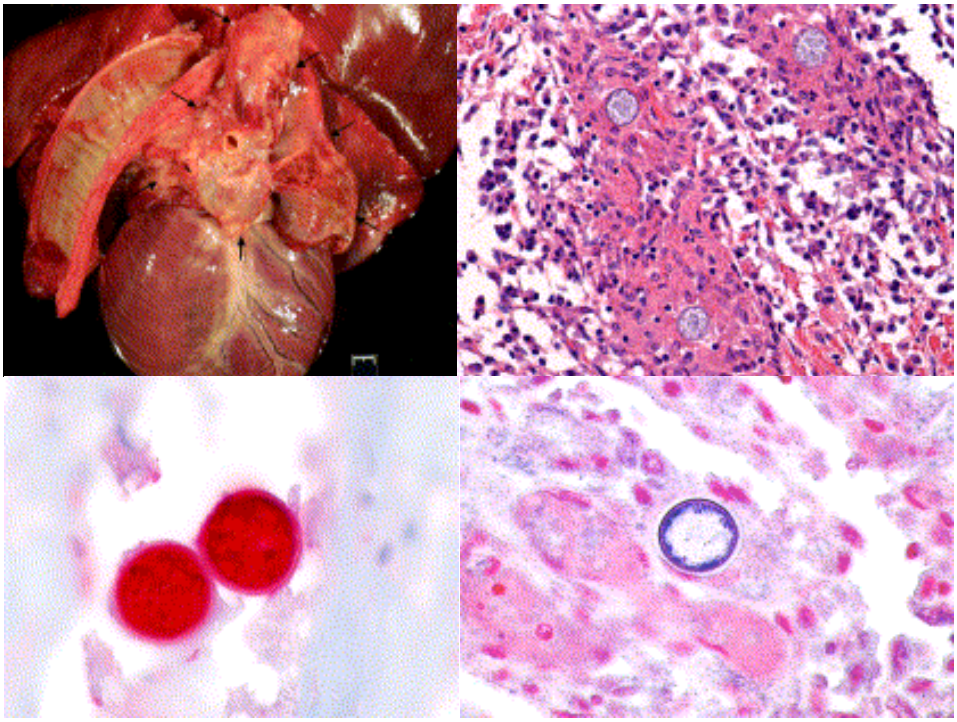
Heinritz *et al.* (2005) carried out a retrospective study to determine the history, clinicopathologic findings, and results of surgery for effusive-constrictive pericarditis associated with Coccidioides immitis infection in 17 client-owned dogs that underwent a subtotal pericardectomy and epicardial excision. Hospital records from May 1999 to June 2003 were reviewed. Data collected included history, clinicopathologic findings, treatments, and outcome. Follow-up information was obtained via recheck examination and by use of standardized telephone interviews with referring veterinarians and owners. All dogs were of large breeds, and most were male (mean age, 4.66 years). Ten dogs had no prior history of C. immitis infection, and 7dogs had chronic infection with C. immitis. Having a chronic C. immitis infection reduced the odds of survival, compared with no previous infection. All dogs had clinical signs of right-sided heart failure. All dogs had serum titers (range, 1:8 to 1:256) for antibodies against C. immitis prior to surgery, and titers were not significantly associated with outcome. Predominant echocardiographic findings were thickened pericardium, reduced right ventricular filling, and pleural or pericardial effusion. All dogs underwent a subtotal pericardectomy and epicardial excision and had fibrosing pyogranulomatous pericarditis in biopsy specimens obtained during surgery. The perioperative mortality rate was 23.5%, and the 2-year postdischarge survival rate was 82%. Surgical treatment via subtotal pericardectomy and epicardial excision was successful at relieving right-sided heart failure in dogs with effusive-constrictive pericarditis secondary to C. immitis infection, but long-term treatment with antifungal agents may still be required.

Shubitz *et al.* (2005) conducted community-based longitudinal and cross-sectional studies to determine the incidence of Coccidioides infection among 124 healthy 4- to 6-month-old seronegative puppies (longitudinal study) and 381 4- to 18-month-old dogs with unknown serostatus (cross-sectional study) residing in a region in which the organism is endemic (Pima and Maricopa counties, Arizona) and estimate the rate of clinical illness. Dogs in the longitudinal study were tested at 6-month intervals for at least 1 year for anticoccidioidal antibodies. Dogs that became ill were evaluated for coccidioidomycosis. Dogs in the cross-sectional study were tested for anticoccidioidal antibodies once, and clinical abnormalities were recorded. 28 of the 104 (27%) dogs that completed the longitudinal study developed anticoccidioidal antibodies. Thirty-two of the 381 (8%) dogs in the cross-sectional study had anticoccidioidal antibodies. Five seropositive dogs in the longitudinal study and 13

seropositive dogs in the cross-sectional study had clinical signs of disease. The remaining seropositive dogs were otherwise healthy and were classified as subclinically infected. Survival analysis indicated that the cumulative probability of infection by 2 years of age was 28%, and the cumulative probability of clinical infection by 2 years of age was 6%. Titers for clinically and subclinically infected dogs overlapped. Results suggested that young dogs living in the study area had a high likelihood of becoming infected with Coccidioides spp, but few developed clinical illness. Serologic testing alone was insufficient for a diagnosis of clinical disease because of the overlap in titers between clinically and subclinically infected dogs.

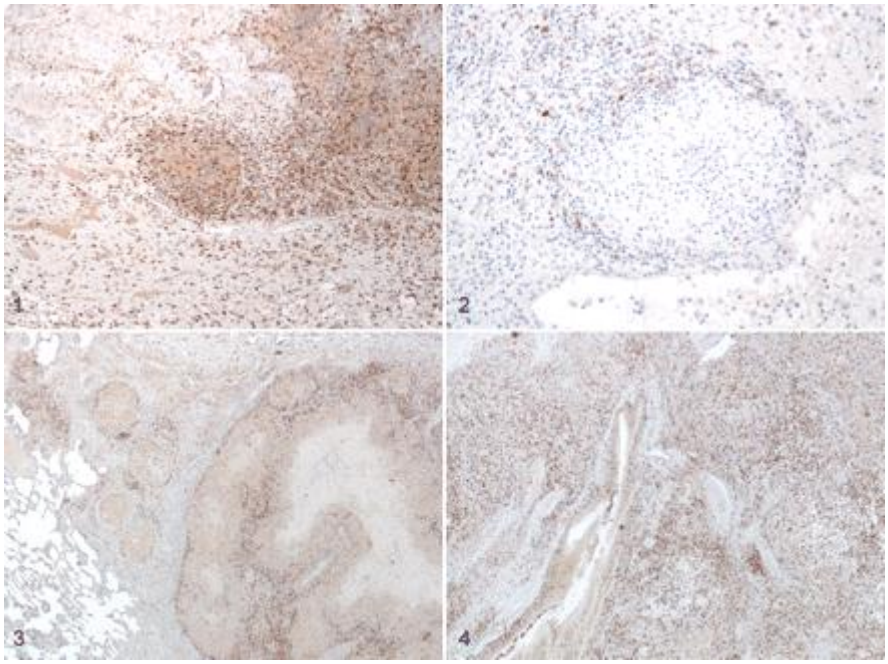
Crabtree *et al.* (2008) carried out a study to determine the relationship of hilar lymphadenopathy tococcidioidomycosis titers for dogs in an endemic area. Thoracic radiographs of 131 dogs from an endemic area were reviewed for evidence of hilar lymphadenopathy. These results were compared with serology results. There was a significant association between hilar lymphadenopathy and a positive serology result ($P < 0.001$). With hilar lymphadenopathy as a predictor of a positive titer result, sensitivity was 28.0%, specificity was 91.5%, the positive predictive value was 43.8%; and the negative predictive value was 84.4%. There was no association between the titer result and gender, age, or weight. The radiographic finding of hilar lymphadenopathy appears to be a useful indicator of coccidioidomycosis infection in an endemic population of dogs supporting the treatment of patients for coccidioidomycosis when hilar lymphadenopathy is present and before obtaining serology results.

Ajithdoss *et al.* (2011) described atypical cases of coccidioidomycosis in 2 dogs presented as heart base masses. An echocardiogram performed in one of the two dogs revealed a large mass at the base of the heart and a computed tomography scan showed that the mass compressed the bronchi, left atrium, aorta and pulmonary arteries. A firm, white or pale yellow mass was found at the base of the heart at necropsy examination in both cases. Microscopical examination of the masses revealed severe, chronic, locally extensive granulomatous or pyogranulomatous inflammation with intralesional spherules consistent with Coccidioides spp. The diagnosis was further confirmed by immunohistochemistry and in-situ hybridization. Coccidioides spp. have been reported to cause pericarditis in dogs, but this is the first description of coccidioidomycosis mimicking a heart-based tumour in dogs.



Ajithdoss *et al.* (2011)

Shubitz *et al.* (2011) characterized the lymphocytic infiltration by studying archived formalin-fixed, paraffin-embedded tissues (subcutis, pericardium/heart, lung, bone, and synovium) from 18 dogs with coccidioidomycosis with immunohistochemistry for CD3 and CD79a. In nearly all lesions, T lymphocytes were more numerous than B lymphocytes and were distributed throughout the lesion with concentration in the periphery of granulomas, whereas B lymphocytes were mostly confined to the periphery of granulomas. The predominance of T lymphocytes in lesions of canine coccidioidomycosis was independent of the tissue evaluated, the number of intralesional organisms, and the nature or severity of the inflammatory response.



1. Synovium; dog No. 8. T lymphocytes are denser in the periphery of granulomas but scattered throughout the inflamed tissue. Anti-CD3 immunohistochemistry with diaminobenzidine as chromogen and hematoxylin counterstain. 2. Synovium; dog No. 8. B lymphocytes are mostly confined to the

periphery of this granuloma. Neutrophils surround the spherule in the center of the granuloma. Anti-CD79a immunohistochemistry with diaminobenzidine as chromogen and hematoxylin counterstain. Lung; dog No. 11. The large granuloma has central coalescing foci of necrosis and is demarcated from adjacent lung by a band of macrophages and fibroblasts. T lymphocytes are most numerous at the periphery of the granulomas. Anti-CD3 immunohistochemistry with diaminobenzidine as chromogen and hematoxylin counterstain. 4. Lung; dog No. 3. This consolidated pulmonary lesion has dense, diffuse T-lymphocytic infiltration with little organization and no distinct borders. The lesion also contains numerous macrophages and neutrophils, with few scattered spherules. Anti-CD3 immunohistochemistry with diaminobenzidine as chromogen and hematoxylin counterstain. **Shubitz *et al.* (2011)**

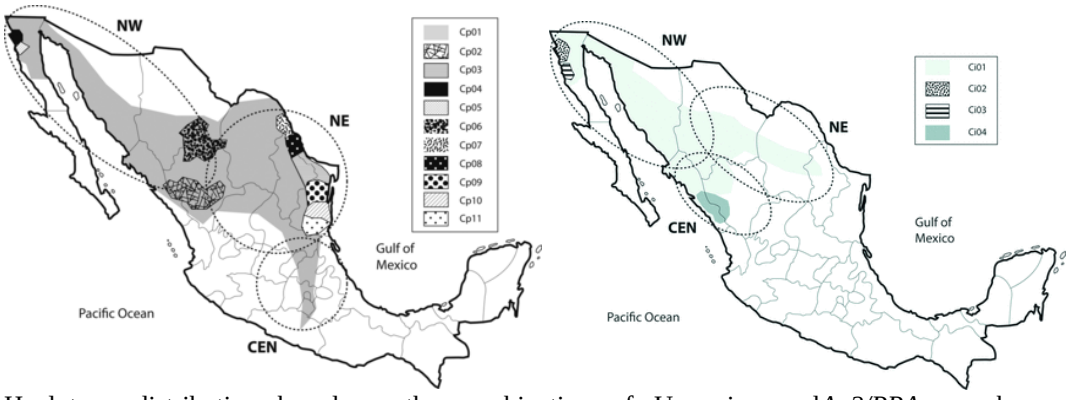
Kirsch *et al.* (2012) evaluated antigen detection as method for rapid diagnosis of coccidioidomycosis in 60 cases diagnosed based on detection of anti-Coccidioides antibodies at titers of 1:16 or more in serum. Controls included dogs with presumed histoplasmosis or blastomycosis, other fungal infections, or non-fungal diseases and healthy dogs. Urine and serum specimens were tested using an enzyme immunoassay for Coccidioides galactomannan antigen. Antibody testing was performed at commercial veterinary reference laboratories. Antigen was detected in urine or serum of 12 of 60 (20.0%), urine only in 2 of 57 (3.5%), and serum only in 11 of 58 (19.0%) dogs with coccidioidomycosis. Antigen was detected in the urine of 3 of 43 (7.0%) and serum of 1 of 37 (2.7%) dogs with histoplasmosis or blastomycosis but not in 13 dogs with other fungal infections (serum, 9; urine, 13), 41 dogswith non-fungal diseases (urine, 41; serum, 18), or healthy dogs (serum, 21; urine, 21). Detection of antigen was an insensitive method for diagnosis of coccidioidomycosis in dogs in which the diagnosis was based primarily upon detection of antibodies at titers of 1:16 or higher, and the highest sensitivity was in serum.

Gautam *et al.* (2013) performed a study is to use the spatial and temporal distribution and clusters of dogs seropositive for VF to define the geographic area in Texas where VF is endemic, and thus presents a higher risk of exposure to humans. The included specimens were seropositive dogs tested at a major diagnostic laboratory between 1999 and 2009. Data were aggregated by zip code and smoothed by empirical Bayesian estimation to develop an isopleth map of VF seropositive rates using kriging. Clusters of seropositive dogs were identified using the spatial scan test. Both the isopleth map and the scan test identified an area with a high rate of VF-seropositive dogs in the western and southwestern parts of Texas (relative risk = 31). This location overlapped an area that was previously identified as a potential endemic region based on human surveys. Together, these data suggest that dogs may serve as sentinels for estimating the risk of human exposure to VF.

Shubitz *et al.* (2013) treated 12 dogs with coccidioidal pneumonia that had been present for an average of three months with 250 mg (5-15 kg) or 500 mg (> 15-30 kg) twice daily for 60 days. Nine dogs completed the course of treatment and seven dogs had improvement in disease based on radiographs, clinicopathological parameters, physical examination findings, and subjective assessment by owners; three dogs had resolution or near resolution of disease. Based on this small study, NikZ shows efficacy to treat naturally acquired coccidioidomycosis and merits further development for trials in humans.

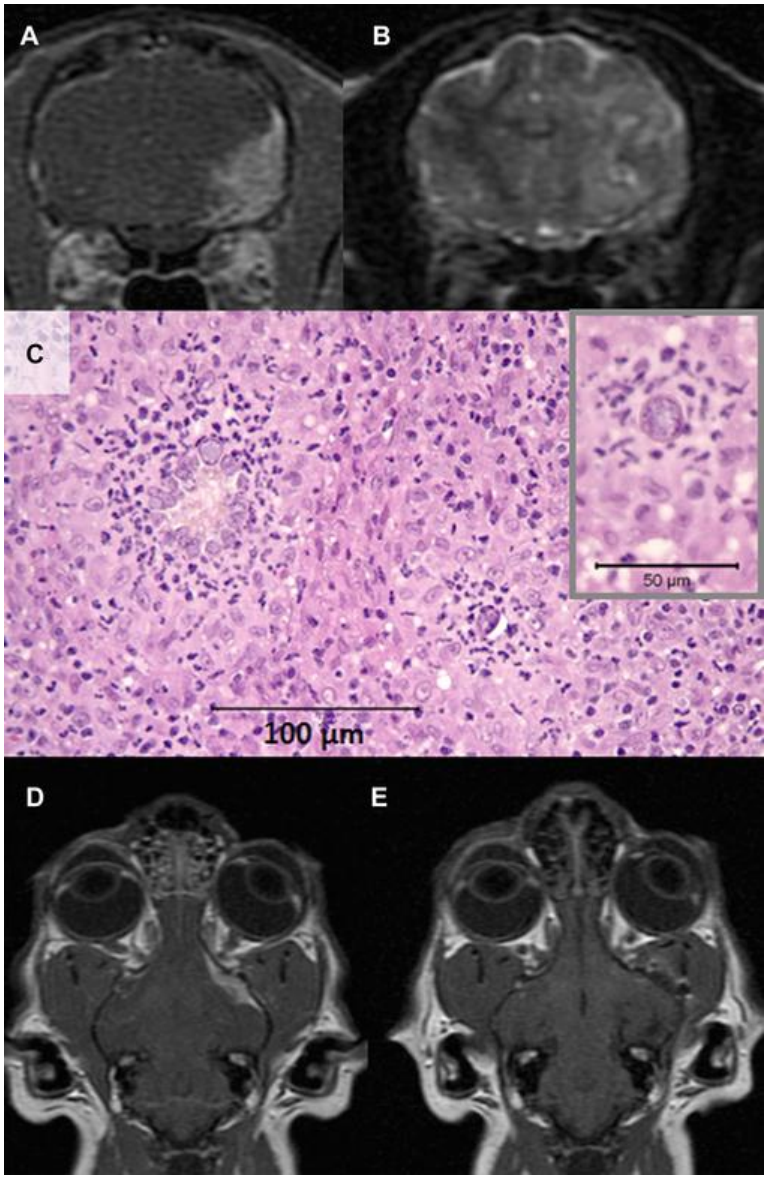
Luna-Isaac *et al.* (2014) identified the predominant Coccidioides species in Mexico, infered their current geographical distribution and explored the correlation between

species and clinical presentation. They collected 154 strains, which were cultured, inactivated, and processed for DNA extraction. Nine microsatellite loci, the Ag2/PRA gene and Umeyama Region were amplified from each isolate. To infer the current geographical distribution of Coccidioides spp. and to establish a correlation between genotype and clinical presentation, they evaluated genetic population structure under the following grouping criteria: putative origin and clinical presentation records. Microsatellite analysis showed that 82% of the isolates corresponded to C. posadasii and 18% were C. immitis. The species identification results obtained using Umeyama region, Ag2/PRA, and microsatellites of five of the isolates were inconsistent with the data collected for the remaining isolates. C. posadasii strains were found primarily in the northeastern region and C. immitis in the northwestern region. However, there was no relationship between clinical presentation and Coccidioides species. The molecular markers used in this study proved to have a high power of resolution to identify the Coccidioides species recovered in culture. While C. posadasii was found to be the most abundant species in Mexico, more detailed clinical records are needed in order to obtain more accurate information about the infections in specific geographical locations.

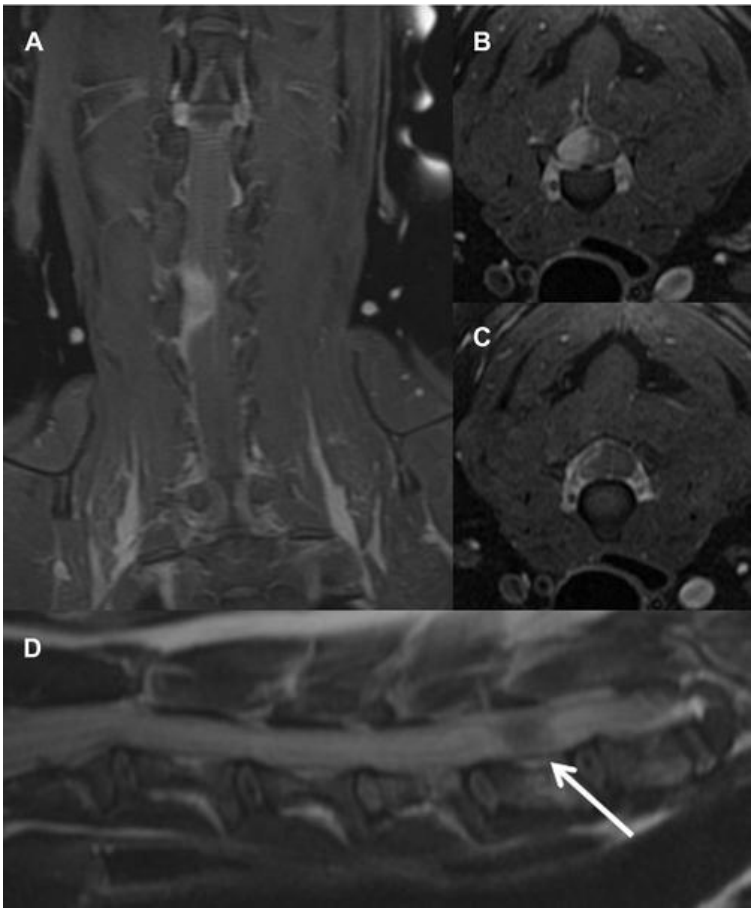


Haplotype distribution based on the combination of U region and*Ag2/PRA* gene by region for *Coccidioides posadasii.* NW, northwest; NE, northeast; CEN, central. Haplotype distribution based on the combination of U region and*Ag2/PRA* gene by regions for *Coccidioides immitis.* NW, northwest; NE, northeast; CEN, central. **Luna-Isaac *et al.* (2014)**

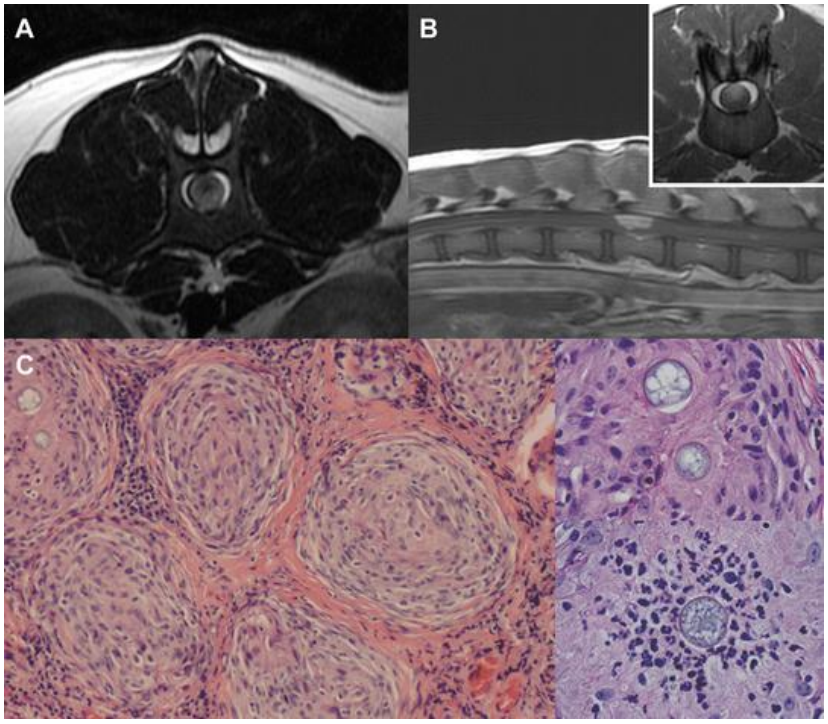
Bentley *et al.* (2015) carried out a cross-sectional retrospective study to describe magnetic resonance imaging (MRI) features and outcome for a group of dogs and cats with solitary CNS Coccidiodes granulomas. Nine canine and two feline cases met inclusion criteria; four diagnosed and treated with surgery and fluconazole and seven diagnosed by serology or cytology and treated medically. Three cases had left Coccidioides endemic areas long before developing neurological disease. The MRI lesions shared many features with neoplastic masses. The extra-axial granulomas often had a lack of a distinct border between the mass and neural parenchyma. Four cases were extra-axial and seven were intra-axial, but distinguishing between extra-axial and intra-axial locations was sometimes challenging. The surgical cases had good outcomes and histology allowed definitive diagnosis. Medically managed patients also had generally good outcomes, with resolution of clinical signs in most cases. Findings indicated that distinction between neoplasia and focal Coccidioides granulomas based on MRI features is likely to be imprecise. Demonstration of the organism by cytology or histology is required for definitive diagnosis.



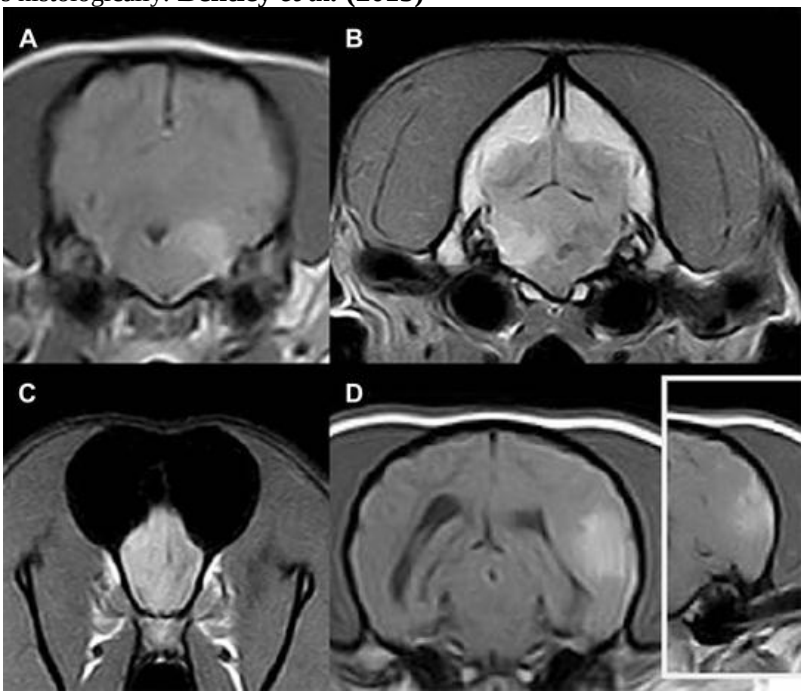
Coccidioides granuloma, caudal frontal lobe of a cat (cat 1). Transverse T1-weighted postcontrast (A) and T2-weighted (B) magnetic resonance images, surgical hematoxylin and eosin histology (C), dorsal T1-weighted postcontrast (D) and matching 5-day postoperative (E) images. The lesion is markedly contrast enhancing and extra-axial. The border between the contrast-enhancing mass and adjacent brain varies from regular and sharply defined to irregular and indistinct (A and D) and dural tails are present. Perilesional T2-hyperintensity (B) is severe and extends to the corona radiata. In (C), note a pyogranulomatous reaction consisting of epithelioid macrophages, multinucleated cells and neutrophils surrounding a cluster of spherules with double-refractile walls; and a spherule (inset) with double-refractile walls and endospores. Postoperative imaging revealed complete excision of the contrast-enhancing mass and resolution of mass effect (E); mild-moderate postoperative contrast enhancement is present in the temporalis muscle and surrounding the foam placed into the craniectomy defect **Bentley *et al.* (2015)**



Cervical spinal *Coccidioides* granuloma in a dog (dog 1). Dorsal T1-weighted postcontrast image (A), consecutive transverse T1-weighted fat saturation postcontrast images at the level of C6 (B and C), and parasagittal T2-weighted image (D). In (A), the lesion appears most likely intradural extramedullary, with an apparently broad-based contact with dura mater. However, in (B and C), the lesion appears to involve spinal cord tissue itself; much of the contrast-enhancing area appears intramedullary. The final interpretation was an intramedullary lesion with difficult differentiation from an intradural-extramedullary lesion; an intramedullary mass was present surgically. In (A–C), the lesion is markedly contrast-enhancing, and the border with the spinal cord tissue is irregular and varies between distinct and indistinct. In (D), the lesion (arrow) is hypointense to normal gray matter; note the extensive perilesional T2-hyperintensity both cranial and caudal. **Bentley *et al.* (2015)**



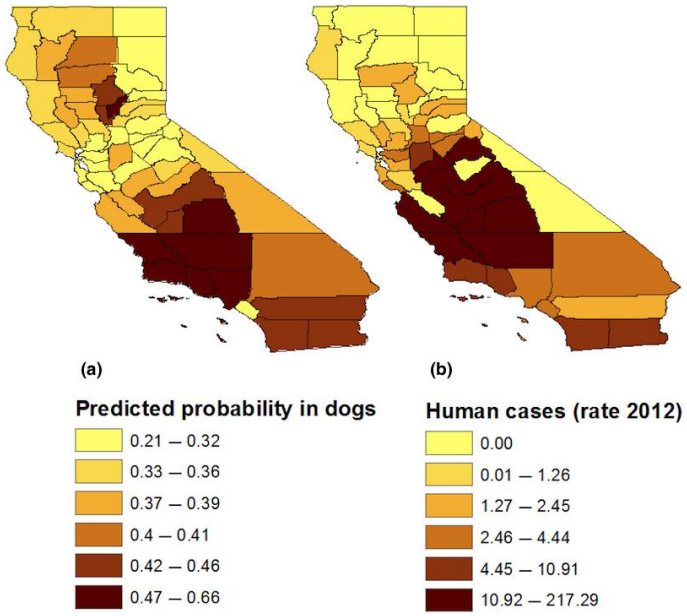
Lumbar spinal *Coccidioides* granuloma in a dog (dog 2). Transverse T2-weighted image at the level of L2 (A) and sagittal T1-weighted postcontrast image with transverse image inset (B). On MRI, this lesion was considered either intradural extramedullary or intramedullary but very superficial. T2-weighted images indicated a T2-hypointense lesion that was intramedullary; however postcontrast T1-weighted images mainly supported an intradural-extramedullary lesion. Note that lesion borders are sharply defined in (B), but are less exact in the inset. Surgical HE histology (C): multiple coalescing nodules of foamy macrophages surrounded by neutrophils and lymphocytes were observed, sometimes centered on large, spheroid organisms with a double refractile wall and clusters of neutrophils. The mass had appeared superficial to the pia mater surgically, and no spinal cord tissue was observed in the biopsy samples histologically. **Bentley *et al.* (2015)**



Intracranial *Coccidioides* granulomas in four dogs, transverse T1-weighted postcontrast magnetic resonance images. Note that none of the lesions have a sharply defined border between the contrast-enhancing granuloma and the adjacent brain tissue; the border varies between distinct and indistinct (A, B, and D) or completely indistinct (C). The granulomas in (A and B) were both considered most likely

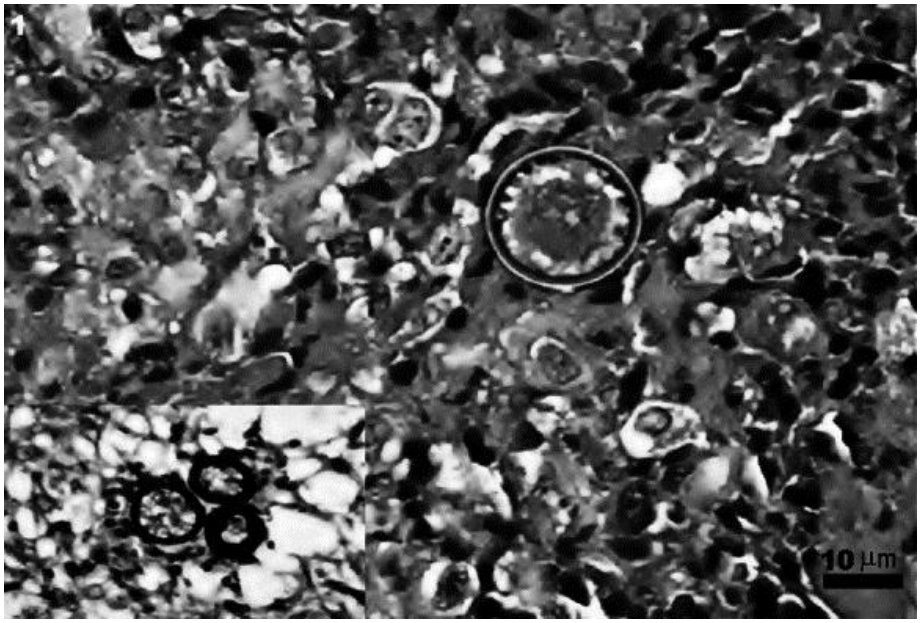
extra-axial, however the granuloma in (A) was also considered likely to be invading the adjacent brainstem and cerebellar tissue. The granulomas in (C and D) were both overall considered intra-axial; however, the distinction between intra-axial and extra-axial was incomplete, with certain images more suggestive of an extra-axial lesion (inset, D). Note also the enhancement of the neighboring meninges in (D). **Bentley *et al.* (2015)**

Grayzel *et al.* (2016) identified 41 dogs seen at the Veterinary Medical Teaching Hospital at the University of California, Davis, between 2005 and 2013 with coccidioidomycosis together with a control population of 79 dogs. Owners were surveyed about potential risk factors including younger age, digging behaviour, and travel to Arizona or the California central valley. Risk factors were analysed using logistic regression analysis. Outcomes were used to generate a risk map for coccidioidomycosis in California. There was a significant correlation between the reported rate of coccidioidomycosis in humans and the risk map for canine coccidioidomycosis in California, supporting the idea of dogs as sentinels for emerging geographic areas for coccidioidomycosis in humans.

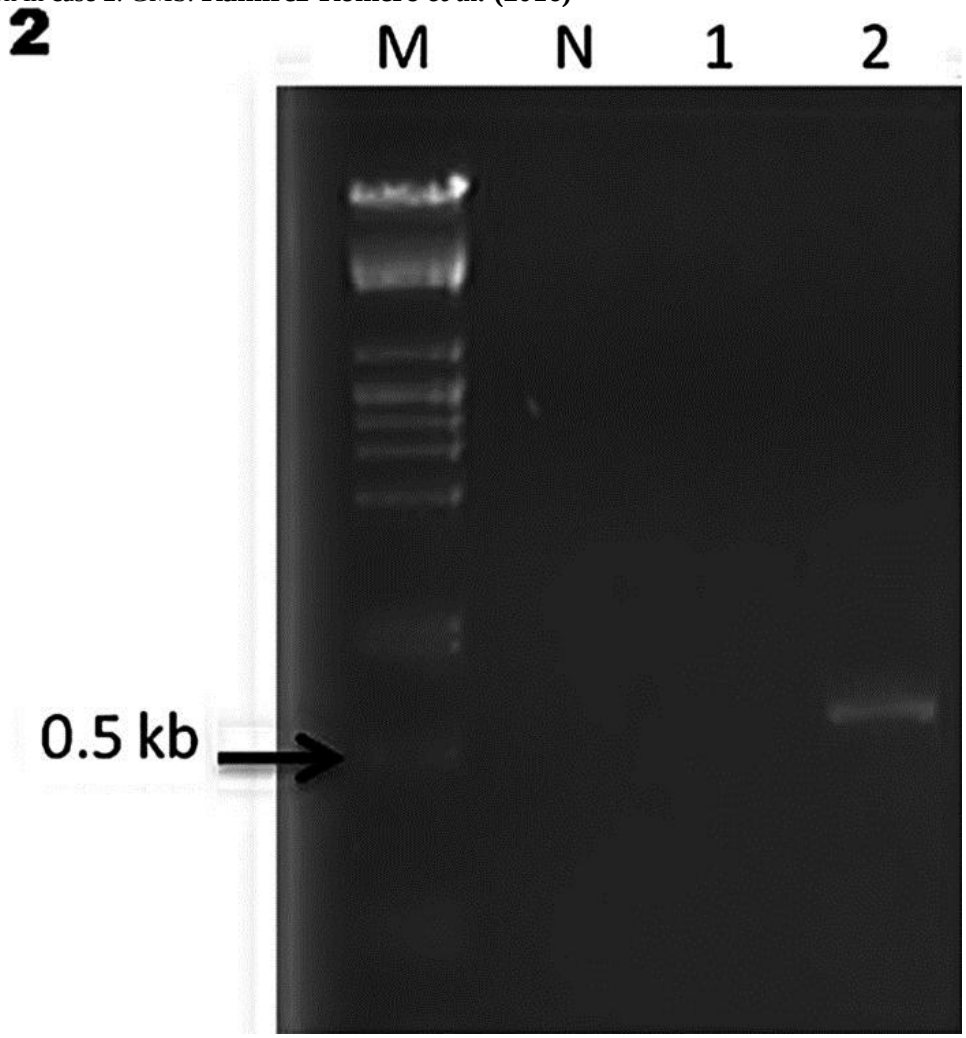


Spatial distribution of the relative predicted probability of coccidioidomycosis in dogs (a) and of the human incidence rate during 2012 (b). Categories were selected using the natural breaks or Jenks optimization method (Jenks, 1967) implemented in ArcGIS10.2, **Grayzel *et al.* (2016)**

Ramírez-Romero *et al.* (2016) described three cases of granulomatous inflammation caused by Coccidioides spp. which were masquerading malignancies in dogs (0.39 %). The presumptive diagnoses in these cases were osteosarcoma, lymphoma and neurofibroma, respectively. A PCR assay employing tissues in paraffin blocks resulted positive for C. posadasii in one of these cases.



Dog with suspicion of neurofibroma. There is one spherule with thick and refractile cell wall. The endospores contained within are ill defined. The inflammatory reaction is composed by epithelioid macrophages and lymphocytes; the proliferation of fibrous connective tissue is prominent. H&E *bar* 10 µm. The *inset* depicts the special stain with three organisms in a pyogranulomatous reaction in case 2. GMS. **Ramírez-Romero *et al.* (2016)**

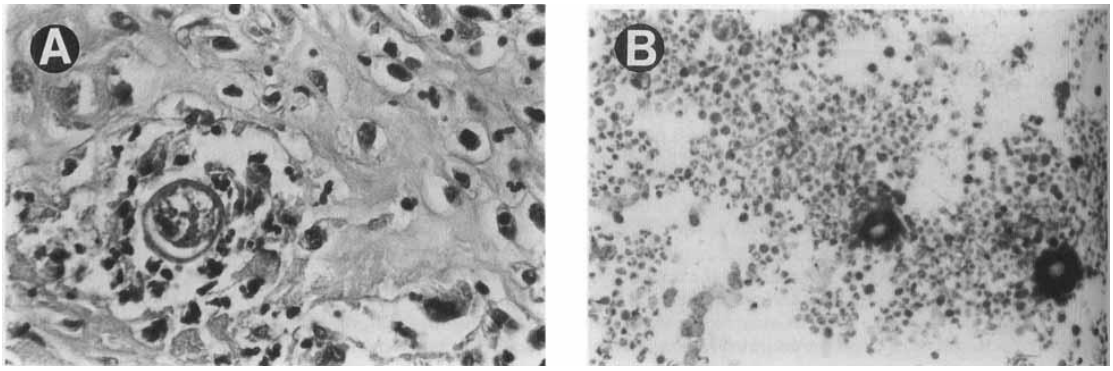


PCR amplification from two paraffin-embedded tissues. *Lane M*, DNA molecular weight marker, *lane N*, negative control. *Lane 1*, sample case 2 and *lane 2*, sample case 3 with an amplicon of 634 pb. The result corresponds to *C. posadasii*, **Ramírez-Romero *et al.* (2016)**

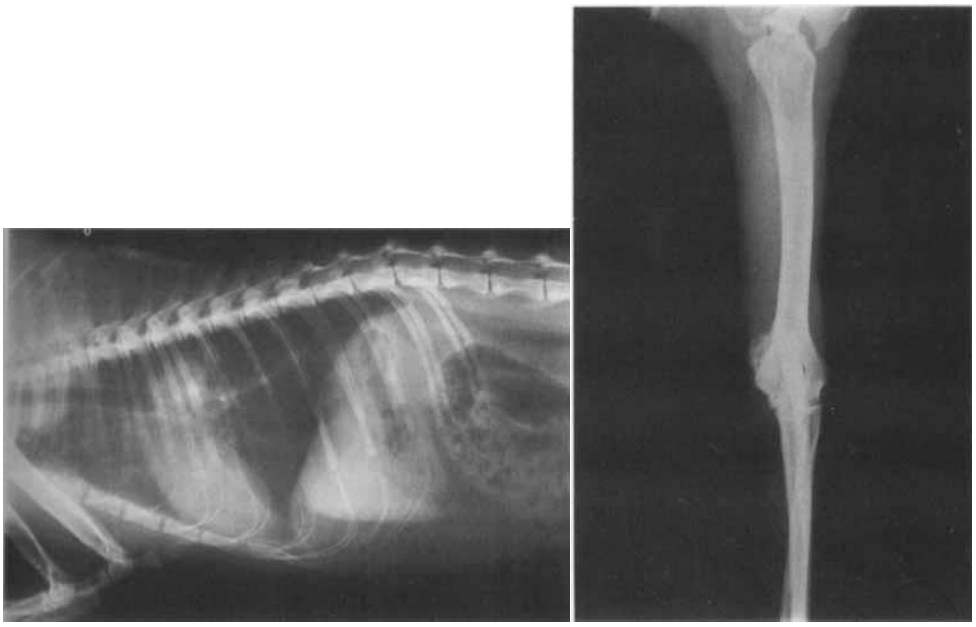
2.5.2. Reports on coccidioidomycosis in cats

Angell *et al.* (1985) performed enucleation of the right eye on a 12-year-old male Persian cat when therapy for uveitis failed. Histologic examination of the anterior and posterior chambers and the vitreous led to a diagnosis of endophthalmitis caused by Coccidioides immitis infection. The primary focus of infection was not determined. Latex particle agglutination, agar gel immunodiffusion, and complement fixation gave negative results for Coccidioides immitis antibody.

Greene and Troy (1995) diagnosed coccidioidomycosis in 48 cats. Forty-one cases were identified within a period of 3 years. Coccidioides immitis was revealed by cytological or histopathological examinations, or culture in 70% of cats. The remaining 30% of cases were diagnosed by appropriate clinical signs, radiographic lesions, and serological test results. The average age of affected cats was 6.2 years with a median age of 5.0 years. Fifty-four percent (n = 26) were female and 46% (n = 22) were male. Domestic shorthaired and longhaired breeds comprised 89% (n = 41) of affected cats. Sixty-seven percent of cases were diagnosed during the 6-month period of December through May. Cats infected with C immitis were presented for evaluation of dermatologic (56%), respiratory (25%), musculoskeletal (19%), and neurological or ophthalmologic signs (19%). Fever, inappetence, and weight loss were present in 44% of the cats. Duration of clinical signs before diagnosis was less than 4 weeks in 85% (n = 42) of cats, with an average of 3.8 weeks and a median of 2 weeks. Agar gel immunodiffusion tests were positive in all 39 cats tested at sometime during the course of their disease. Hyperproteinemia (greater than 7.9 g/dL) was present in 52% (10/23) of cases. The majority of cats (n = 39) were negative for feline leukemia virus. Antibodies to feline immunodeficiency virus were absent in the 19 cats tested. Ketoconazole was the most common antifungal agent used to treatcats with coccidioidomycosis. Duration of treatment ranged from less than 1 week to 43 months. Thirty-two cats are currently asymptomatic, with or without treatment. Eleven cats died or were euthanized. **Greene and Troy (1995)**

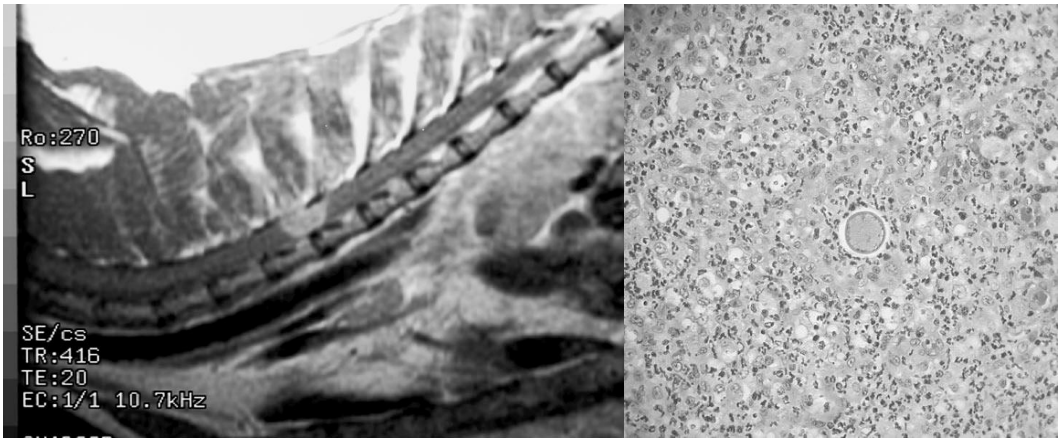


[A) Histopathology of a lymph node showing a granulomatous reaction and the *Cimmiris* organism (Silver stain; original magnification 1000 x). [B) Cytology of a draining skin lesion depicting *Cimmifis* organism [H&E stain: original magnification 400 X). **Greene and Troy (1995)**



Lateral thoracic radiograph in a cat with respiratory distress.Note the marked hilar lymphadenopathy.Anteroposterior view of the distal humerus in a cat withan osteoproductive lesion due to coccidioidomycosis. **Greene and Troy (1995)**

Foureman *et al.* (2005) reported a 6-year-old 5.5-kg female spayed domestic short-haired cat from Tucson, AZ, with a ˜5-day history of progressive pelvic limb weakness. Magnetic resonance image (MRI, T1 weighted image with gadolinium contrast) showed contrast-enhancing lesion in the spinal cord at the level of the 3rd and 4th thoracic vertebrae. Histopathology of mass removed from the spinal cord of showed the prominent fungal spherule on a background of neutrophils and The case was diagnosed as Spinal cord granuloma due to Coccidioides immitis.



Magnetic resonance image (MRI, T1 weighted image with gadolinium contrast) showing contrast-enhancing lesion in the spinal cord at the level of the 3rd and 4th thoracic vertebrae (0.5 T Signa MRI unit).Histopathology of mass removed from the spinal cord of this cat. Note the prominent fungal spherule on a background of neutrophils and macrophages. **Foureman *et al.* (2005)** Abstract

Gaidici and Saubolle (2009) reported an unusual case of coccidioidomycosis in the arm of a 37-year-old veterinary technical assistant complaining of an initial right thumb swelling without pulmonary symptoms. The patient had been bitten on the hand by skinny, stray cat she was examining at an animal clinic approximately 2 weeks previously. The bite wound at first formed only a small eschar but then

progressed to increased erythema, swelling, and tenderness. The cat had died shortly after having bitten the patient. A necropsy was performed, and splenic masses were evaluated by histopathology as numerous "pyogranulomas" with massive numbers of spherules present. The final diagnosis was multifocal granulomatous splenitis and disseminated disease, with *Coccidioides* spp. as the etiologic organisms. The patient responded to fluconazole therapy and remained asymptomatic at 2 months after cessation of therapy.

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3. Histoplasmosis in cats and dogs

3.1. Introduction:

- Histoplasmosis is the most commonly diagnosed major systemic mycosis in dogs and the second most commonly reported fungal infection in cats. The causative organism, ***Histoplasma capsulatum***, is endemic in 31 of the 48 contiguous US states and has a worldwide distribution. Histoplasma organisms enter the body via inhalation or, possibly, ingestion. They are phagocytized by macrophages and can be disseminated via the bloodstream or lymphatic system to the reticuloendothelial and gastrointestinal (GI) systems and, sometimes, the bones, skin, eyes, or brain. Clinical signs are often nonspecific, including lethargy, weight loss, and inappetence, although respiratory or GI signs may help localize the infection. Definitive diagnosis requires identification of H. capsulatum on cytology or histopathology. However, antigen testing may be useful in animals in the future. Itraconazole is the treatment of choice. The prognosis is fair for animals with pulmonary histoplasmosis and guarded to poor for those with GI or disseminated disease (**Lin Blache, 2011**).

- Histoplasmosis is distributed in tropical, subtropical and temperate zones of the world. The disease is one of the imported mycoses in Japan. To date, although more than 30 human and one canine case of histoplasmosis have been reported in Japan, some including that of the canine might have been infected domestically, since the patients have no history of going abroad. The pathogen of histoplasmosis is thus believed to be present in our country **(Sano *et al.*, 2001)**.

- The lesions of **histoplasmosis in dogs in Japan** differ from those in dogs in North America. Affected dogs in Japan have had multiple granulomatous or ulcerated foci in skin or gingiva and have not had pulmonary or gastrointestinal lesions. **(Ueda *et al.*, 2003)**.

- Histoplastnosis is caused by the dimorphic fungus *Histoplasma capsulatum* which grows intracellularly as a yeast form with an affinity for cells of the monocyte macrophage system. The disease in animals and man varies from a benign local infection of the respiratory tract to a fatal generalised disease with extensive dissemination of the organism, especially in immunocompromised patients. The majority of infections are asymptomatic or mild. Human and dog appear to be the species most susceptible to clinical histoplasmosis.

- In Australia a small number of human cases of clinical histoplasmosis have been reported and skin test surveys have shown a low prevalence of human Although there have been two suspected cases of histoplasmosis reported in animals in Australia, both in dogs, neither case could be confirmed by culture or immunohistocheniistry.

- In dogs it is usually a disease of the pulmonary, gastrointestinal or lymphoreticular system. Infection occurs by inhalation of the spores from the mycelial (hyphal) form of the fungus which naturally grows in soil, particularly soil enriched with the guano of bats and birds.' There is also evidence of primary gastrointestinal infection in dogs without detectable pulmonary manifestations.

3.2. Clinical signs (Guptill and Gingerich, 2008)

The clinical signs seen in dogs and cats with histoplasmosis vary depending on which form of infection has taken root.

3.2.1. Pulmonary histoplasmosis

Acute pulmonary histoplasmosis in dogs and cats is thought to be uncommon. In dogs, this form is characterized by a rapid onset of dyspnea and cyanosis. Dogs with chronic pulmonary histoplasmosis are presented for evaluation of a mild, chronic cough and a history of weight loss and inappetance. Coughing may be due to partial airway obstruction secondary to hilar lymphadenopathy. Most affected cats have disseminated disease, and, even with evidence of pulmonary involvement, they seldom cough. Other clinical signs of respiratory tract involvement include dyspnea and tachypnea.

3.2.2. Canine disseminated histoplasmosis

Acute disseminated histoplasmosis affects multiple organs, with a history of illness of only a few days' duration in experimental animals. Gastrointestinal involvement was reported in 28 of 36 (78%) dogs with chronic disseminated histoplasmosis. Large bowel diarrhea, characterized by hematochezia, mucus, and tenesmus, is common. With disease progression into the small intestine, diarrhea may become watery and voluminous, and protein-losing enteropathy may occur. In addition to gastrointestinal signs, common nonspecific clinical signs of chronic disseminated histoplasmosis in dogs include weight loss, inappetence, and fever of unknown origin that is nonresponsive to antibiotic therapy. Abnormal lung sounds, with or without accompanying cough or dyspnea, are noted in fewer than 50% of dogs with disseminated histoplasmosis. Infiltration of the organism into other organs, including the liver, spleen, and bone marrow, may result in hepatomegaly, splenomegaly, or pallor associated with anemia. Less commonly reported clinical findings of canine disseminated histoplasmosis include:

- Vomiting
- Peripheral lymphadenopathy
- Polyarthropathy or fungal osteomyelitis
- Ulcerated dermal nodules, sores on footpads, or draining abscesses
- Neurologic signs, including seizures and vertical nystagmus
- Oral lesions, including gingival nodules and lingual erosions
- Conjunctivitis, chorioretinitis, retinal detachment, or optic neuritis
- Icterus
- Pleural and peritoneal effusion

3.2.3. Feline disseminated histoplasmosis

Clinical signs of feline disseminated histoplasmosis are often chronic and nonspecific. Weight loss, pale mucous membranes, lethargy, pyrexia, anorexia, and dehydration were the predominant findings cats with disseminated histoplasmosis. Granulomatous chorioretinitis occurs, possibly because of *H. capsulatum* in the choroid and retina. Additional ocular involvement may include retinal hemorrhage, optic neuritis, and fungal granulomas. About one-third of affected cats may have lymphadenopathy, splenomegaly, or hepatomegaly, occasionally accompanied by icterus. In contrast to

dogs, primary intestinal histoplasmosis is unusual in cats; in one cat, this form of histoplasmosis caused vomiting and watery diarrhea with hematochezia. Cutaneous lesions, infrequently reported, are nodular or ulcerated and may exude serosanguineous fluid. Neurologic signs also occur. Rare clinical findings include nasal polyps and oral and lingual ulceration.

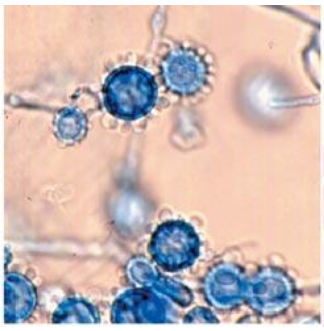
3.3. Aetiology

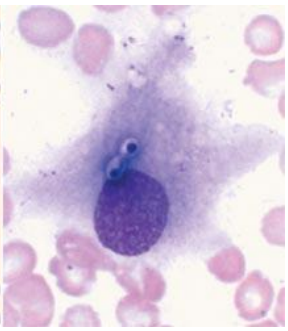
Histoplasma capsulatum Darling 1906

Synonyms: *Cryptococcus capsulatus* Castellani et Chalmers, 1910-
Posadasia capsulate Moore 1934-
Histoplasma pyriforma Dodge 1935
Perfect stage: *Emmonsiella capsulate* KWON-CHUNG 1972

On Sabouraud dextrose agar, at temperature below 35C, the fungus is slow growing, usually requiring 2-6 weeks. The growth initially appears moist and waxy, then aerial mycelium develops , which is gray to white in colour and turns to buff or dark with age. Microscopically, the hyphae are small, hyaline and septate. They bear both micro- and macroconidia. The microconidia are small, round, sessile or stalked, 2-6 microns in diameter. The macroconidia, which are diagnostic, are round to pear-shaped, 8-14 microns in diameter, tuberculate and born on narrow conidiophores. In tissues, the fungus exists in the form of small, round or oval yeast-like cells, 1-4 microns in diameter. They are intracellular, often filling the cytoplasm of mononuclear and occasionally polymorphonuclear cells.


H. capsulatum at 25^{o}C

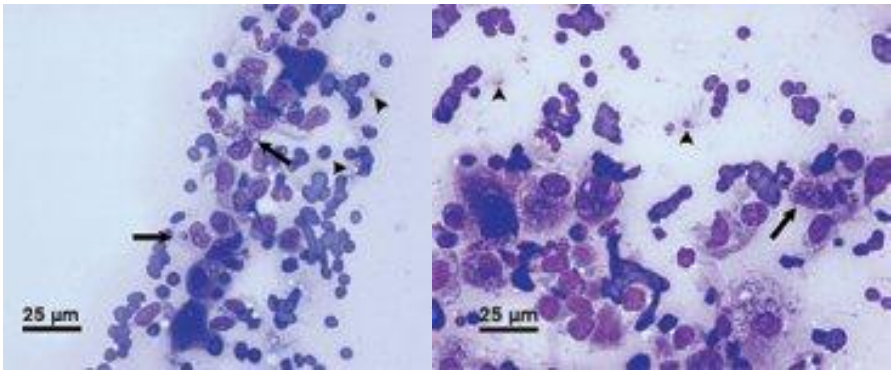

tuberculate macroconida


intracellular yeast cells

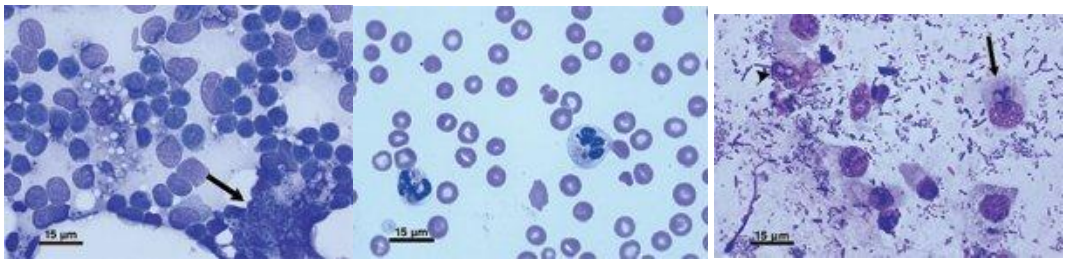
3.4. Diagnosis

3.4.1. Direct microscopic examination:

Pus, discharges and biopsy material are examined after Giemsa staining. The yeast cells are found within monocytes or macrophages. Cells are ovoid,with the bud at the smaller end, with cell wall as a halo of unstained material. In equine histoplasmosis, microscopical examination of Giemsa-stained smear from the pus reveals the presence of double-contoured , spherical, oval to pear-shaped yeast cells, 2.5-3.5 by 3-4 microns with granular cytoplasm.



A liver aspirate from a cat. Three dark-purple hepatocytes are seen among multiple macrophages, some of which contain yeast (arrows). Several yeast are noted free in the background (arrowheads) of erythrocytes and a small amount of finely granular protein (Diff-Quik, 60X), A lung aspirate from a cat. Multiple foamy activated macrophages are noted, including a single giant cell on the far left. One macrophage contains several yeast (arrow). Several yeast are noted in the background (arrowheads) of erythrocytes and a small amount of finely granular protein. Also note the morphologically normal respiratory epithelial cells with their tufts of apical cilia to the right of the micrometer (Diff-Quik, 60X). (**Guptill and Gingerich, 2008**)



A lymph node aspirate from a cat. A single large macrophage is densely packed with yeast (arrow), and numerous yeast organisms are free in the background. Note the clear area surrounding the yeast, caused by shrinkage that occurs during fixation. Numerous small lymphocytes and a few bare nuclei are also present (Diff-Quik, 100X).A canine peripheral blood smear demonstrating two segmented neutrophils that contain several budding yeast (modified Wright's stain, 100X). A rectal scraping from a dog. A mixture of individualized epithelial cells and macrophages are seen in a moderately heavy background of mixed bacteria. Yeast are seen within macrophages (arrow) and are free in the background (arrowhead) (Diff-Quik, 100X). (**Guptill and Gingerich, 2008**)

3.4.2. Isolation and identification:

Samples are cultured on blood agar, brain heart infusion agar and Sabouraud agar and incubated both at room temperature and at 37 C. The fungus grows slowly at room temperature and may need a period of 6-12 weeks. Plates incubated at 37 should be examined for the growth of yeast colonies. Microscopically, the tuberculate macroconidia is characteristic for *H. capsulatum*, but similar structure is formed by Sepedonium which is not diphasic fungus. The yeast phase is differentiated from Paracoccidioides because of the lack of the mariner's wheel and from Blastomyces, which form broad-based budding cell. Conversion from mould to yeast phase and vice versa is diagnostic.

3.4.3. Serology

The EIA (antigen), a radioimmunoassay for histoplasma polysaccharide antigen, has been developed. This is a proprietary test so the evaluation of the results have been questioned. Urine testing, which detects antigens to *H. capsulatum,* is the most sensitive test for disseminated histoplasmosis. It is also used to monitor antigens levels during treatment. It is however less useful in chronic histoplasmosis

3.4.4. Histopathology

Histoplasma capsulatum var. *capsulatum* in tissue is an oval 2- to 4-μm yeast that may show narrow-based buds. With H&E stain, the basophilic yeast cytoplasm is separated from the surrounding tissue by a clear zone corresponding to the cell wall. The cell wall is highlighted with GMS and PAS stains. Because the yeasts are initially ingested by macrophages, they appear to be clustered. African histoplasmosis shows similar clustering inside phagocytic cells (particularly large multinucleated giant cells), but the yeasts are larger (8 to 15 μm in diameter) than with *H. capsulatum* and may be pigmented.

3.5. Reports:

3.5.1. Reports on histoplasmosis in dogs:

Silva-Ribeiro *et al.* (1987) mentioned that 7 of 73 mongrel dogs in Rio de Janeiro gave positive results in skin tests with a polysaccharide mycelial antigen from ***Histoplasma capsulatum***. Five of the positive reactors were necropsied and four of them had disseminated histoplasmosis proved by histopathology and culture. Four healthy puppies exposed for 10 min to soil at the site of a known outbreak of histoplasmosis developed symptoms and died 7-14 days after exposure with fulminant histoplasmosis. These experiments show the value of dogs as epidemiological indicator of histoplasmosis and as experimental models for the disease.

Clinkenbeard *et al.* (1988a) diagnosed **disseminated histoplasmosis** in a 10-year-old dog that had chronic diarrhea, weight loss, fever, and anemia. The diagnosis was based on detection of Histoplasma organisms in circulating neutrophils, monocytes, and eosinophils. The dog had severe histoplasmal fungemia, which may have been caused by treatment with prednisolone.

Clinkenbeard *et al.* (1988b) reported diarrhea, intestinal blood loss, anemia, and lethargy as predominant clinical findings in 12 dogs with disseminated histoplasmosis. Young dogs were affected most commonly, with 6 dogs being 1 to 3 years old. A diagnosis of **disseminated histoplasmosis** was established on the basis of histologic or cytologic detection of Histoplasma organisms in intestinal or rectal mucosa in 7 dogs, in circulating leukocytes in 5 dogs, in bone marrow in 3 dogs, and in multiple tissues at necropsy in 1 dog (4 dogs had Histoplasma organisms detected in greater than 1 site). Anemia was detected in 10 dogs (PCV less than 20% in 3 dogs), and the anemia was inadequately regenerative or nonregenerative in 7. Hypoalbuminemia was detected in 9 dogs, and serum albumin concentrations were low (less than 1.0 g/dl) in 4 of the 9 dogs. Of

5 dogs treated with ketoconazole, 2 were in remission for greater than or equal to 1 year. Corticosteroid therapy may have exacerbated the disease in 4 dogs. Histoplasma infection of multiple organs was detected in 5 necropsied dogs.

Davies and **Colbert (1990**) reported highly symptomatic pulmonary histoplasmosis with diffuse infiltrates occurring simultaneously in a man and a dog

Kowalewich *et al.* (1993) identified ***Histoplasma capsulatum*** organisms by cytologic evaluation in the **thoracic and abdominal effusions** of a 5-year-old sexually intact male Cocker Spaniel that was referred because of anorexia and lethargy. Treatment with **amphotericin B and ketoconazole** was instituted. The dog developed respiratory arrest, a complication of the disseminated disease, and died. Necropsy findings included pleural effusion, hepatomegaly, and enlarged tracheobronchial, hilar, mediastinal, and mesenteric lymph nodes. Granulomas containing periodic acid-Schiff (PAS)-positive yeast-like organisms identified as H capsulatum were seen in the lungs, liver, and lymph nodes. The lymphatic vessels were dilated, and fibrosis of the portal and periportal regions of the liver was noticed. Identification of Histoplasma organisms by cytologic examination of pleural and abdominal effusions is a rare laboratory finding and can provide a minimally invasive and inexpensive definitive diagnosis of histoplasmosis.

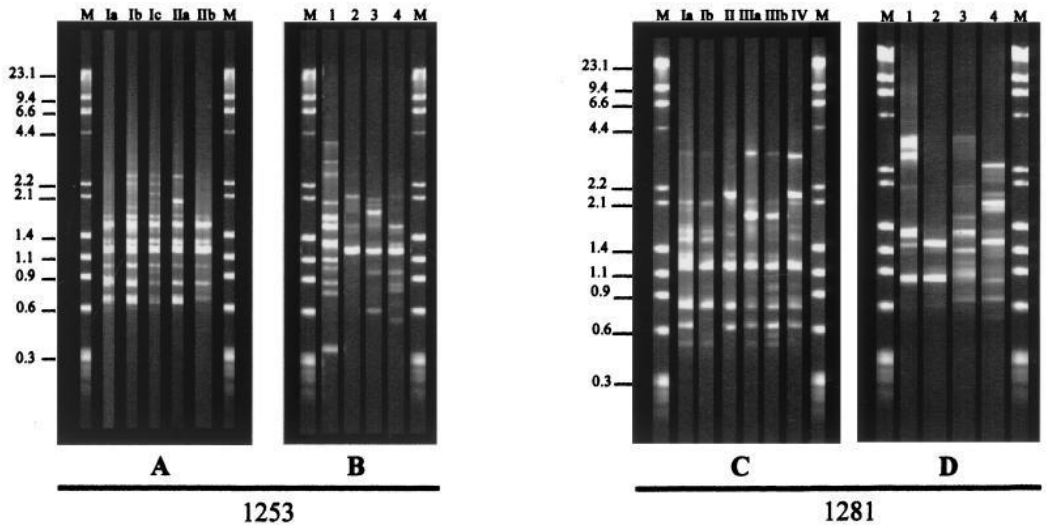
Mackie *et al.* (1997) described what they believed to be the first confirmed case of histoplasmosis in a non-human animal in Australia. In August 1995 a formalin-fixed skin biopsy was collected from the foot of a 10-year-old male Rotnveiler-Doberman cross dog from Gordonvale, south of Cairns. The dog had a 12- month history of multiple dermal nodules up to 2.5 cm diameter, some of which were ulcerated. The lesions had failed to resolve despite several extended courses of treatment with a number of antibiotics. The dog had also suffered moderate weight loss and intermittent diarrhoea. The dog had never travelled out of Australia. The owner kept chickens in his backyard but the dog had not had access to other specific avian habitats or to bat caves. Histologically there was a severe pyogranulomatous deep dermatitis with infiltration of much of the superficial dermis and to a lesser extent the deep dermis by macrophages and smaller numbers of neutrophils. Within the inflammatory reaction there were numerous round to oval yeasts with frequent narrow based buds, most of which appeared to be within macrophages. The organisms were approximately 2 to *4* pm in diameter and consisted of a spherical, lightly basophilic central body surrounded by a clear halo. The cell wall was selectively stained using Gomori's methcnaniine-silver method. Replicate sections of skin were examined by direct imrnunofluorcscence staining using conjugates prepared at the Centers for Disease Control. The yeasts stained using the conjugate for *H capsulatum* but failed to stain using the conjugates for *Cryptococcus neoformans."* Serum collected from the dog in January 1996 was found to contain the histoplasmosis diagnostic M precipitin when tested by the immunodiffusion test (Irnm?', Immuno-Mycologics Inc, Norman, OK, The skin lesions on this dog resolved completely following a 6-month course of treatment with ketaconazole at 20 mg/kg/day. The dog also gained weight and appears to be now in good health, 8 months after treatment ceased.

Kagawa *et al.* (1998) reported an 8-year-old, female mongrel dog with granulomatous lesions in the skull **skin and gingiva** of the left mandible. The lesions were macroscopically seen as grayish white papular granulomas, and microscopically consisted to numerous swollen macrophages and a few neutrophils without fibro-

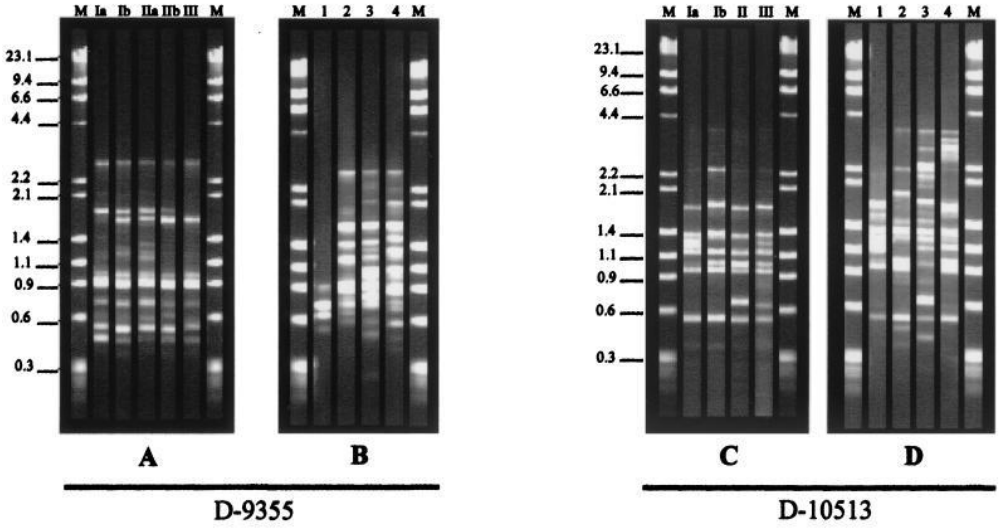
caseous necrosis. Macrophages contained many small oval or round-shaped yeast-like cells and a few rod-shaped organisms indicating a narrow based budding in their cytoplasm. The yeast-like cells were 2-5 microns (average 3.5 microns) in diameter, and appeared as a central, spherical, lightly basophilic body surrounded by a clear zone or "halo". The cell wall and central body were stained by the periodic acid-Shiff, Grocott's methenamine silver impregnation, or Gridley fungus method. **Immunohistochemically,** yeast-like cells were positive to anti-histoplasma yeast antibody, and rod-shaped organisms were positive to anti-histoplasma mycelial antibody.

Schulman *et al.* (1999) carried out a study to examine use of corticosteroids in treating 16 dogs with airway obstruction secondary to hilar lymphadenopathy caused by chronic histoplasmosis. Records for dogs with airway obstruction examined from January 1979 through December 1997 were reviewed. Dogs were included in the study if they had hilar lymphadenopathy documented radiographically and bronchoscopically, had serum antibodies against ***Histoplasma capsulatum***, and did not have organisms in any cytologic or histologic samples. Dogs were assigned to groups on the basis of treatment given (5dogs, corticosteroids only; 5 dogs, corticosteroids and antifungal medication; 6 dogs, antifungal medication only). Clinical signs resolved in < 1 week in dogs treated only with corticosteroids. In dogs treated with corticosteroids and an antifungal medication, improvement was evident in a mean of 2.6 weeks. In 5 of 6 dogs treated with only an antifungal medication, clinical signs resolved in a mean of 8.8 weeks. Dogs receiving corticosteroids did not develop active or disseminated histoplasmosis.

Muniz *et al.* (2001) characterized 13 environmental, 7 animal, and 28 clinical H. capsulatum isolates by using a **PCR**-based random amplified polymorphic DNA **(RAPD) assay**. DNA fingerprinting of these soil, animal, and clinical specimens was performed with four primers (1253, 1281, D-9355, and D-10513) and generated amplicons with considerable polymorphism. Although all of the isolates exhibited more than 80% genetic relatedness, they could be clustered into four to six genotypes for each primer. The RAPD profiles of ***H. capsulatum*** isolated from Rio de Janeiro State could be distinguished from those of the U.S. strains included in this study (Downs, G222B, G-186B, and FLS1) by showing less than 70% similarity to each primer. The genetic polymorphisms between H. capsulatum strains isolated from animals and soil obtained in the same geographic areas were 100% similar, suggesting that an environmental microniche could be acting as a source of infection for animals and the local human population.



Representative RAPD profiles of *H. capsulatum* isolates from Rio de Janeiro State, Brazil (A and C), and U.S. strains in classes 1, 2, 3, and 4 (B and D) with primers 1253 and 1281, respectively. Lanes M, DNA molecular size marker (Roche Biochemicals). The values on the left are molecular sizes in kilobases. **Muniz *et al.* (2001)**

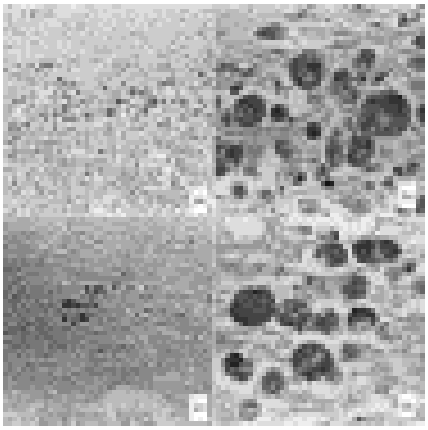


Representative RAPD profiles of *H. capsulatum* isolates from Rio de Janeiro State, Brazil (A and C), and U.S. strains in classes 1, 2, 3, and 4 (B and D) with primers D-9355 and D-10513, respectively. Lanes M: DNA molecular size marker (Roche Biochemicals). The values on the left are molecular sizes in kilobases. **Muniz *et al.* (2001)**

Sano *et al.* (2001) examined skin biopsies from two dogs in Tokyo and Kumamoto, and found fungal elements 1-2 or 2-4 microEm in diameter in the macrophages. The homology of DNA sequences for the ITS rRNA gene were correspondent to Ajellomyces capsulatus at a rate of more than 97.4%. Therefore, the two dogs were diagnosed as having been infected with Histoplasma capsulatum which is the anamorph of A. capsulatus. Since the dogs had no history of having been outside Japan and had not been brought from an endemic area, they might have been infected domestically. Further epidemiological surveys on canine histoplasmosis may be able to estimate autochthonous human cases in Japan.

Ueda *et al.* (2003) introduced a polymerase chain reaction (**PCR**) diagnosis of canine histoplasmosis and the characteristic of disease in Japan. The surgically removed skin ulcerate samples from a 5-years-old female Shiba-inu native to Japan

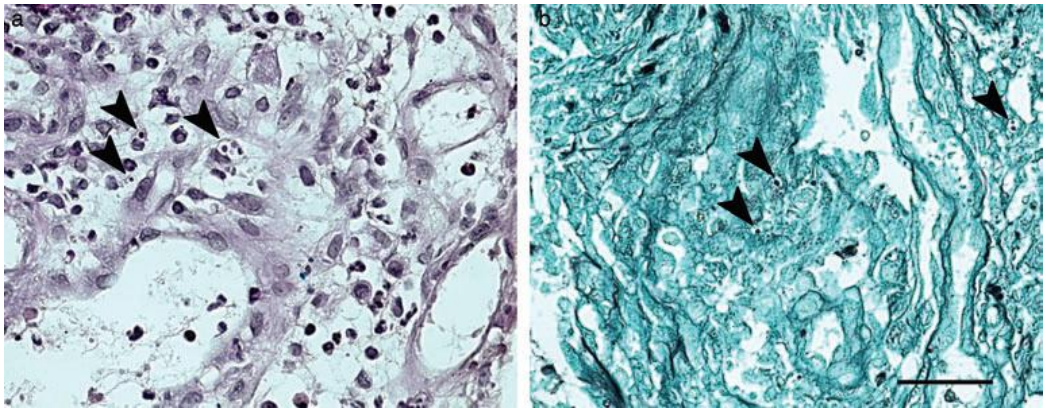
without traveling out of the country were evaluated. Tissue samples had many yeast-like organisms in the macrophages. DNA was extracted from paraffin-embedded tissue samples. A nested PCR technique was applied. The detected sequence of the internal transcribed spacer of ribosomal RNA gene had 99.7% in homology with Ajellomyces capsulatus (the teleomorph of Histoplasma capsulatum). Clinical manifestations, historical background of equine epizootic lymphangitis in Japan, and a human autochthonous case ofhistoplasmosis farciminosi indicated that this dog might have been infected with H. capsulatum var. farciminosum as a heteroecism.



Ueda *et al.* (2003)

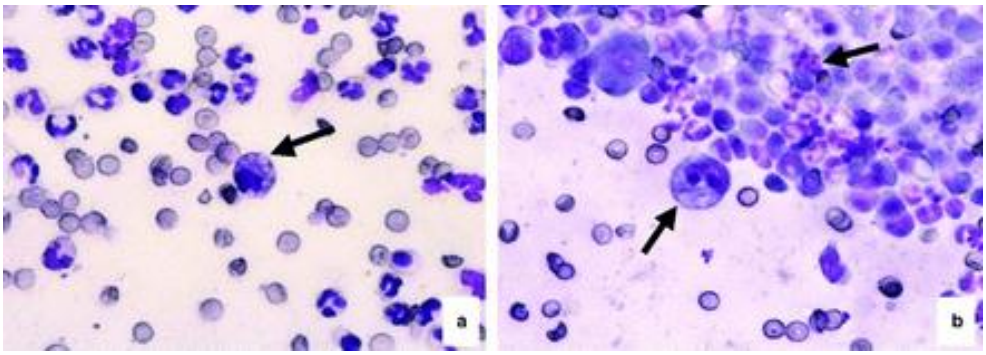
Nishifuji *et al.* (2005) reported a 5-year-old male Siberian husky bred outdoor in Tokyo with a interdigital granulomatous lesions in the left hind limb. The dog had no apparent pulmonary or gastrointestinal involvement. **Histopathological** analysis of the **skin** lesions demonstrated yeast-like organisms predominantly within macrophages. Sequence analysis of fungal **ribosome RNA gene** isolated from a paraffin sample revealed a 100% homology with the teleomorph of Histoplasma capsulatum. The present case may support the concept of primary cutaneous canine histoplasmosis as an endemic phenotype recognized in Japan.

Swelling, multiple draining as well as interdigital granulomatouschanges in the left hindlimb. **Nishifuji *et al.* (2005)**

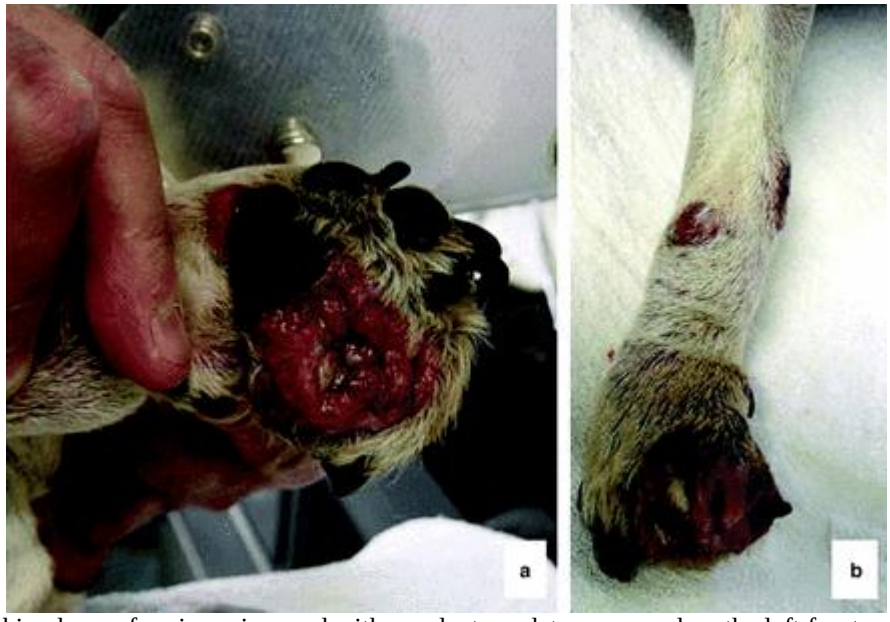


Histopathological findings of the granulomatous skin lesions. Tissue sections were stained by Periodic acid-Schiff (a) and Gomori's methenamide silver (b) stains. Note spherical fungal cells (arrowheads) found in macrophages. Bar indicates 100 lm. **Nishifuji *et al.* (2005)**

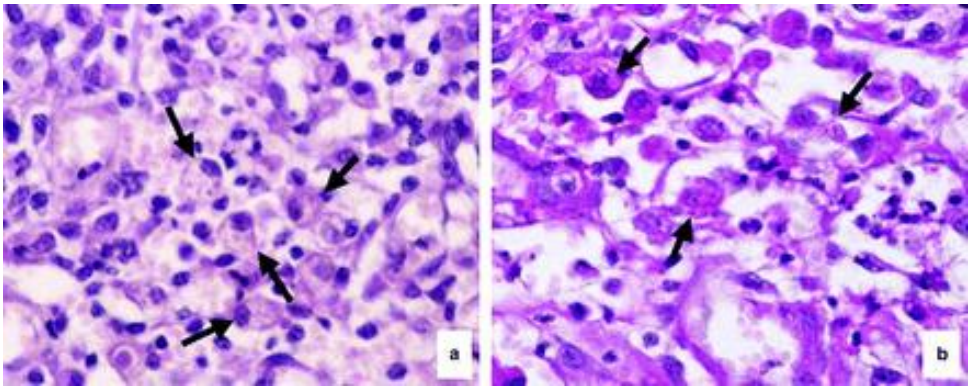
Murata *et al.* (2007) diagnosed a recent of canine histoplasmosis of **disseminated** infection accompanied by carcinoma in Japan, by clinical characteristics, **histopathological examination**, **chest radiographs**, ocular fundoscopy and **molecular biological** data. The clinical manifestations were not limited to cutaneous symptoms but were referable to disseminated infection, similar to human autochthonous cases. The partial sequences of the internal transcribed spacer (ITS1/2) regions of the ribosomal DNA genes of this and other Japanese canine histoplasmosis strains were 99-100% identical to the sequence AB211551 derived from a human isolate in Thailand, and showed a close relationship to the sequences derived from Japanese autochthonous systemic and cutaneous human cases. The phylogenetic analysis of 97 sequences of the ITS1/2 region disclosed six genotypes. The genotypes derived from Japanese autochthonous human and dog cases belonged to the cluster consisting of Histoplasma capsulatum var. capsulatum and H. capsulatum var. farciminosum sequences, indicating that these varieties might cause not only cutaneous but also systemic histoplasmosis, regardless of their host species.



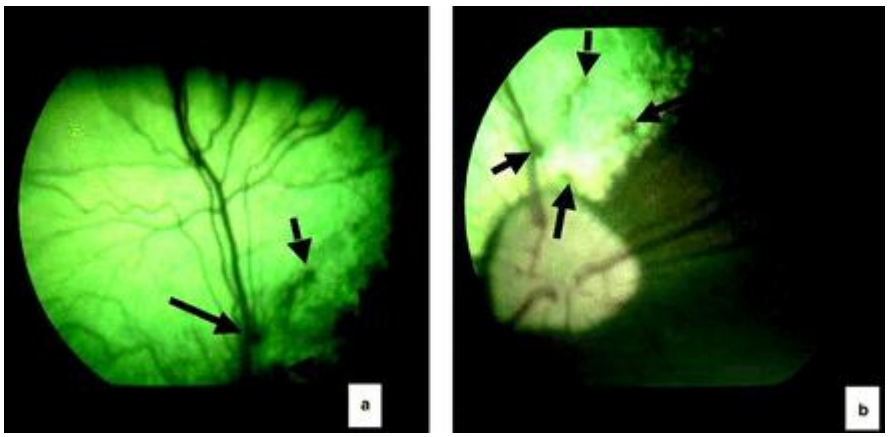
Pus stamps of Case 1 stained with Giemsa solution showing macrophages containing small yeast cells, 1–4 μm in diameter (arrows); ×400. **Murata *et al.* (2007)**



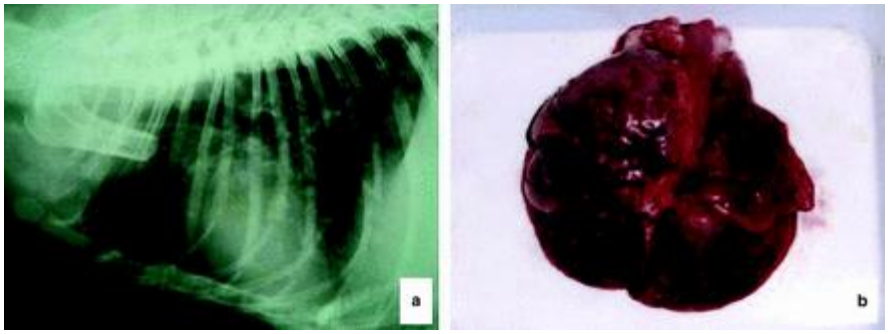
Multiple skin ulcers of various sizes and with purulent exudates appeared on the left front paw of Case 2 (a and b). **Murata *et al.* (2007)**



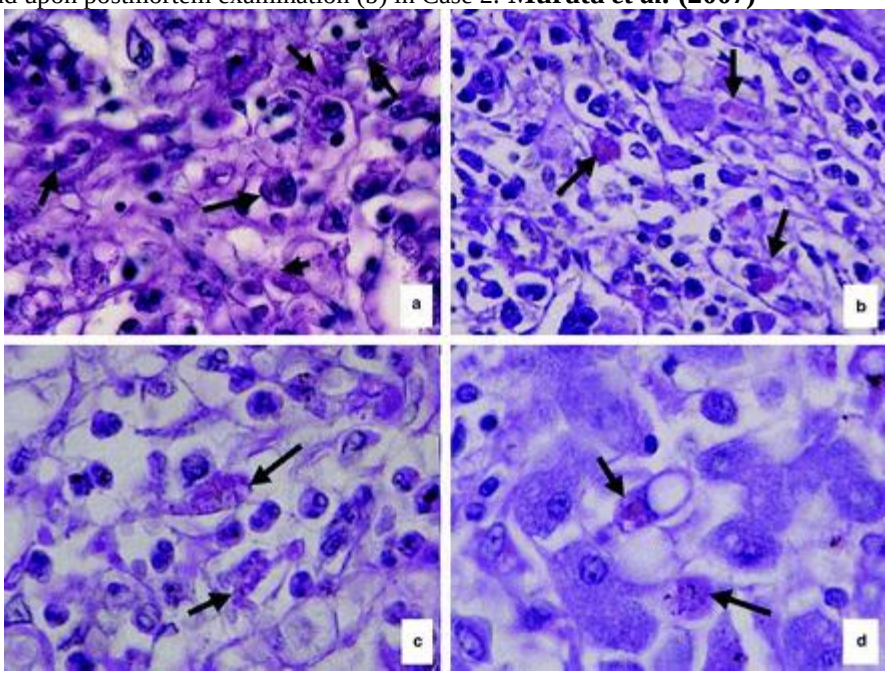
Histopathological observation of the skin biopsy sample of Case 2 showed macrophages in the granulomatous tissue containing yeast-like cells, 1–4 μm in diameter (arrows): (a) hematoxylin-eosin (HE); (b) periodic acid Schiff (PAS); ×400. **Murata *et al.* (2007)**



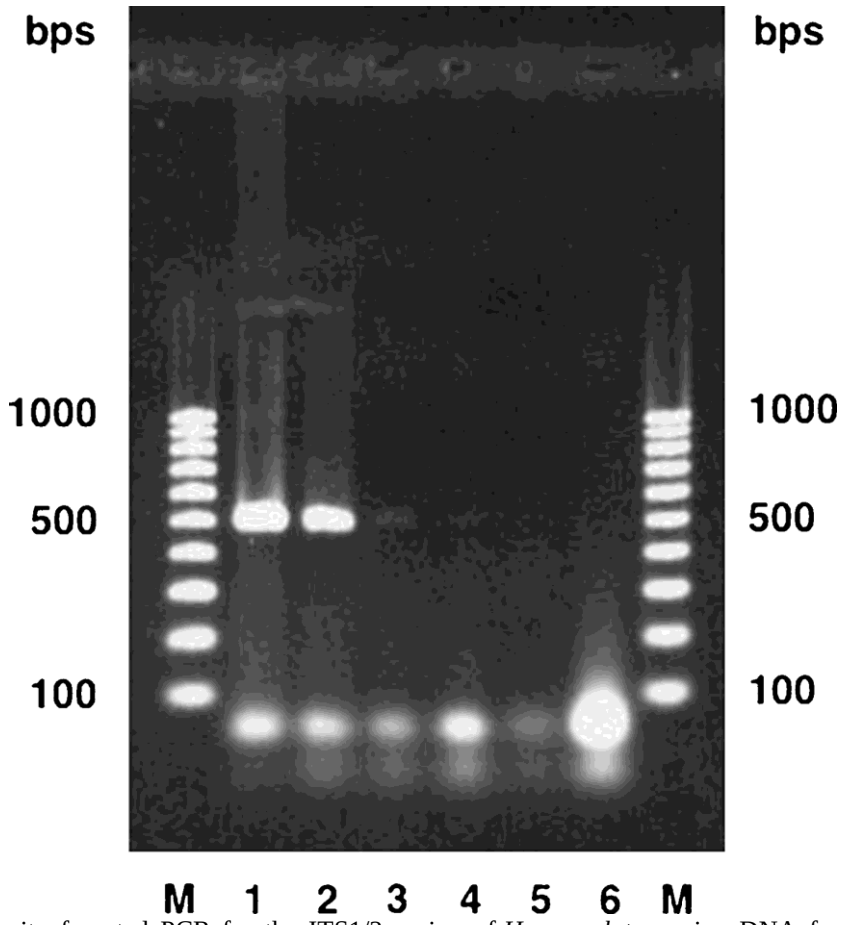
The right ocular fundus (a and b) observed by indirect othalmoscopy indicated retinochoroiditis with granules in Case 2 (arrows). **Murata *et al.* (2007)**



A chest x-ray showed multiple foci 3 days before death (a), and abundant rice-sized nodules in the lungs found upon postmortem examination (b) in Case 2. **Murata *et al.* (2007)**

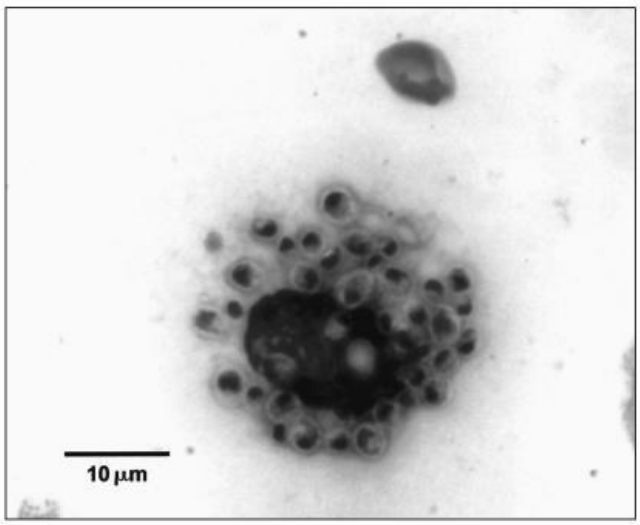


PAS-positive yeast-like cells (arrows) in the neoplastic tissue in the lung (a), spleen (b), liver (c and d) in Case 2, ×400. **Murata *et al.* (2007)**



Detection limit of nested PCR for the ITS1/2 region of *H. capsulatum* using DNA from pus 9. M, marker; 1, DNA from pus; 2, fungal DNA at 2.5 ng; 3, 250 fg; 4, 25 fg; 5, 2.5 fg; and 6, negative control. **Murata *et al.* (2007)**

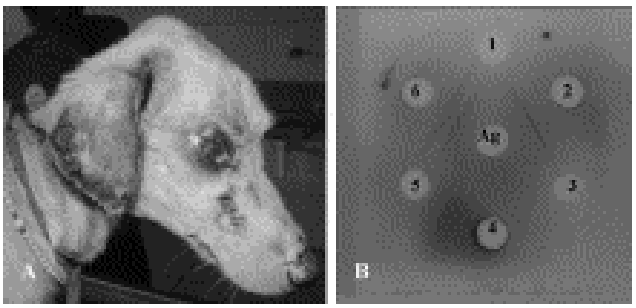
Tyre *et al.* (2007) presented a young dog with a history of chronic diarrhea, anorexia, and weight loss. ***Histoplasma capsulatum*** was suspected, based on **cytologic examination** of lymph node aspirates and peritoneal fluid, and confirmed by **fungal culture**.



Wright-Giemsa stained cytologic sample of a peritoneal fluid macrophage containing intracytoplasmic *Histoplasma capsulatum*organisms. Bar = 10 µm.

Clemans *et al.* (2011) reported a 4-year-old spayed female Golden Retriever was with an acute edema and erythema in the left hind limb and an **inguinal mass**, and a 5-year-old female Jack Russell Terrier (dog 2) with a recurring **retro-peritoneal mass**. Diagnostic imaging in dog 1 revealed abnormal tissue surrounding the larger vessels and ureters and complete occlusion of the left limb veins. Surgery resulted in incomplete removal of the mass. **Histologic examination** revealed fibrosing pyogranulomatous inflammation. Results of a Histoplasma antigen test were positive, and reanalysis of the tissues revealed yeast cells indicative of **Histoplasma capsulatum**. Histologic examination in dog 2 revealed fibrosing pyogranulomatous inflammation. The mass recurred 8 months later; exploratory abdominal surgery at that time resulted in substantial hemorrhage from the adhered caudal aorta. Histologic examination of tissue sections from the second surgery revealed yeast cells consistent with Blastomyces dermatitidis. Both dogs had temporary improvement after surgery. Full clinical resolution required treatment for fungal disease. Dog 1 was treated with itraconazole, then **fluconazole** (total treatment time, 23 weeks). Dog 2 was treated with fluconazole for 36 weeks.

Cordeiro *et al.* (2011) investigated the serologic evidence of H. capsulatum in dogs, considering that these animals can act as sentinels for histoplasmosis. A total of 224 serum samples from dogs were tested for antibodies against ***H. capsulatum*** through **immunodiffusion**. A total of 128 (57.14%) samples were positive for leishmaniasis by indirect immunofluorescence assay and four (1.78%) samples were positive for antibodies against H. capsulatum. Immunological evidence of the co-existence of histoplasmosis and leishmaniasis indogs living in urban areas was observed. Diagnosis and clinical management of these diseases in endemic areas should be improved by veterinarians.

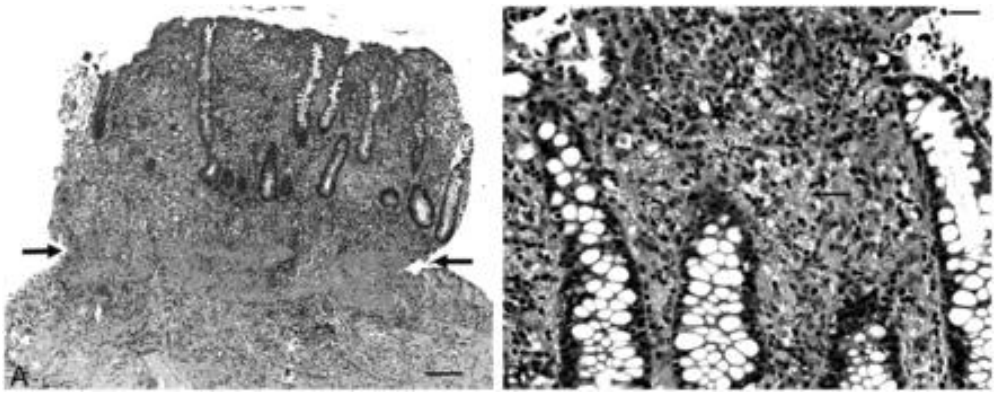


Cordeiro *et al.* (2011)

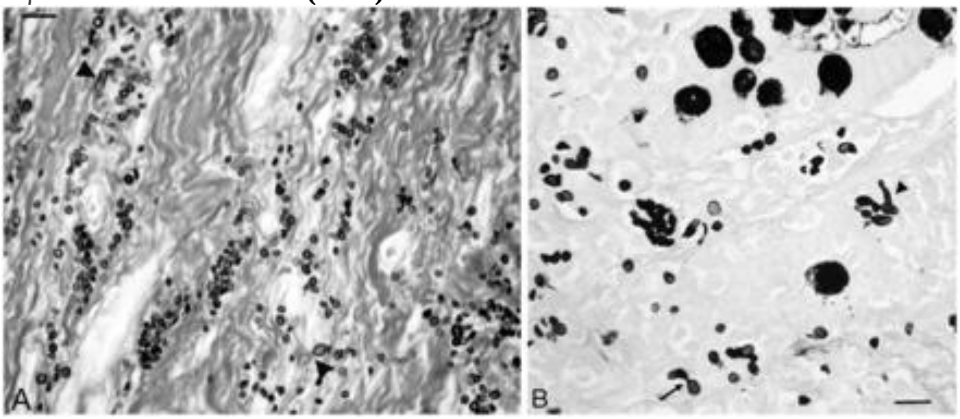
Gilor *et al*. (2011) diagnosed **disseminated histoplasmosis** and disseminated intravascular coagulopathy in a 7 mo old, female spayed mixed-breed dog. The dog improved transiently with supportive care, but deteriorated shortly after initiation of antifungal therapy. The dog was subsequently euthanized. At necropsy, marked granulomatous vasculitis was identified in all affected organs. The tunicae and laminae of the arteries and arterioles were obscured by epithelioid macrophages and multinucleated giant cells admixed with necrotic material. Intracytoplasmic yeast were present within some of these macrophages. To the authors' knowledge, this is the first reported case of granulomatous vasculitis associated with Histoplasma capsulatum in a dog.

Pratt *et al.* (2012) reported a dog with disseminated histoplasmosis. the dogs did not originate from, or had traveled to, typical regions endemic for histoplasmosis. The diagnosis was established from **histopatholog**y and either **polymerase chain reaction** (PCR), **cytology and culture**. The dog was euthanized without treatment.

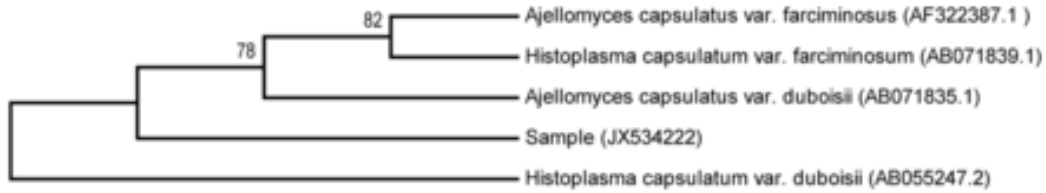
Schumacher *et al.* (2013) reported a 12-year-old intact male Miniature Schnauzer dog with **intestinal histoplasmosis.** Chronic diarrhea was unresponsive to empirical treatment. A laparotomy was performed, and formalin-fixed biopsies of duodenum, jejunum, and colon were subjected **histologic examination**, that revealed a severe, diffuse, granulomatous enteritis and colitis with intralesional yeast and hyphal forms. Grocott methenamine silver stains revealed short, aseptate hyphae co-mingled with 2-8 µm, oval to round yeast organisms consistent with ***Histoplasma capsulatum***. The atypical presentation of both yeast and hyphal forms prompted identification of the organism. Direct **sequencing of a polymerase chain reaction** product from paraffin-embedded intestinal samples confirmed the presence of ***Ajellomyces capsulatus*** with a homology over 99% to several sequences in GenBank. Ajellomyces capsulatus is the holomorphic name for H. capsulatum. Therefore, the mycelial form of a dimorphic fungus such as H. capsulatum can coexist with yeast cells within lesions of histoplasmosis. Following diagnosis, the dog was treated with **itraconazole** for 6 months and has improved.



Dog, colon. A, the lamina propria of the colon is infiltrated by marked numbers of inflammatory cells that separate and regionally efface colonic glands. The inflammation regionally extends deep into the submucosa (arrows demarcate mucosa from submucosa). Hematoxylin and eosin (HE) stain. Bar = 100 µm. B, numerous intralesional yeast organisms accompany the inflammatory cells (arrows). The organisms have a spherical or crescentic yeast body surrounded by a 2–8 µm, clear capsule. HE stain. Bar = 20 µm. **Schumacher *et al.* (2013)**

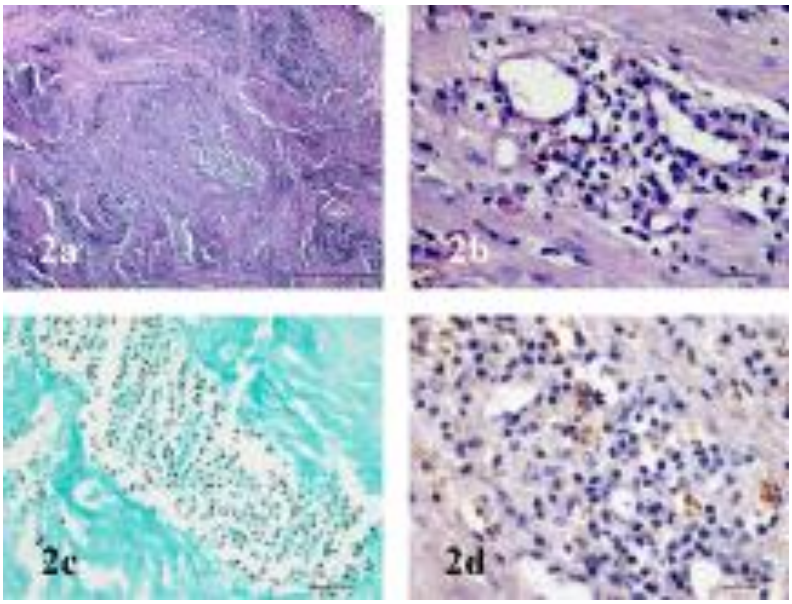


Dog, colon. A, most regions contained aggregates and clusters of yeasts occasionally accompanied by elongated, segmented, hyphal-type structures (arrowheads). Grocott methenamine silver (GMS) stain. Bar = 20 µm. B, occasionally, the organisms exhibited branching (arrowhead) and narrow-based budding (arrow). GMS stain. Bar = 25 µm. **Schumacher *et al.* (2013)**



Evolutionary relationship to other known *Histoplasma*/*Ajellomyces* species. The evolutionary history of the isolated sequence and few other closely related sequences was inferred using the maximum parsimony method. Numbers adjacent to the nodes are bootstrap values ≥70%. Phylogenetic analyses were conducted in MEGA5.14 GenBank accession numbers of the sequences are given in parentheses next to the sequence names. **Schumacher *et al.* (2013)**

Reginato *et al.* (2014) presented a 7-year-old intact male mixed dog with canine **neurological histoplasmosis** . Abnormal clinical signs consisted of non-ambulatory paraparesis, hind limbs hypertonia and severe thoracolumbar pain. Magnetic resonance imaging demonstrated an isointense in T1 and T2 WI epidural lesion, with good contrast enhancement, extending from T-10 to T-13. Laminectomy was carried out to remove the epidural mass. **Histological examination** revealed a pyogranulomatous lesion characterized by numerous macrophages containing yeast-like Grocott and PAS-positive bodies. Immunohistochemistry and PCR performed on formalin-fixed paraffin-embedded tissue confirmed Histoplasma capsulatum as the causative agent. ***H. capsulatum*** has a worldwide distribution in temperate and subtropical climates but its presence as an autochthonous fungus in Europe is now recognized. To the authors' knowledge this is the first report of canine histoplasmosis in Italy with lesion confined to the central nervous system.



Reginato *et al.* (2014)

Cunningham *et al.* (2015) conducted a study to evaluate the sensitivity and specificity of an antigen **enzyme immunoassay (EIA)** on urine samples for the diagnosis of histoplasmosis in dogs. This retrospective medical records review included canine cases with urine samples submitted for Histoplasma EIA antigen assay between 2007 and 2011 from three veterinary institutions. Cases for which urine samples were submitted for Histoplasma antigen testing were reviewed and compared to the gold standard of finding Histoplasma organisms or an alternative diagnosis on cytology or histopathology. Sensitivity, specificity, negative predictive

value, positive predictive value, and the kappa coefficient and associated confidence interval were calculated for the EIA-based Histoplasma antigen assay. Sixty cases met the inclusion criteria. Seventeen cases were considered true positives based on identification of the organism, and 41 cases were considered true negatives with an alternative definitive diagnosis. Two cases were considered false negatives, and there were no false positives. Sensitivity was 89.47% and the negative predictive value was 95.35%. Specificity and the positive predictive value were both 100%. The kappa coefficient was 0.9207 (95% confidence interval, 0.8131-1). The Histoplasma antigen EIA test demonstrated high specificity and sensitivity for the diagnosis of histoplasmosis in dogs.

3.5.2. Reports on histoplasmosis in cats:

Gwin *et al.* (1980) reported a cat with disseminated histoplasmosis and multifocal inflammatory lesions in the posterior segment of the eyes. Histologic examination revealed lesions of active choroiditis (cat) in association with numerous Histoplasma capsulatum.

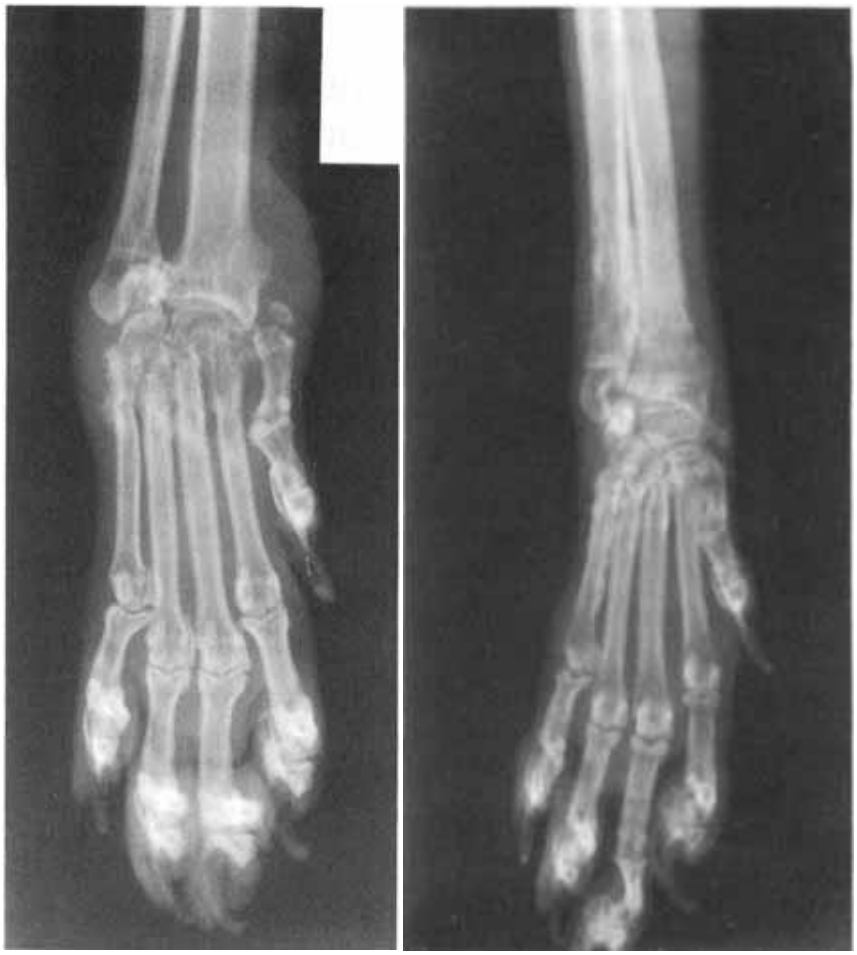
Percy (1981) reported a 12-year-old, castrated male cat with a history of respiratory disease developed bilateral endophthalmitis, retinal detachment, and granulomatous choroiditis, and unilateral glaucoma. Other lesions included granulomatous optic neuritis, myositis involving extraocular muscles, and focal retinitis. Light and electron microscopy showed many intracellular organisms, interpreted to be Histoplasma. Granulomatous inflammatory lesions and organisms were present in lung, liver, lymphoid tissues, and adrenals.

Kabli *et al.* (1986) reported 17 cases of histoplasmosis involving 2 dogs and 15 cats in the Upper Rio Grande Valley of El Paso since 1978. The diagnosis, based on clinical signs and radiographic findings, was confirmed by one or more of the following laboratory procedures: demonstration of intracellular Histoplasma capsulatum yeast cells in tissue, positive serology, or isolation of H. capsulatum from various organs of necropsied animals. H. capsulatum was isolated also from a bat cave and soil in the vicinity of some of the houses where the affected animals had resided. Skin-tests of 97 persons for histoplasmosis indicated a 14% positive prevalance in this locale.

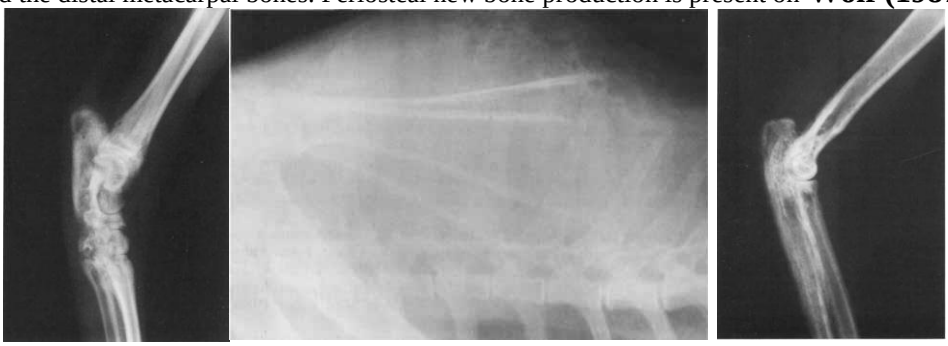
Clinkenbeard *et al.* (1987) mentioned that anemia, weight loss, lethargy, fever, anorexia, and interstitial lung disease were the predominant clinical findings in 12 cats with **disseminated histoplasmosis**. Some cats were examined because of dysfunction or lesions of bone, eyes, or skin. In most cases, the clinical signs were observed by the owner for 4 weeks or less before seeking veterinary care. Young cats were most commonly affected, with 7 of the 12 cats less than or equal to 1 year old. Identification of Histoplasma organisms in bone marrow aspirates was used to confirm the diagnosis of histoplasmosis in 11 of the 12 cats. Histoplasma infection of multiple organs was found at necropsy. In this study, disseminated histoplasmosis had a higher prevalence in cats than in dogs at the same veterinary medical teaching hospital. Feline disseminated histoplasmosis was not associated with FeLV infection. Treatment was attempted in 7 of the 12 cats.

Wolf (1987) reported **disseminated Histoplasma capsulatum infection** in 7cats with osseous lesions as the primary manifestation. The major clinical signs in these cats were related to the bony lesions and included lameness, bone pain, and soft tissue swelling of limbs and joints. Other clinical and pathologic findings were similar to previously reported forms of disseminated histoplasmosis in the cat. The radiographic appearance of the lesions was

predominantly osteolytic; periosteal and endosteal new bone production was present in some cases. Infection occurred primarily in bones of the appendicular skeleton with a predilection for sites below the elbow and stifle joints.



Anterior-posterior radiographof the carpus from cat 4 demonstrating soft tissue swelling and osteolysis of the carpal bones with collapse ofthejoint spaces. There is punctate osteolysis of the distal radius and severe osteolysis of the proximal metacarpal bones with associated periosteal reaction. Anterior-posterior radiograph of the carpus from cat 5 demonstrating irregular osteolysis of the distal radius and ulna and the distal metacarpal bones. Periosteal new bone production is present on **Wolf (1987)**



Lateral radiograph of the tarsus from cat *2* illustrating osteolysis of the tibia, tarsus, and proximal metatarsal bones with collapse of the joint spaces and periosteal new bone production. Lateral radiograph of the scapulae from cat 5 demonstrating "punched out," lytic areas on the proximal dorsal border seen in two cats. Lateral radiograph of the elbow from cat 5 illustrating patchy osteolysis interspersed in a more productive pattern of periosteal and endosteal bone reaction in the proximal radius and ulna and the distal humerus. **Wolf (1987)**

Hodges *et al.* (1994) treated 8 cats with histoplasmosis with **itraconazole** at 5 mg/kg per dose PO bid. There were multiple sites of infection, and 2 of the catshad hypercalcemia that

was attributed to the histoplasmosis. All 8 cats were eventually cured, but 2 cats experienced recurrences of disease after completion of therapy, requiring 2 to 3 additional months of itraconazole. There were no clinically relevant adverse effects during treatment. Although a limited number of cats were treated.

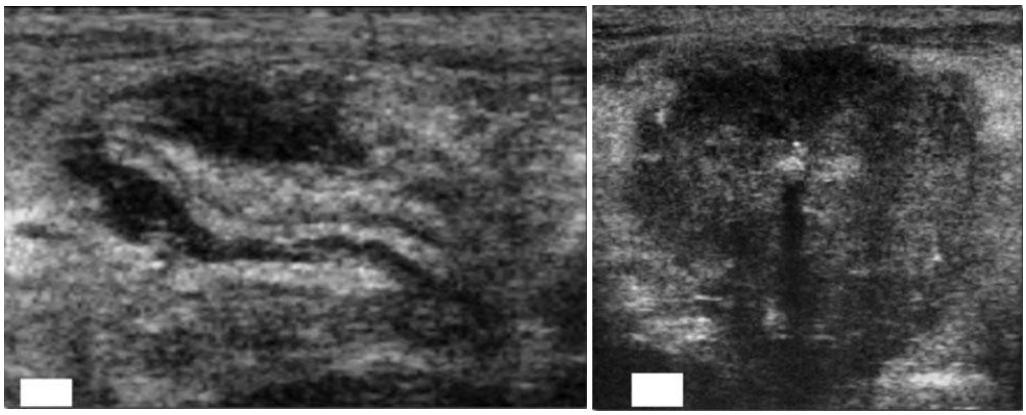
Johnson *et al.* (2004) described infection with histoplasmosis in two indoor cats from central California, an area not considered to be endemic for the disease. Systemic mycotic infections should be considered as differential diagnoses in any cat with compatible clinical signs, regardless of travel history or residence, especially if the cat is presented within a recognized endemic region.

Vinayak *et al.* (2007) examined a 7-year-old domestic shorthair cat with a 2-month history of decreased appetite and weight loss because of paraparesis of 1 week's duration that had progressed to paraplegia 3 days earlier. Neurologic examination revealed normo- to hyperreflexia and absence of deep pain sensation in the hind limbs and thoracolumbar spinal hyperesthesia. Neuro-anatomically, the lesion was located within the T3 through L3 spinal cord segments. Biochemical analysis and cytologic examination of CSF revealed no abnormalities. Radiography revealed narrowing of the T11-12 intervertebral disk space and intervertebral foramen suggestive of intervertebral disk disease. Myelography revealed an extradural mass centered at the T12-13 intervertebral disk space with extension over the dorsal surfaces of T11-13 and L1 vertebral bodies. A right-sided hemilaminectomy was performed over the T11-12, T12-13, and T13-L1 intervertebral disk spaces, and a space-occupying mass was revealed. Aerobic bacterial culture of samples of the mass yielded growth of a yeast organism after a 10-day incubation period; histologically, ***Histoplasma capsulatum*** was identified. Treatment with **itraconazole** was initiated. Nineteen days after surgery, superficial pain sensation and voluntary motor function were evident in both hind limbs. After approximately 3.5 months, the cat was ambulatory with sling assistance and had regained some ability to urinate voluntarily.

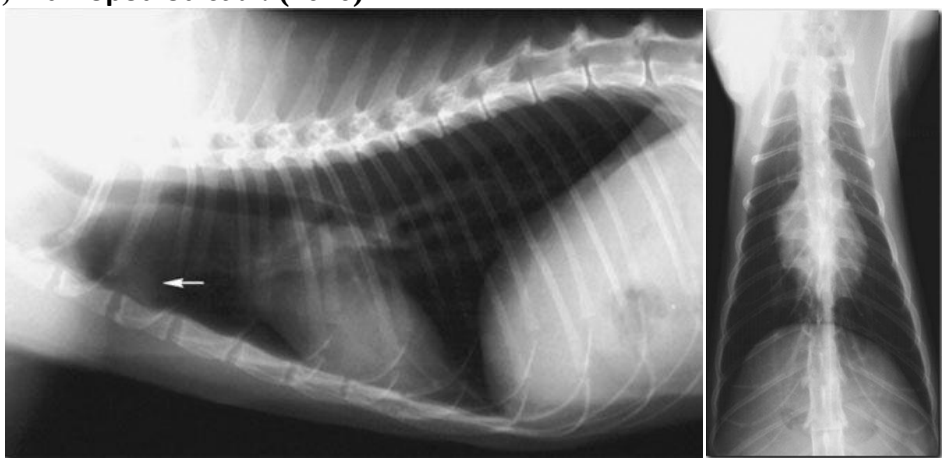
Kobayashi *et al.* (2009) carried out cytological, histopathological and immunohistochemical examinations on a presumed 10-year-old Japanese cat showing vomiting and emaciation. On cytologic examination of the mass of the upper **abdominal cavity**, many yeast-like organisms were detected in the macrophages. At necropsy, the upper part of colon was markedly dilated with a thickened wall. The lung did not show significant changes. **Histologically**, severe necrotic and granulomatous lesions were observed in the colon. In the colonic lesion, the cytoplasm of the macrophages contained yeast-like organisms with irregularly shaped dots, and the cell walls of these organisms were stained black by Grocott-Gomori methenamine-silver stain. Immunohistochemically, they were found to be positive for anti-histoplasma yeast antibody. This is the first report of feline histoplasmosis in Japan.

Mavropoulou *et al.* (2010) presented **disseminated histoplasmosis** in a cat with a history of vomiting, decreased appetite and weight loss. Abnormal findings were poor body condition, pale mucous membranes, dehydration and a palpable abdominal mass. Abdominal ultrasound showed lymph node enlargement, a mass of uncertain origin, thickening of the muscularis layer of the small bowel, focal thickening of the ileum with loss of layering and free peritoneal fluid. Cytology revealed a piogranulomatous infiltrate and numerous macrophages containing oval or round yeast-like cells 2 to 5 microm diameter with a central, spherical, lightly basophilic body surrounded by a clear halo, compatible with ***Histoplasma capsulatum***, within the cytoplasm. Post-mortem examination revealed cavity effusions, granulomatous nodules in lungs, intestine and omentum, thickened intestinal walls and intestinal

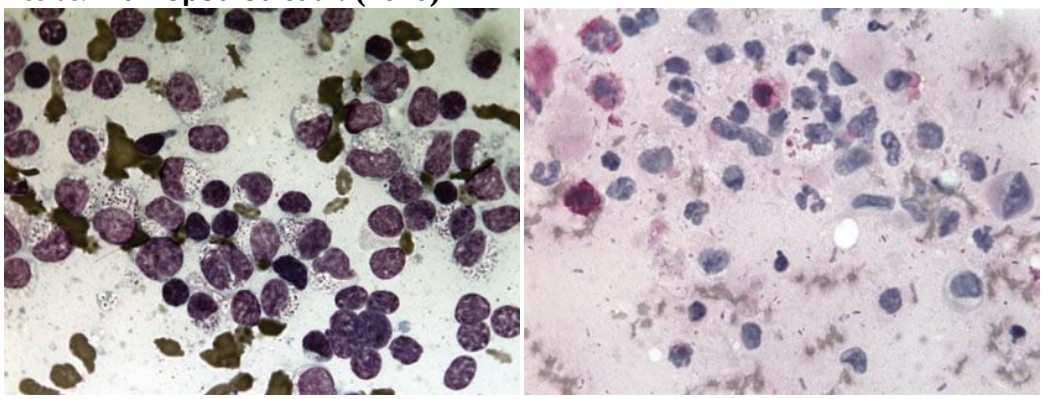
perforation. Staining with Grocott and immunohistochemistry (IHC) revealed numerous organisms within the granulomatous reaction. H. capsulatum has a worldwide distribution in temperate and subtropical climates. To the author's knowledge, this is the first report of feline histoplasmosis in Europe.



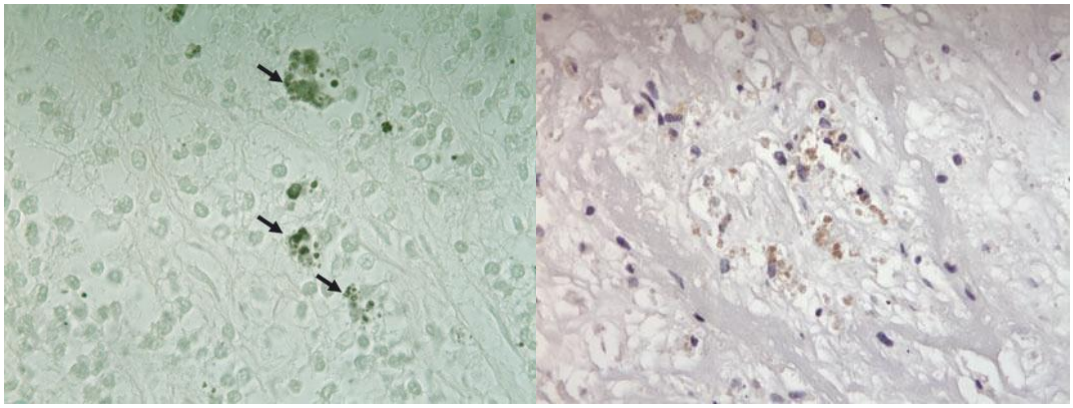
Ultrasound examination of the abdomen of the cat with disseminated histoplasmosis (a–c). Focal thickening of a jejunal loop with loss of layering (a). Mesenteric hypoechoic, heterogeneous rounded mass (b) **Mavropoulou *et al.* (2010)**



Radiographic examination of the thorax of the cat with disseminated histoplasmosis. No pulmonary parenchyma abnormalities were obvious at the time of the examination. Enlargement of the retrosternal lymph node can be seen on the lateral view (arrow), as a soft tissue opacity dorsal to the cramial stomebrae. **Mavropoulou *et al.* (2010)**



(a) Fine-needle aspirate from the abdominal mass in a cat with disseminated histoplasmosis. There are numerous intracellular bodies within macrophages. Note the central, spherical, lightly basophilic body surrounded by a clear halo. Diff-Quick, (b) Fine-needle aspirate from the abdominal mass in a cat with disseminated histoplasmosis. Intracellular bodies stain positive with periodic acid Schiff stain (PAS) **Mavropoulou *et al.* (2010)**



Grocott staining of same mass. Note the clusters of small, black organisms in unstained macrophages, Anti-*H. capsulatum* immunohistochemistry. Notice the numerous positive-staining fungal bodies scattered within the abdominal mass **Mavropoulou *et al.* (2010)**

Tamulevicus *et al.* (2011) reported a 4 yr old, spayed female domestic shorthair with a 2 mo history of weight loss, anorexia, and diarrhea. Skin fragility was noted on presentation and a large skin tear measuring 5 cm × 5 cm was obvious over the dorsal cervical region. The patient was previously treated with short-term prednisone that was discontinued 6 wk before presentation. Initial diagnostics (complete blood count and biochemistry) did not indicate an endocrine disorder, the most common cause of acquired feline skin fragility. Necropsy revealed diffuse histoplasmosis (most significantly affecting the **skin)**, epidermal atrophy, dermal collagen separation, and infiltration in the dermis and subcutis by inflammatory cells containing yeast organisms consistent with Histoplasma spp. Infiltrative fungal infection should be considered as a potential cause of acquired feline skin fragility.

Aulakh *et al*. (2012) reported 22 cases of feline histoplasmosis seen at the Virginia-Maryland Regional College of Veterinary Medicine Teaching Hospital between 1986 and 2009. The median age of affected cats was 9 yr (mean, 8.8 yr). Female domestic shorthairs were more commonly affected. The clinical presentation of most cases was nonspecific. The most common presenting complaints included weakness, lymphadenopathy, weight loss, and anorexia. Less frequent clinical signs included vomiting, diarrhea, blindness, and lameness. Less than half of the cats had clinical evidence of pulmonary disease on admission. Anemia and hypoalbuminemia were common laboratory abnormalities. An interstitial pattern was the most common radiographic pattern observed with pulmonary disease. Diagnosis was based on identification of the organism on cytology or histopathology. Fifteen of the 22 cats were treated, and **itraconazole** was the most common antifungal agent prescribed. Median duration of the antifungal treatment was 5 mo for cats that survived to discharge. Overall survival at time of discharge for cats in this study was 55%.

Cook *et al.* (2012) carried out retrospective study to compare results of a urine antigen assay with standard diagnostic methods incats with clinical signs suggestive of histoplasmosis. Antigenuria was detected in 17/18 cats with a histopathologic or cytopathologic diagnosis ofhistoplasmosis. This preliminary evaluation of the Histoplasma urine antigen test suggests it may be a useful aid in diagnosing this disease in cats.

Brilhante *et al.* (2012) described clinical and epidemiological aspects of three cases of feline histoplasmosis and compared them to previously described cases. A detailed mycological identification and antifungal susceptibility profile of each isolate are presented. Secondarily, a serological survey for anti-Histoplasma antibodies was performed with domestic and wild cats. Diseased animals presented nodular to ulcerated skin lesions and respiratory disorders as main clinical signs. H. capsulatum var. capsulatum was isolated and the strains showed to be susceptible to antifungal drugs

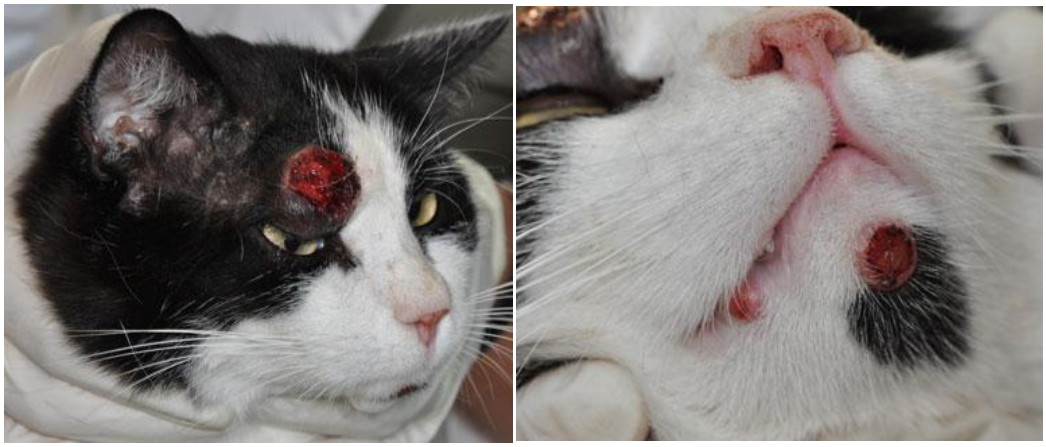
Reinhart *et al.* (2012) performed a study to compare the outcomes of cats with histoplasmosis treated with **fluconazol**e to those treated with **itraconazole**, and to evaluate possible sources of exposure for affected cats. Medical records from feline patients with confirmed histoplasmosis (n = 32) at Kansas State University were systematically reviewed and follow-up was performed by owner telephone interview. Cats treated with fluconazole (n = 17) had similar mortality and recrudescence rates when compared with cats treated with itraconazole (n = 13). Thus, fluconazole may be a viable alternative therapy for the treatment of feline histoplasmosis. Eleven cats were housed strictly indoors and possible sources of exposure reported for these cats included potted plants (5/11) and unfinished basements (6/11).

Taylor *et al*. (2012) presented a case of feline disseminated histoplasmosis in a 10 yr old domestic longhair with a 2.5 mo history of recurrent hematuria. Abdominal ultrasound examination demonstrated a thickened urinary bladder, abdominal lymphadenopathy, and a thickened and rounded spleen. Cytologic examination of fine-needle aspirate samples revealed ***Histoplasma capsulatum*** organisms in the urinary bladder wall and spleen. The cat was treated with **itraconazole** (10 mg/kg per os q 24 hr for 2.5 wk). The cat was euthanized after 19 days of treatment because of lack of improvement.

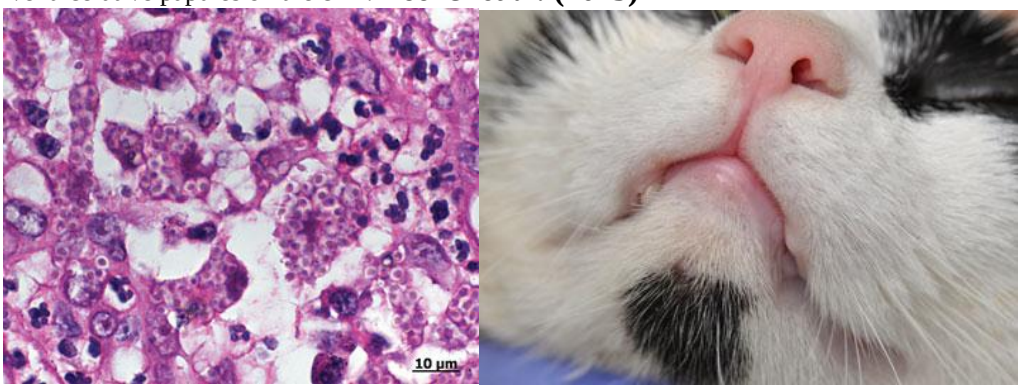
Arunmozhi *et al*. (2013) examined one isolate and five formalin-fixed paraffin-embedded (FFPE) tissue samples received from six of 15 suspected cases of histoplasmosis in cats residing in areas not known to be endemic for H. capsulatum. Polymerase chain reaction (**PCR**) amplification and sequence analysis of the rDNA ITS-2 region confirmed the diagnosis of ***H. capsulatum***. Results of molecular analysis indicated that the H. capsulatum recovered from the cats were most closely related to the North American-1 clade, but clustered separately outside this clade, suggesting that the H. capsulatum infecting the animals may represent a separate clade or phylogenetic species. This study also demonstrated the utility of obtaining valuable molecular subtype data directly from archived FFPE tissue blocks, particularly when a fungus culture was not performed or is otherwise unavailable.

Fischer *et al.* (2013) reported a 6-year-old male castrated outdoor cat with a history of **skin lesions** evolving over 1 month and consisting of multiple papules and nodules on the head and neck. General examination was unremarkable. Cytological examination of the ulcerated nodules revealed a pyogranulomatous infiltrate, with numerous macrophages containing oval yeast-like cells, 2-5 μm in size, with a central, lightly basophilic core surrounded by a clear halo. A tentative diagnosis of fungal infection was made, and skin biopsy specimens were taken. **Histological examination** confirmed the cytology findings, and Grocott staining showed numerous organisms suggestive of Histoplasma within macrophages. Thoracic radiographs, abdominal ultrasound and routine laboratory testing were unremarkable. Fungal

culture of a nodule was negative. PCR of total DNA extracted from the infected tissue and subsequent sequencing confirmed the diagnosis of H. capsulatum var. capsulatum. Surgical excision of the other nodules was performed, and the cat was treated with **oral itraconazole** 5 mg/kg once daily; 12 weeks after initial consultation, no lesions were visible. No recurrence was observed during an 8 month follow-up period.

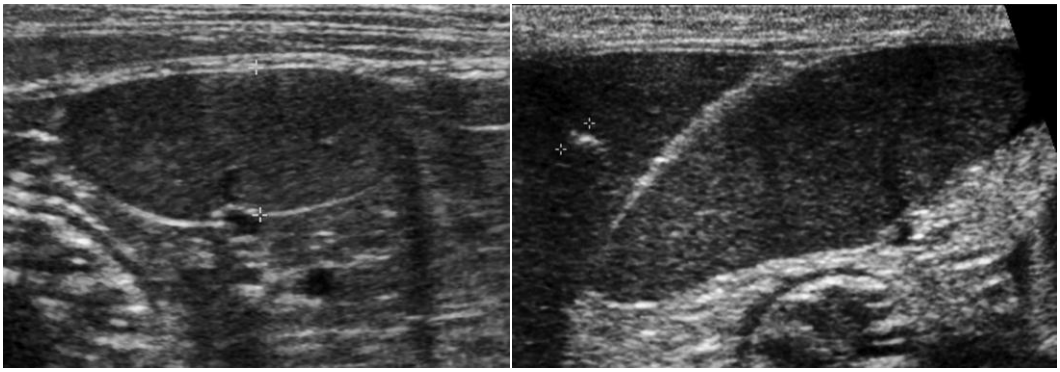


Pretreatment picture of the lesions. Note the large ulcerated nodule on the right eyelid and several erosive–ulcerative papules on the chin. **Fischer *et al.* (2013)**

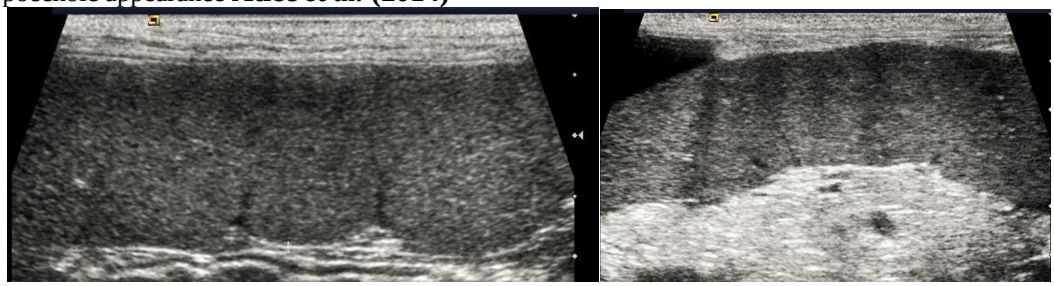


Dermal pyogranulomatous inflammation, with large numbers of Histoplasma within macrophages. Haematoxylin and eosin staining. After surgical excision of several nodules and systemic therapy with itraconazole (5 mg/kg) for 6 weeks, lesions resolved. **Fischer *et al.* (2013)**

Atiee *et al.* (2014) performed a retrospective review of splenic ultrasound images from 15 cats confirmed to have histoplasmosis by splenic aspirates. Size, echotexture, echogenicity, margin appearance, presence of nodules, and the overall shape of the spleen were reported in each case. **Splenomegaly** was documented in all cases (15/15) and a hypoechoic appearance of the spleen was documented in 14/15 of cases. The spleen was diffusely and uniformly affected in 14/15 (six homogenous and eight with a subtle mottled appearance) and had discrete nodules in 1/15 cats.



Ultrasound image of feline a spleen with *Histoplasma capsulatum* found on aspiration. Transverse plane of the head of the spleen showing rounded borders, with the width measurement made adjacent to splenic vein on the mesenteric side. Ultrasound image of feline spleen with *Histoplasma capsulatum* found on aspiration. Comparison between the echogenicity of the liver and the spleen depicting the hypoechoic appearance **Atiee *et al.* (2014)**



Ultrasound image of feline spleen with *Histoplasma capsulatum* found on aspiration. Enlarged body of the spleen with homogenous echotexture and rounded, bulging borders on the mesenteric surface. Ultrasound image of feline spleen with *Histoplasma capsulatum* found on aspiration. This is an enlarged spleen showing a subtly mottled appearance. There is peritoneal fluid adjacent to the spleen (arrowhead). **Atiee *et al.* (2014)**

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4. Paracoccidioidomycosis (PCM)

4.1. Introduction (Martinez,2015)

- Paracoccidioidomycosis is an endemic fungal disease acquired exclusively in Latin American countries, and that presents a greater prevalence in South America. Its etiological agent is the dimorphic fungus *Paracoccidioides brasiliensis,* which causes an infection that may progress to systemic granulomatous disease with tegumentary and visceral disease. *P. lutzii* is another species recently identified within the genus *Paracoccidioides,* whose endemic area involves the Midwest and North regions of Brazil. The characteristics of the disease caused by *P. lutzii* are still poorly known.
- *Paracoccidioides brasiliensis* is soil saprophyte, which has a transitory and short saprophyte phase and its survival is related to the ability to infect the host.
- Natural infection with *P. brasiliensis* occurs in men and animals and is acquired by the respiratory route after inhalation of fungal conidia suspended in air.
- Transmission of the disease through the skin or the mucosa is unlikely due to the low number of fungal propagules inoculated subcutaneously in small traumas. A case of accidental percutaneous inoculation in the laboratory only caused a local granulomatous reaction. There is no evidence of human transmission of paracoccidioidomycosis.
- Paracoccidioidomycosis disease manifests as two main clinical forms that are epidemiologically distinct.

    - The acute/subacute form commonly affects children and young adults who tend to show more disseminated lesions.
    - The chronic form of the disease is more common among adult men who present lesions usually involving the oral mucosa, the airways and the lungs.

- Autochthonous cases of paracoccidioidomycosis are limited to the American continent, approximately between 23°N and 35° S, i.e., from Mexico to Argentina. About 80% of the cases reported occurred in Brazil, and most of the remaining ones occurred in Venezuela, Colombia and Argentina. Autochthonous human paracoccidioidomycosis has not been reported in some countries such as Chile, Guyana, Surinam, French Guyana, Belize and Nicaragua.
- *Paracoccidioidomycosis in non-endemic countries:* At least 60 cases of paracoccidioidomycosis have been reported and diagnosed in countries outside Latin America. The cases were observed in the United States of America, Canada, Spain and other European countries, the Middle East, Japan and Africa. All 60 cases were considered to be imported paracoccidioidomycosis since the patients had reported visiting or working in South or Central American countries. Some of them had left the endemic area already presenting clinical manifestations of the mycosis, but

most of them had shown lesions after at least five years of permanence in non-endemic countries. These cases are indicators of endemic sites of the mycosis.

- The skin test with the intradermal application of *P. brasiliensis* antigens is used to assess delayed hypersensitivity and is the method most frequently used to detect asymptomatic infection with the fungus. There are limitations to the interpretation of skin test results and to the comparison of studies. Different *P. brasiliensis* antigens lead to different rates of reactivity. There is also evidence of cross-reactivity when the skin test is applied to people infected with *Histoplasma capsulatum*, although infection with both fungi should be considered.
- The search for anti-*P. brasiliensis* antibodies in population samples has also demonstrated the presence of paracoccidioidomycosis-infection

  - The disease and particularly *P. brasiliensis* infection have been demonstrated in different species of domestic and wild animals. An armadillo species (*Dasypus novemcinctus*) is more associated with the eco-epidemiology of paracoccidioidomycosis, being frequently infected or showing histopathological changes suggestive of disease caused by *P. brasiliensis*. Armadillos dig and produce aerosols with soil particles and are probably infected by inhaling fungal conidia in suspension in the air, as is the case in humans.
  - Regarding domestic animals, paracoccidioidomycosis was confirmed by isolation of the fungus and/or histopathological examination in two dogs from the Brazilian Southeast which showed lymphadenomegaly. Dogs are susceptible to experimental infection,
  - There is some evidence that dogs can be naturally infected by Paracoccidioides brasiliensis in endemic areas of paracoccidioidomycosis
  - Infection by *Paracoccidioides. brasiliensis* in canines has been investigated in different Brazilian regions, demonstrating significant positivity possibly due to the habit of these animals to sniff and dig the soil, contributing to understanding the fungal transmission, ecology and epidemiology.
  - Serological surveys or skin tests with *P. brasiliensis* antigens have revealed the existence of paracoccidioidomycosis-infection in cats, dogs, chickens, pigs, cattle, horses, sheep, goats, rabbits and in monkeys and in other free or captive wild animals. Dogs living in the rural area have a higher rate of infection than dogs living in the urban area.

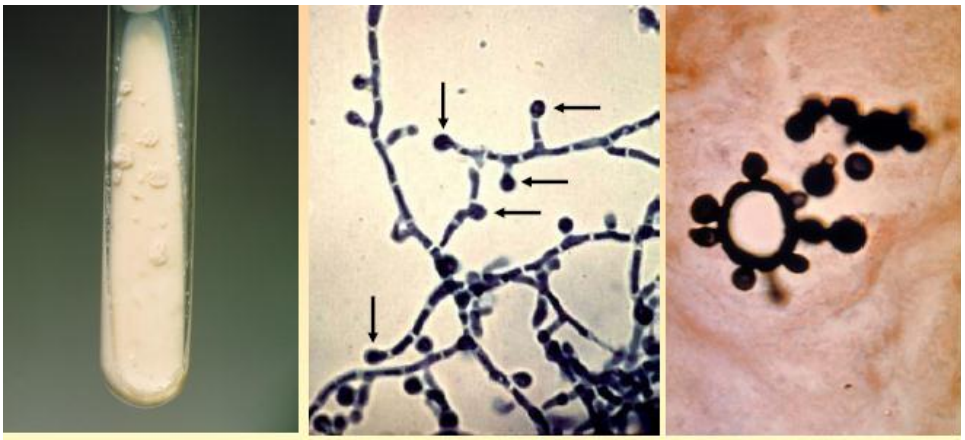
4.2. Aetiology

Paracoccidioides brasiliensis (SPLENDORE) ALMEIDA 1930)

Synonyms: *Zymonema brasiliense* SPLENDORE 1912
Zymonema histosporocellularis ALMEIDA 1914
Coccidioides brasiliensis ALMEIDA 1929
Coccidioides histosporocellularis FONSECA 1932
Paracoccidioides cerebriformis MOORE 1935
Paracoccidioides tenuis MOORE 1935
Lutziomyces histosporocellularis FONSECA FILHO 1939

*Blastomyces brasilien*sis CONANT et HOMMEL 1941
Aleurisma brasiliensis AROEIRA et BOGLIORI 1951
Perfect stage: unknown

Paracoccidioides brasiliensis is the cause of paracoccidioidomycosis (South American blastomycosis). *Paracoccidioides brasiliensis* is a thermically dimorphic fungus. The mould phase grows slowly and matures within 3-4 weeks. The colonies vary from glabrous, leathery, brownish, flat ones with a tuft of aerial mycelium to wrinkled, folded, floccose to velvety, white, beige to pink forms. Microscopically, the hyphae are hyaline and septate and may be sterile or carry few terminal conidia. All cultures produce intercalary chlamydospores.. The mould phase is easily converted to yeast phase when subcultered and incubated at 37 C. The yeast phase grows slowly, producing wrinkled, folded, glabrous and whitish colonies. Microscopically, the yeast cells are 2-30 microns in diameter, thin-walled, oval or irregular in shape and produce multiple, thin-necked, round buds which develop from all areas of the mother cell.



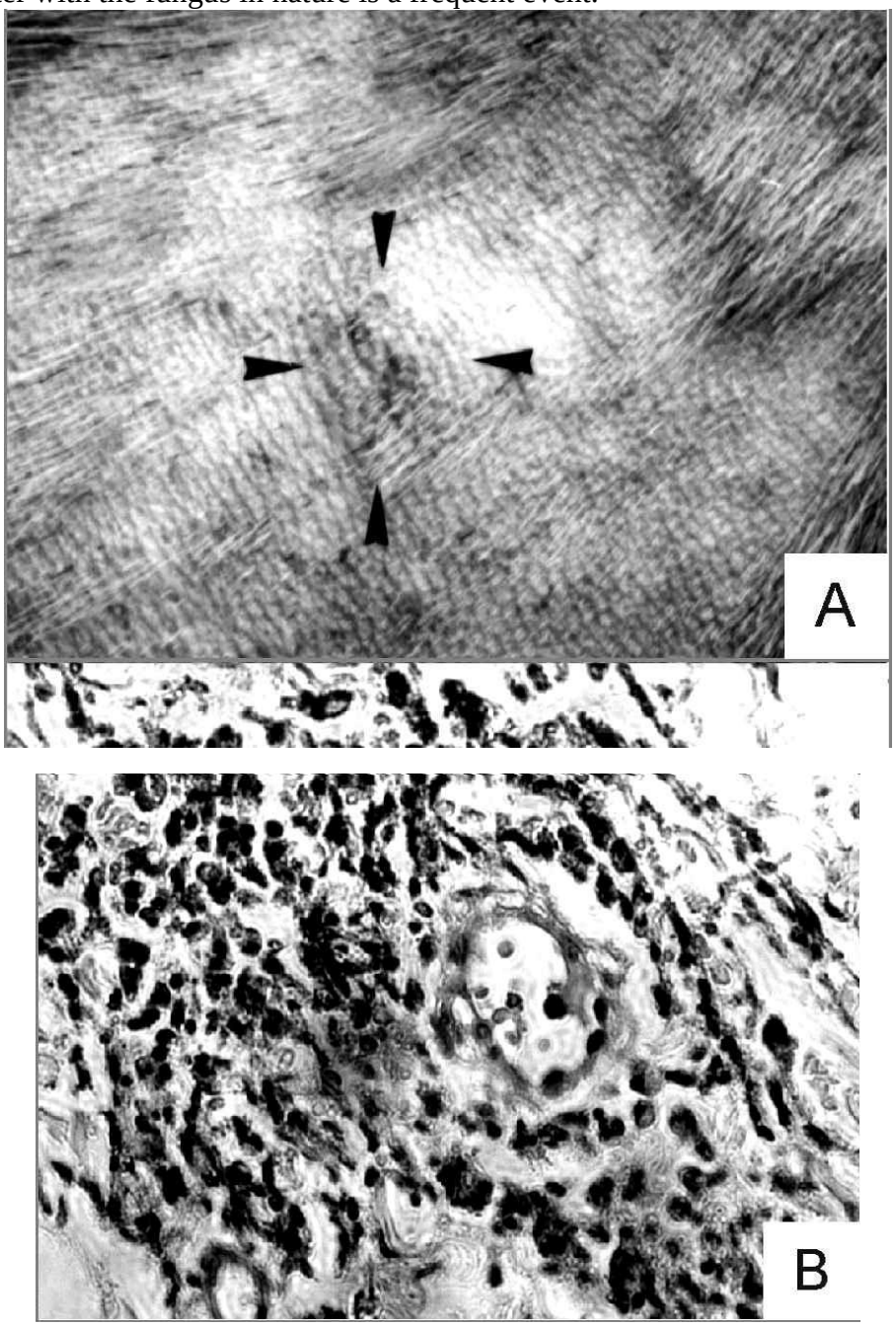
P. brasiliensis at 25^{o}C *terminal conidia* *multiple-budding yeast cells*

4.3. Reports of Paracoccidioidomycosis

4.3.1. Reports of Paracoccidioidomycosis in dogs

Ono *et al.* (2001) analyzed sera from 305 dogs by enzyme-linked immunosorbent assay (**ELISA**) to determine presence of the antibody anti-gp43, which reacts to a specific antigen of ***Paracoccidioides brasiliensis***. The dogs were divided into three groups according to their origin: urban dogs (animals with little or no contact with rural areas); suburban dogs (from the urban outskirts); and rural dogs. There was a significant difference between groups ($P < 0.05$). Rural dogs reacted positively in 89.5% of cases, followed by suburban (48.8%) and urban dogs (14.8%). There were no differences between male and female dogs. In an attempt to verify the feasibility of skin testing with gp43 to determine sensitization against P. brasiliensis in dogs, suburban ($n = 61$) and rural ($n = 21$) dogs were tested, showing positivity of 13.1 and 38.1%, respectively. Six dogs that had higher ELISA titers and also showed strong reactions in skin testing were killed in an attempt to isolate P. brasiliensis. The fungus was not detected by culture or histopathological analysis in these dogs, suggesting that dogs have a natural resistance or that they encounter an inoculum level that is insufficient to cause disease. These results indicate that ELISA and skin testing can be

useful in the epidemiological study of paracoccidioidomycosis in dogsand that encounter with the fungus in nature is a frequent event.

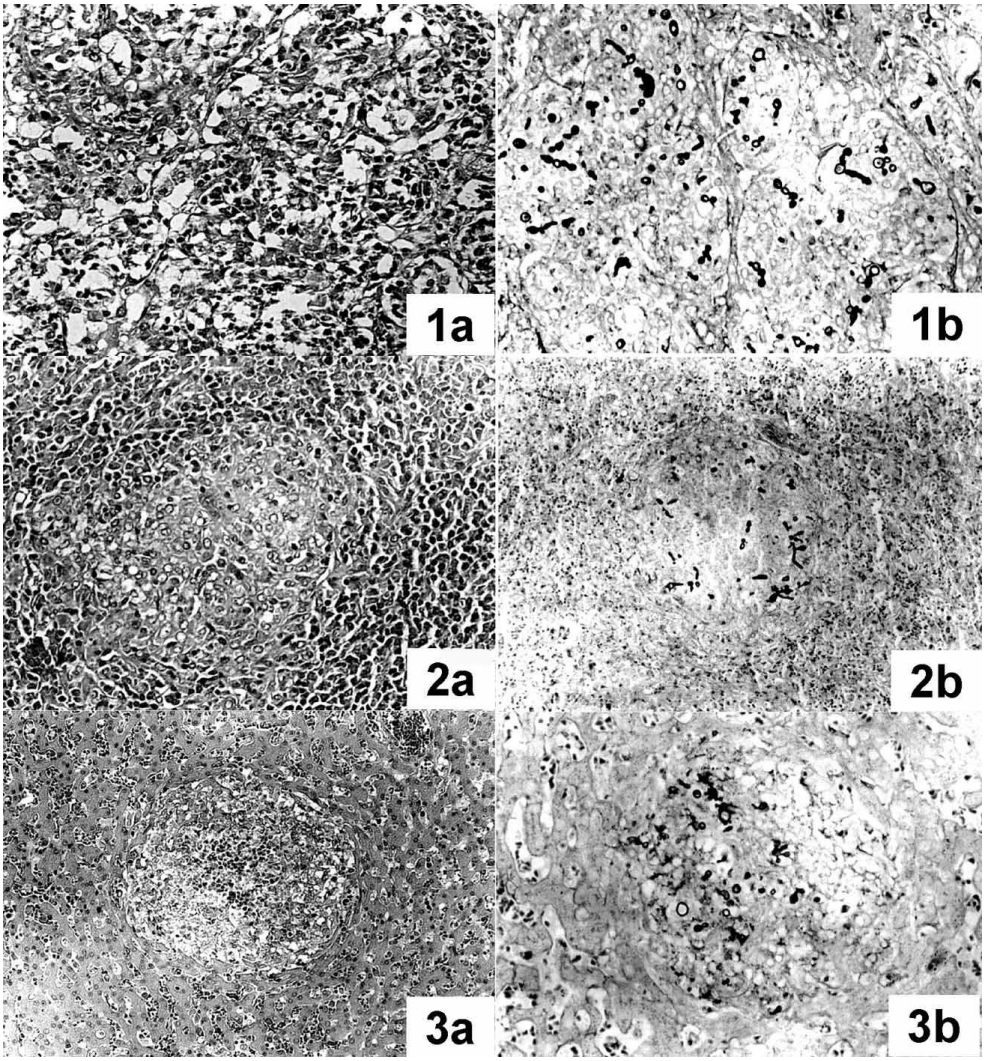


(A) Site of intradermal skin test with gp43 in a dog immunized with *P. brasiliensis*. (B) DTH after skin test showing in • ammatory in.ltrates with mononuclear and polymorphonuclear leukocytes.

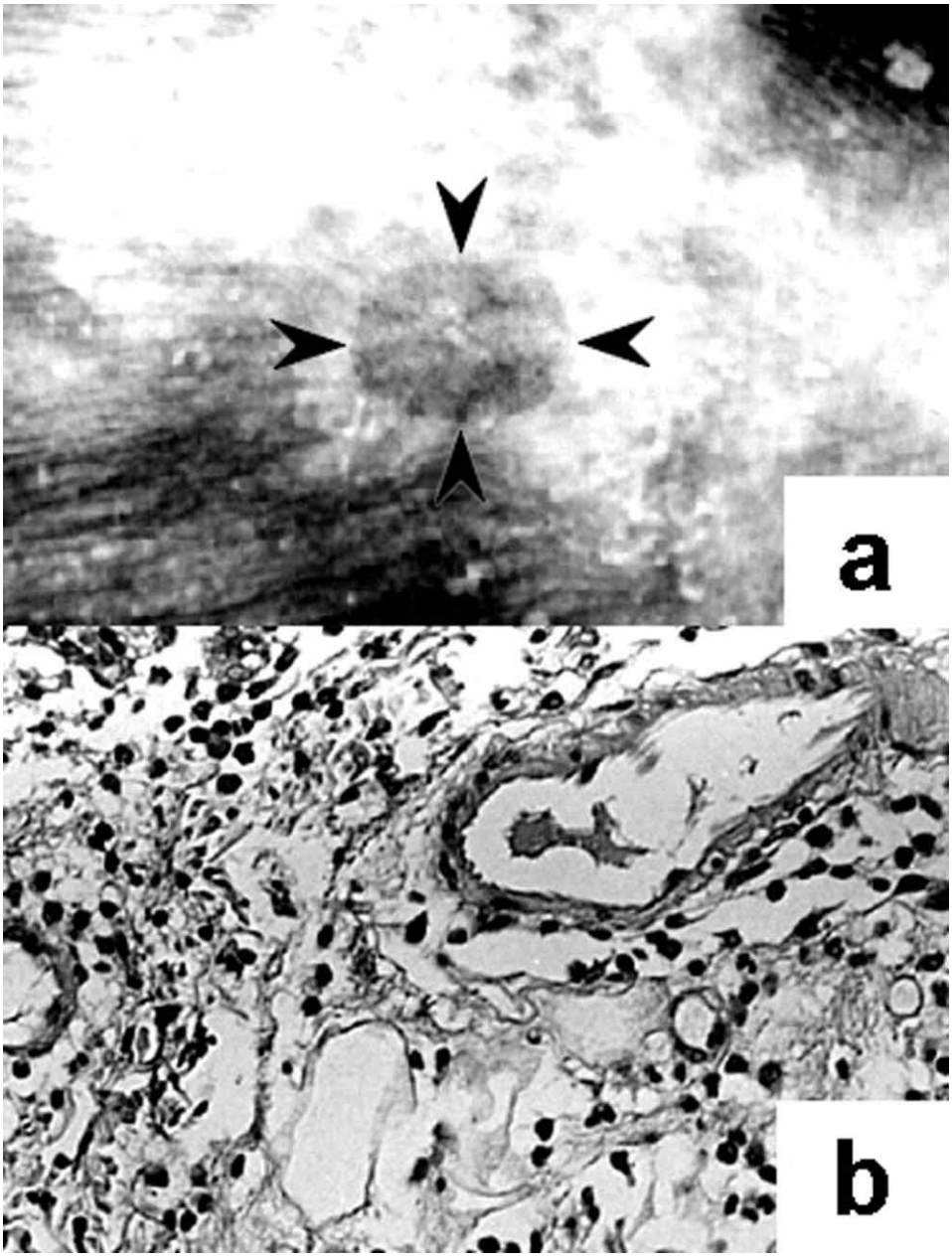
Ono *et al.* (2001)

Ono *et al.* (2003) evaluated the susceptibility of dogs to experimental infection by paracoccidioidomycosis. Puppies were inoculated with Paracoccidioides brasiliensis by an intravenous route and two out of four died 1 week postinoculation, showing, at histopathological analysis, granulomas in the lungs, spleen and liver. P. brasiliensis was isolated from these organs. The animals that survived the infection showed a strong reaction when skin was tested with gp43, a specific antigen of P. brasiliensis. These animals were killed at 1 and 5 months after infection, and no lesions, macroscopic or microscopic, were observed in the lungs, spleen or liver;

furthermore no P. brasiliensis culture was obtained from these organs. These results suggested that dogs can develop paracoccidioidomycosis and reinforces the importance of this animal as a sensitive indicator of P. brasiliensis in the environment.



Lung (1), spleen (2) and liver (3) from a puppy infected experimentally with Paracoccidioides brasiliensis. Tissues were stained with hematoxylin and eosin (a) and with Grocott (b). The lung shows more extensive lesions, with several granulomas and a high number of fungus cells. The spleen and liver show sporadic granulomas with lower number of fungus cells (200_/). **Ono *et al.* (2003)**

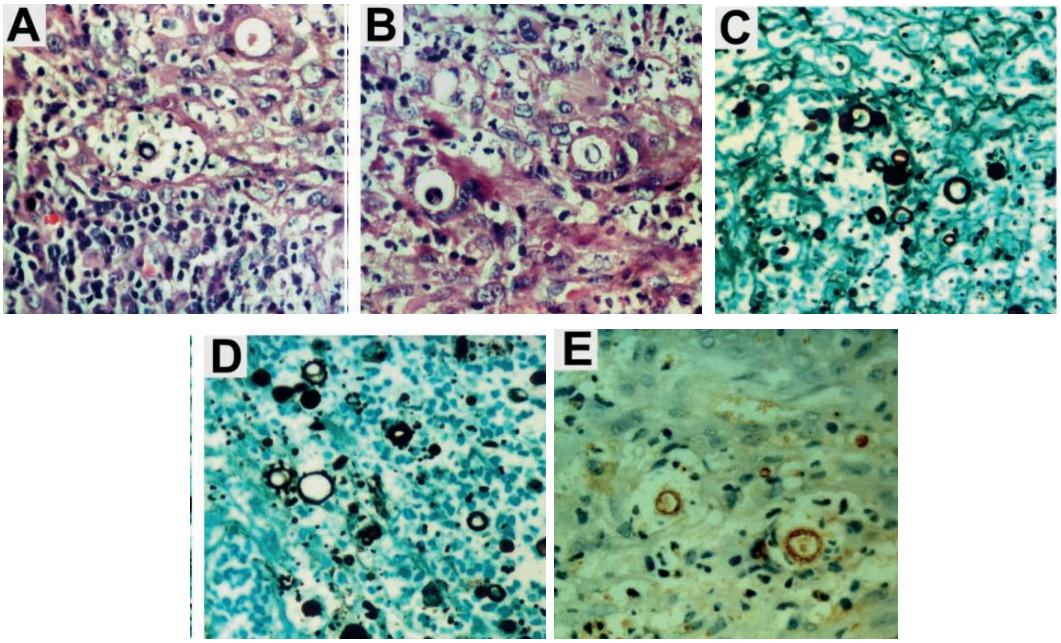


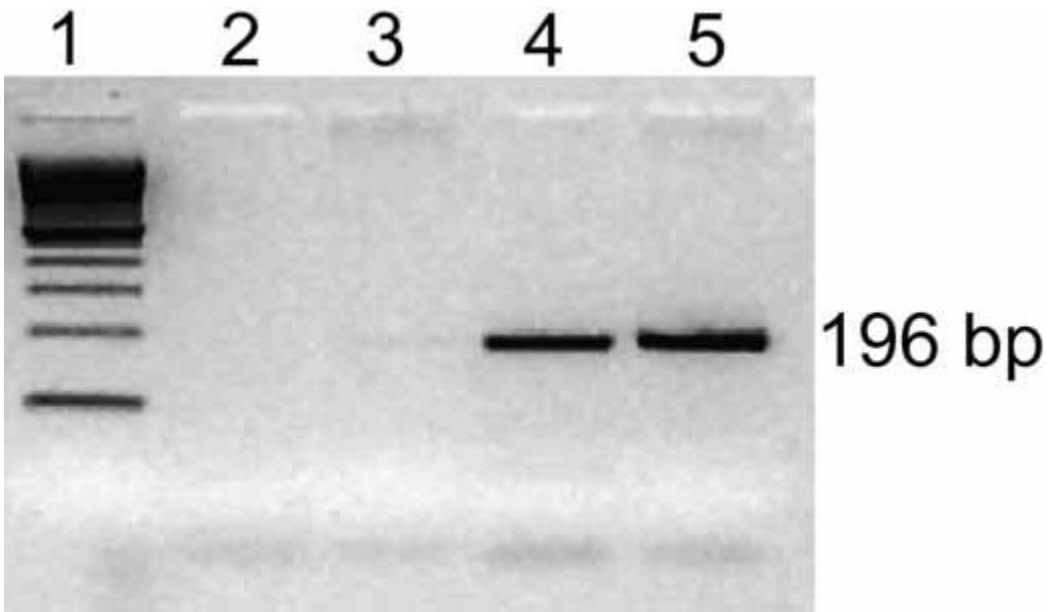
a, Skin test with P. brasiliensis -specific gp43 antigen in a puppy infected with P. brasiliensis. b, Perivascular inflammatory infiltrate with mononuclear and polymorphonuclear leukocytes (H.E.) (200_/). **Ono *et al.* (2003)**

Eisele *et al.* (2004) performed a study to evaluate the immune response of young dogs experimentally infected with Paracoccidioides brasiliensis. Six dogs were infected intravenously with P. brasiliensis and one control dog was inoculated with sterile saline. The infected animals were sacrificed in groups of two at 1, 6 and 12 months after infection. During the experimental period, the immune responses of the dogs to the fungus were followed by **ELISA** (IgM and IgG), by the immunodiffusion test and by the skin test with gp43. After killing the dogs, samples from several organs were submitted to histopathological analysis (H&E and Grocott stains) but the fungus was not observed in any tissue. Attempts to isolate the fungus from these tissue samples were also unsuccessful. All infected dogs, except one, reacted positively to the immunodiffusion and skin tests. All infected dogs showed a humoral immune response to the gp43 antigen detected by ELISA. The IgM and IgG

response peaked by the first and second month, respectively. It was concluded that young dogs appear to be resistant to the development of paracoccidioidomycosis.

Ricci *et al.* (2004) presented the case of a female adult Doberman that developed cervical lymphadenomegaly. Histopathological examination of a cervical biopsy specimen revealed active PCM, with an epithelioid, granulomatous inflammation containing numerous yeast-like, multiple budding fungal forms. The diagnosis of PCM was confirmed by immunohistochemistry using a specific antibody anti-gp43 and by nested PCR using primers for the amplification of the gp43 gene region. This is the first report of PCM disease occurring in a dog, an animal that has been shown to play an important role in the natural history of North American blastomycosis.





PCR. Lane 1, 100-bp molecular-weight marker; lane 2, template-free DNA, lane 3, negative control (H. capsulatum); lane 4, P. brasiliensis gp43 amplicom (195 bp) from a human biopsy; lane 5, P. brasiliensis gp43 amplicom (196 bp) from the canine biopsy. **Ricci *et al.* (2004)**

Silveira *et al.* (2006) carried out a study to detect antibodies against Paracoccidioides brasiliensis in dogs seropositive and seronegative for leishmaniasis. Sera from 836 dogs (449 positive and 387 negative to leishmaniasis) were analysed by ELISA and the immunodiffusion test using gp43 and exoantigen, respectively. The analysis of the 836 serum samples by ELISA and the immunodiffusion test showed a positivity of 67.8 % and 7.3%, respectively, for P. brasiliensis infection. The dogs positive to leishmaniasis showed a higher reactivity to gp43 (79.9%) and exoantigen (12.7%) than the negative ones (54.0% and 1.0%, respectively). The higher reactivity to P. brasiliensis antigens may be due to cross-reactivity or a co-infection of dogs by Leishmania and P. brasiliensis. The lower correlation (0.187) observed between reactivity to gp43 and Leishmania antigen reinforces the latter hypothesis.

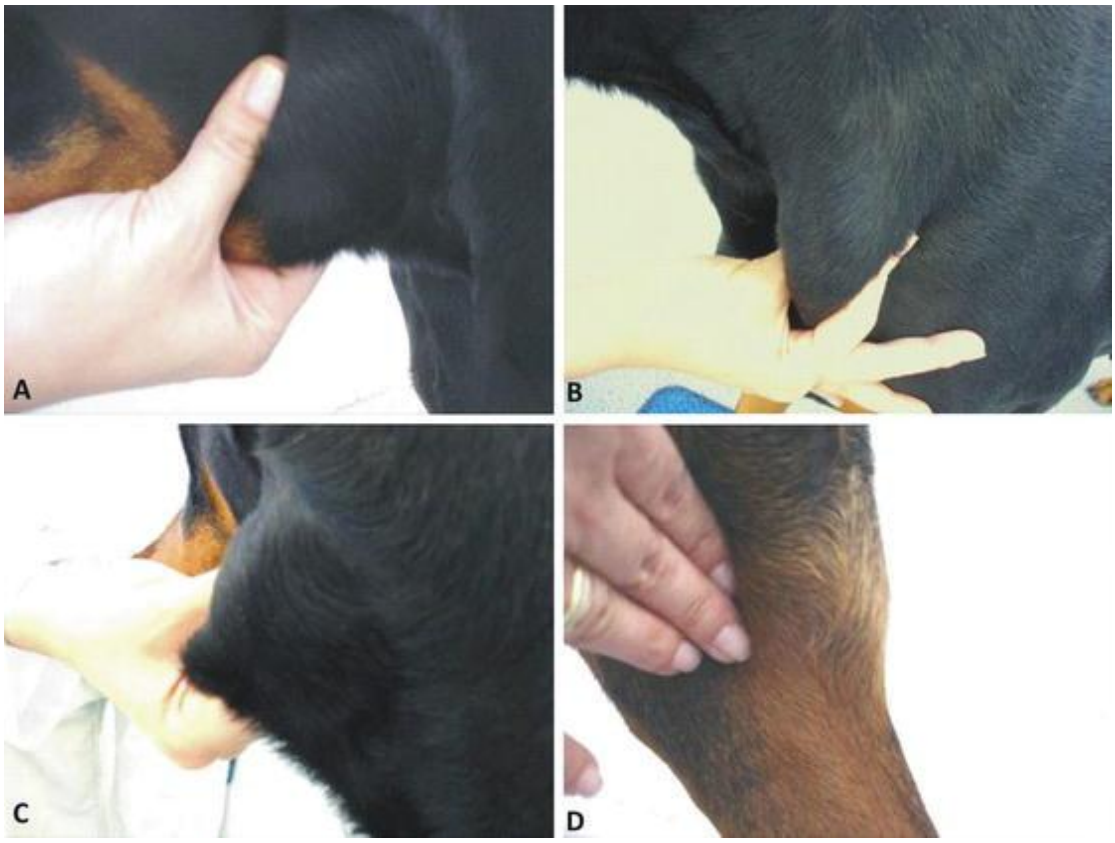
Theodoro *et al.* (2008) determined the sequences of the PRP8 intein from P. brasiliensis isolates to be belonging to the three described genetic groups and two unidentified isolates and analyzed in order to check their functionality and usefulness for species identification. All the isolates presented a full-length intein, although the Endonuclease domain seemed to be inactive due to substitutions in the second essential aspartic acid residue. Phylogenetic analysis by Maximum-Parsimony, Maximum Likelihood, and Bayesian analysis clearly separated the isolates from the three species and revealed a significant difference between the Pb01 isolate and the remaining ones. The Pb01 isolate did not belong to any of the groups previously described since it presented a high divergence level compared to the three different genetic groups, corroborating some previous studies that suggested this isolate as a new species of Paracoccidioides.

Bianchini *et al.* (2009) carried out a study to evaluate the activation of the dog alternative complement pathway by P. brasiliensis. Initially, the ability of erythrocytes of guinea pig, rabbit, sheep, chicken and swine to activate the dog alternative pathway was evaluated. The guinea pig erythrocytes showed the greatest capacity to activate dog alternative pathway. The alternative (AH50) hemolytic activity was evaluated in 27 serum samples from healthy dogs and the mean values were 87.2 AH50/ml. No significant differences were observed in relation to sex and age. The alternative pathway activation by P. brasiliensis was higher in serum samples from adult dogs when compared to puppies and aged dogs ($p \leq 0.05$). This is the first report of dog alternative complement pathway activation by P. brasiliensis and suggests that it may play a protective role in canineparacoccidioidomycosis.

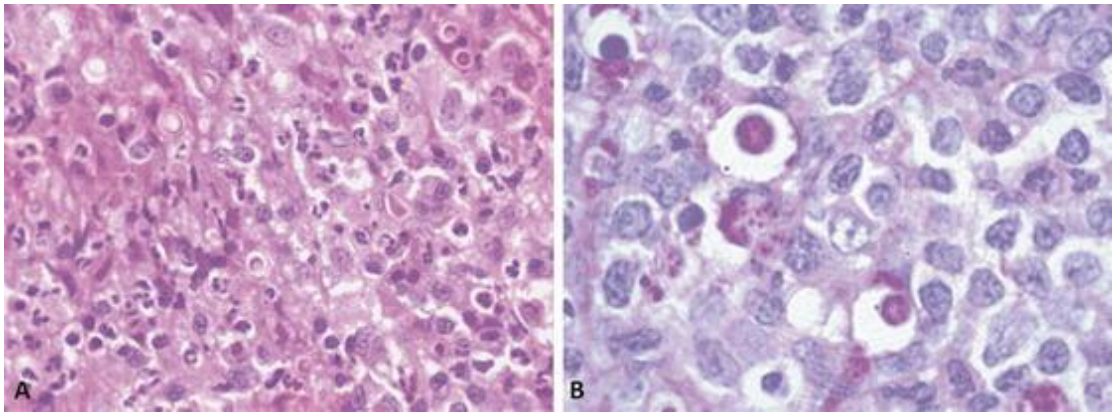
Fontana *et al.* (2010) performed a survey to evaluate canine infection with P. brasiliensis in 149 urban and 126 rural dogs using **ELISA and intradermal tests** with the gp43 antigen of P. brasiliensis in Uberaba, Minas Gerais state of Brazil. Forty-one out of 149 urban dogs were euthanatized and had their lungs, liver and spleen removed. One slice from each viscera was processed for histopathological examination and the remaining was homogenized and then cultivated on mycobiotic agar at room temperature and Fava-Netto medium at 35 degrees C and observed for 12 weeks. Of urban dogs, 75 (50.3%) were small adult females, 56 (36%) were strays, while 93 (64%) had been donated to the municipal zoonosis control center. Nine (6.2%) had a positive intradermal test without statistical differences regarding gender, race, nutritional status or origin. No colonies with microscopic or morphology appearances resembling P. brasiliensis were isolated, nor granulomatous process or fungal structures were observed from histopathological examination. Eighty (53.6%) of the urban dogs presented seroreactivity, without statistical differences regarding gender, race, nutritional state, origin, or positive intradermal test. Of 126 rural dogs,

102 (80.5%) presented antibodies against gp43 antigen, and this was statistically significant in relation to the reactivity detected in urban dogs (P = 0.0001). Thus, dogs are commonly infected with P. brasiliensis, but they probably present natural resistance to develop paracoccidioidomycosis.

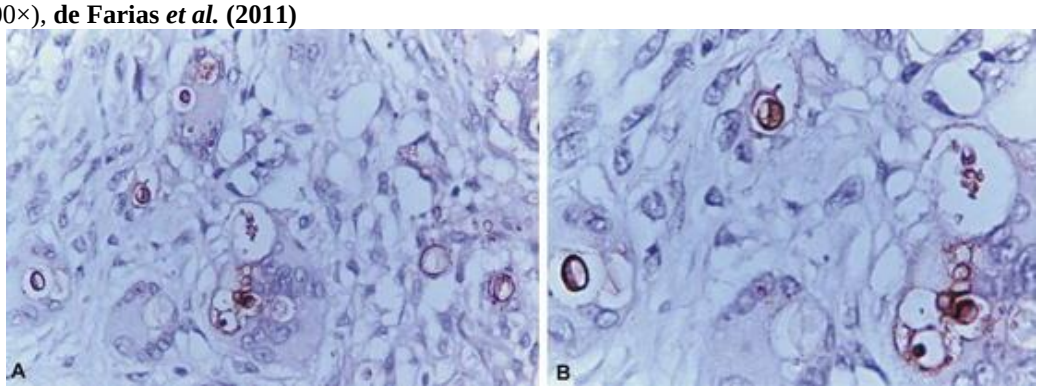
de Farias *et al.* (2011) reported the second case of naturally acquired PCM in a 6-year-old female dog that presented emaciation, **lymphadenomegaly**, and **hepatosplenomegaly**. Biochemical and pulmonary radiographic evaluation did not reveal any abnormalities. PCM was diagnosed by clinical findings, culturing, immunohistochemistry, and histopathology of popliteal lymph node. The fungus was recovered from popliteal lymph node, and the **molecular** analysis showed respective sequencing similarities of 99 and 100% for 803 nucleotides of the Gp43 gene and 592 nucleotides from the ITS-5.8S region of Paracoccidioides brasiliensis. Immunohistochemistry revealed severe lymphadenitis and presented numerous yeasts, which reacted against the gp43 antibody. Histopathology revealed a severe granulomatous lymphadenitis associated with numerous single or multiple budding yeasts. After diagnosis, the dog was successfully treated with **itraconazol** for 2 years.



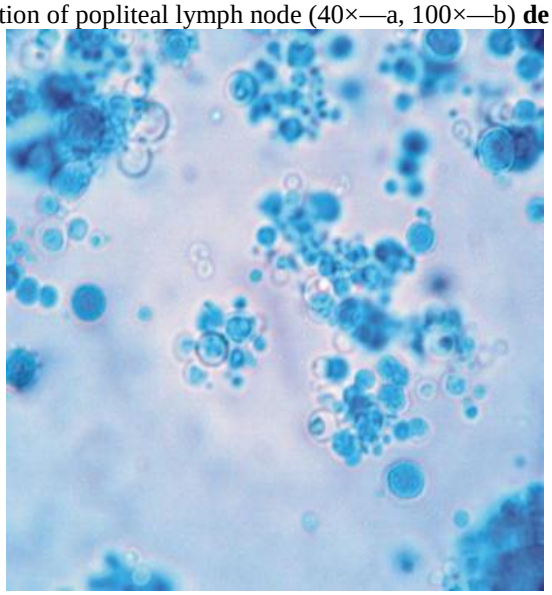
Generalized lymphadenomegaly in a six-year-old female Doberman. **a** Submandibular lymph node; **b**prescapular lymph node; **c** inguinal lymph node; **d** popliteal lymph node, **de Farias *et al.* (2011)**



a Histological fragment of popliteal lymph node stained with hematoxylin and eosin showing *oval structures*(40×). b Histological fragment of popliteal lymph node stained with periodic Acid-Schiff showing the same poorly stained *oval structure* surround by neutrophils, macrophages and giant cells (100×), **de Farias *et al.* (2011)**



Yeasts of *P. brasiliensis* stained through an antibody directed against the protein *Gp43* in immunohistochemical section of popliteal lymph node (40×—a, 100×—b) **de Farias *et al.* (2011)**


Microscopy showing many globous yeast cells stained by lactophenol cotton blue smear (40×) **de Farias *et al.* (2011)**

Teles *et al.* (2016) investigated infection by ***P. brasiliensis*** in dogs from Southern Brazil. Indirect **ELISA** was used to detect antibodies against P. brasiliensis gp43. One hundred and ninety-six stray and semi-domiciled dogs from the municipalities of Pelotas and Capão do Leão, Rio Grande do Sul were included in this study. P. brasiliensis infection was detected in 58 animals (29.6 %) with no significant difference for gender, age and breed. Seropositive animals were detected in all

neighborhoods in the city of Pelotas as well as in the neighboring municipality Capão do Leão. The detection of antibodies against gp43 in dogs suggests the presence and wide distribution of the fungus in Pelotas and Capão do Leão, warning for the possibility of PCM disease in dogs as well as in humans from this region.

4.3.2. Reports of Paracoccidioidomycosis in cats

Gonzalez *et al.* (2010) reported a male Persian cat with persistent fever, anorexia, weakness, hypopyon, nystagmus, and intention tremors. The hemogram showed severe neutropenia and laboratory analysis on cerebrospinal fluid (CSF) smears revealed abundant yeast cells compatible with Paracoccidioides brasiliensis. Urinalysis demonstrated persistent funguria and an increased urine protein-to-creatinine ratio (UPC) in addition to mild azotemia. Long-term therapy with oral fluconazole was effective in controlling the nervous system signs. Funguria was resolved with subcutaneous administration of diluted amphotericin B in a large volume of saline solution for a period of 12 weeks during the second year after initial diagnosis. Throughout 5 years of treatment, no adverse effects were observed and tolerance to the drugs was normal. Due to development of progressive uremic syndrome the animal was euthanased.

Oliveira *et al.* (2013) carried out a study was to evaluate infection of **cats** by ***Paracoccidioides brasiliensis***. Serum samples of 136 cats from rural (n = 86) and urban areas (n = 50) were analyzed by indirect ELISA and immunodiffusion test using P. brasiliensis gp43 and exoantigen as antigens, respectively, and an overall reactivity of 31.6 % was observed by ELISA although no reactivity was detected by immunodiffusion. The positivity observed in animals living in rural areas (48.8 %) with free access to soil was significantly higher ($P < 0.0001$) than among urban animals (2 %) with limited access to soil, although no significant difference was observed in relation to age or sex. The high rates of positivity observed in cats from rural areas suggest that not diagnosed cases of this mycosis may be occurring in cats living in endemic areas for human paracoccidioidomycosis. This is the first report showing serological evidence of P. brasiliensis infection in cats.

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5. Sporotrichosis

5.1. Introduction

Sporotrichosis is a mycotic infectious disease that is generally acquired by traumatic inoculation of contaminated materials especially from plant debris or through bites and scratches from diseased animals, such as domestic cats. It affects the skin, lymphatic system, and other organs in the warm-blooded host. Etiological agents are embedded in the plant-associated order Ophiostomatales. With essential differences between possible outbreak sources and ecological niche, host-environment interactions are classic determinants of risk factors for disease acquisition. Sporotrichosis outbreaks with zoonotic transmission, such as those that are ongoing in southern and southeastern Brazil, have highlighted the threat of cross-species pathogen transmission. Sporothrix brasiliensis has emerged as a human threat owing to the intimate contact pattern between diseased cats and humans in endemic areas. **Montenegro *et al.* (2014)**

Feline sporotrichosis, which is caused by species of the Sporothrix schenckii complex, is endemic to Rio de Janeiro, Brazil. More than 4000 cases of the disease were diagnosed at Fundação Oswaldo Cruz, Brazil, between 1998 and 2012. Sporotrichosis in cats has been reported in several countries, but nowhere has an outbreak of animal sporotrichosis been as large as that seen in Brazil. The clinical

manifestations of the disease range from an isolated skin lesion that can progress to multiple skin lesions and even fatal systemic involvement. Nodules and ulcers are the most common types of lesions, and respiratory signs and mucosa involvement are frequent. The definitive diagnosis depends on isolation of the etiologic agent in culture. Cytology, histopathology, and serology are useful tools for preliminary diagnosis. Severe pyogranulomatous inflammatory infiltrate, high fungal load, and extension of lesions to mucosa, cartilage, and bone in the nose of cats are indicative of an agent of high virulence in this endemic region. Itraconazole is the drug of choice, while, in refractory cases, amphotericin B or potassium iodide might be alternative treatments; however, recurrence after discharge may occur. Sporotrichosis persists as a neglected disease in Rio de Janeiro, and the treatment of cats remains a challenging and long-term endeavor **(Gremião *et al.*, 2015)**

- Sporotrichosis is a subcutaneous mycosis with worldwide distribution, especially in tropical and subtropical areas.
- Sporotrichosis is an acute or chronic subcutaneous mycosis of humans and other mammals, especially cats, caused by pathogenic species of ***Sporothrix schenckii*** complex revealed by gene sequencing:

1. Sporothrix albicans
2. Sporothrix brasiliensis
3. Sporothrix globosa
4. Sporothrix luriei
5. Sporothrix mexicana
6. Sporothrix schenckii

- Molecular studies have demonstrated a high level of intraspecific variability. Components of the S. schenckii cell wall that act as adhesins and immunogenic inducers, such as a 70-kDa glycoprotein, are apparently specific to this fungus. The main glycan peptidorhamnomannan cell wall component is the only O-linked glycan structure known in S. schenckii. It contains an α-mannobiose core followed by one α-glucuronic acid unit, which may be mono- or di-rhamnosylated. The oligomeric structure of glucosamine-6-P synthase has led to a significant advance in the development of antifungals targeted to the enzyme's catalytic domain in S. schenckii. **López-Romero et al. (2011)**

- Sporotrichosis, caused by the Sporothrix schenckii fungal complex, is a zoonotic mycosis distributed worldwide.
- Sporothrix propagules present on soil and plant debris may be traumatically inoculated into the cutaneous/ subcutaneous tissues of the warm-blooded host. An alternative route involves direct animal-animal and animal-human transmissions through deep scratches and bites of diseased cats.
- Zoonotic transmission is described with cats being the main animal species involved. The occurrence of severe feline sporotrichosis with high fungal levels demonstrates

the susceptibility of cats to this disease and the importance of studying its pathogenesis.

- Over the last decades, large epidemics of sporotrichosis occurred in Brazil due to zoonotic transmission, and cats were pointed out as key susceptible hosts.
- The diagnosis solely rests on the isolation of Sporothrix schenckii in culture. On pathologic examination, causative organisms are rarely seen. Staining with fluorescent-labeled antibodies may aid in visualizing the cigar-shaped yeast forms;
- Topical therapy is not effective. Potassium iodide is an effective treatment for sporotrichosis, but this agent has not been subjected to specific treatment trials comparing its efficacy against azoles or allylamine alternatives.
- Itraconazole is generally safe and well tolerated, and the relapse rate is low. Terbinafine could be another therapeutic alternative to treat the disease

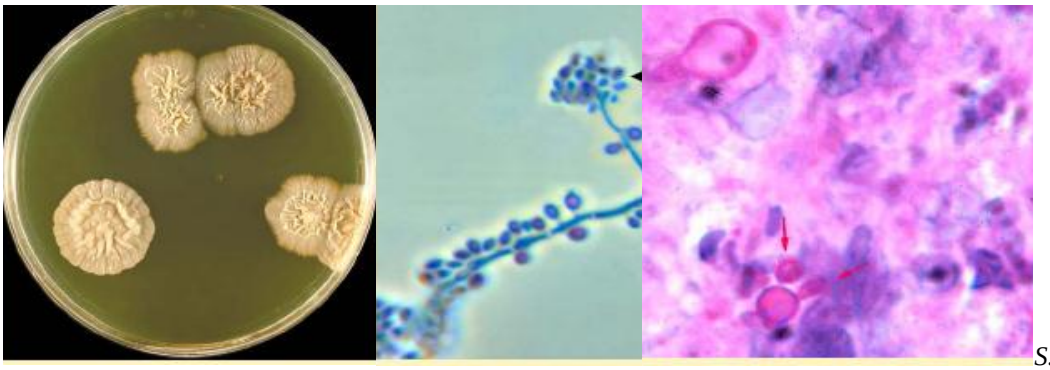
5.2. Aetiology

5.2.1. *Sporothrix schenckii* HEKTOEN et PERKINS 190

Synonyms *:* *Sporotrichum schenckii MATRUCHOT1910*
Sprotrichum beurmannii MATRUCHOT et RAMOND 1905
Sporotrichum asteroids SPLENDORE 1909

Perfect stage: *Ceratocystis stenocera*

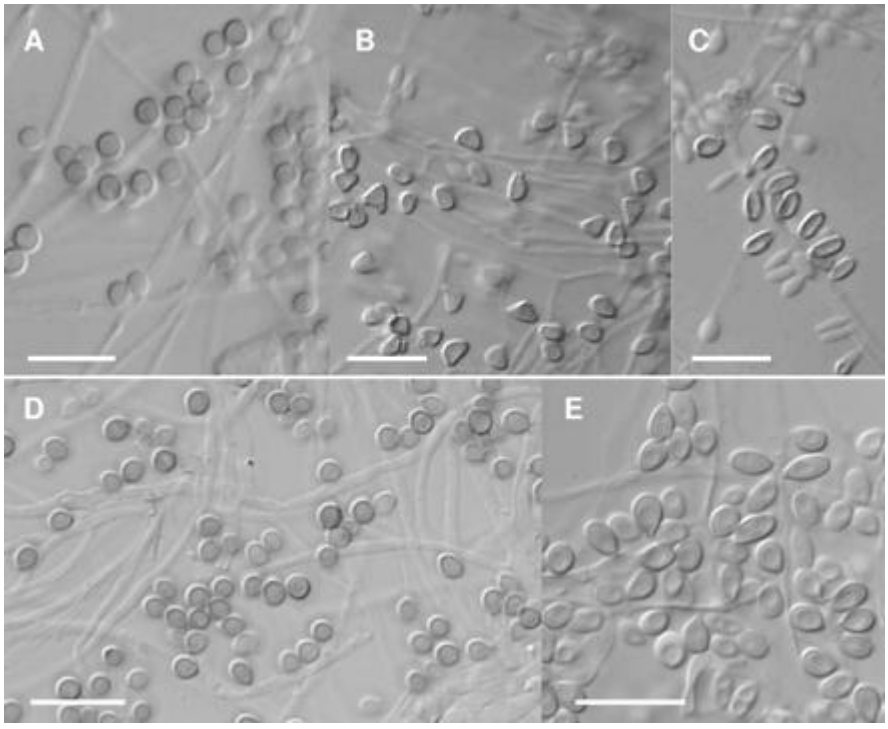
S. schenckii is a thermically dimorphic fungus. On Sabouraud dextrose agar at 25 C colonies develop in 3-5 days, at first blackish and shiny but become fuzzy with age as aerial hyphae are produced. Initially, the colony is moist, glabrous and yeast-like , but becomes tough, wrinkled and folded in time. Microscopically, thin, branching, septate hyphae and small, 3-5 microns, conidia are seen. The conidia are delicately attached to the distal tapering ends of slender conidiophores. The conidia are arranged in flower-like clusters. At 37 ^{o}C, on media containing high concentration of sugars the organism grows in the yeast phase. Conversion to the yeast phase requires 3-5 days. The yeast colony is pasty and grayish Microscopically, the yeast cells are variable in shape, but often fusiform, 1-3 by 3-10 microns, with multiple buds. The elongated yeast cells, resembling cigars with buds, are characteristic.



S. schenckii at 25^{o}C flower-like clusters of conidia cigar-shaped yeast cells in tissues

5.2.2. *Sporothrix brasiliensis* Marimon, Gené, Cano & Guarro, Journal of Clinical Microbiology 45 (10): 3203 (2007)

Colonies on PDA attain a diameter of 15 to 38 mm after 21 days of incubation at 30°C. Conidiogenous cells are usually terminal or intercalary on more or less differentiated conidiophores, slightly swollen, and produce conidia sympodially on a few denticles. Sympodial conidia are usually hyaline to subhyaline, obovoidal, and 2 to 6 μm long by 1 to 4 μm wide. Sessile conidia are brown to dark brown, thick walled, globose to subglobose, and 2.5 to 5 μm long by 2 to 3 μm wide. The maximum growth temperature is 37°C (5 to 10 mm in diameter after 21 days). The fungus does not grow at 40°C and is unable to assimilate sucrose and raffinose.



Morphology of the sessile conidia of the *S. schenckii* species complex. (A) *S. brasiliensis* CBS 120339 (clade I). (B and C) *S. schenckii* (clade II) and FMR 8608 (clade IIa) (B) and FMR 8677 (clade IIb) (C). (D) *S. globosa* CBS 120340 (clade III). (E) *S. mexicana* CBS 120341 (clade IV). Bars, 10 μm.

5.3. Reports

5.3.1. Dogs

Aetiology

- ***Sporothrix schenckii*, Bernstein *et al.* (2007, Cafarchia *et al*. (2007), dos Santos** et al. (2007), **de Miranda *et al.* (2009), Madrid *et al.* (2011), Miranda *et al.* (2011), Madrid *et al.* (2012)**
- ***Sporothrix brasiliensis*, Guterres *et al*. (2014)**

Clinical

- **Cutaneous sporotrichosis, Schubach *et al.* (2006), Bernstein *et al.* (2007, dos Santos** et al. (2007)**, de Miranda *et al.* (2009), Madrid *et al.* (2012)**
- **Lymphocutaneous sporotrichosis, Cafarchia *et al*. (2007), Crothers** *et al.* (2009)
- **Disseminated sporotrichosis**.**Crothers** *et al.* (2009)**, Madrid *et al.* (2012)**
- **osteoarticular sporotrichosis**, **Goad and Goad (1986)**

- Nasal **sporotrichosis, Shany (2000), Cafarchia *et al*. (2007), Whittemore** *et al*. (2007)
- claw bed **sporotrichosis, Sykes *et al.* (2001)**

Treatment

- ketoconazole, **Goad and Goad (1986)**, **Cafarchia *et al*. (2007)**, **Crothers** *et al.* (2009)
- itraconazole, **Shany (2000), Sykes** *et al.* (2001), **Bernstein *et al.* (2007, Whittemore** *et al*. (2007)**, Crothers** *et al.* (2009)**, Guterres *et al*. (2014)**
- (1-3) β-glucan along with itraconazole **, Guterres *et al*. (2014)**

5.3.2. Cats

Aetiology

Sporothrix schenckii , Kovarik *et al.* (2008), Schubach *et al.* (2008), Crothers *et al.* (2009), Reis *et al*. (2009), Weingart *et al.* (2010), Fernandes *et al.* (2011), Gremião *et al.* (2011, Borges *et al.* (2013), dos Santos *et al.* (2013, Rodrigues *et al.* (2013, Pereira *et al.* (2014), Teixeira *et al.* (2014)

- ***S. brasiliensis*** Rodrigues *et al.* (2013, Montenegro *et al.* (2014), Pereira *et al.* (2014), Teixeira *et al.* (2014), Kano *et al.* (2015), Brilhante *et al.* (2016)

Zoonotic aspects

- Kovarik *et al.* (2008), Reis *et al*. (2009), Cordeiro *et al*. (20,11), Rees and Swartzberg (2011), Borges *et al.* (2013)

Clinical

- Cutaneous, Schubach *et al.* (2008), Crothers *et al.* (2009), Gremião *et al.* (2011, Chaves *et al.* (2013), dos Santos *et al.* (2013, Gremião *et al.* (2015)
- cutaneous-lymphatic Crothers *et al.* (2009), Madrid *et al*. (2010)
- Nasal, Gremião *et al.* (2015)
- Claws, Borges *et al.* (2013)
- disseminated sporotrichosis. Crothers *et al.* (2009), Pohlman *et al.* (2014)
- chronic parapreputial wound. Weingart *et al.* (2010)
- Supp granuloma, Miranda *et al.* (2013

Diagnosis

- Cytological and mycological , dos Santos *et al.* (2013, Gremião *et al.* (2015), Jessica *et al.* (2015), de Souza *et al*. (2016), Miranda *et al.* (2016)
- ELISA Fernandes *et al.* (2011)
- Molecular , Rodrigues *et al.* (2013, Montenegro *et al.* (2014), Teixeira *et al.* (2014), Kano *et al.* (2015), Kano *et al.* (2015)

Treatment

- intralesional amphotericin, Gremião *et al.* (2011
- itraconazole, Hirano *et al.* (2006), Madrid *et al*. (2010), Pereira *et al.* (2010) , Weingart *et al.* (2010), Cordeiro *et al*. (2011), Gremião *et al.* (2011, Brilhante *et al.* (2016) , de Souza *et al*. (2016)

- ketoconazole, Crothers *et al.* (2009), Pereira *et al.* (2010), Brilhante *et al.* (2016)
- fluconazole (one cat), Crothers *et al.* (2009), Brilhante *et al.* (2016)

5.4. Reports on sporotrichosis in dogs:

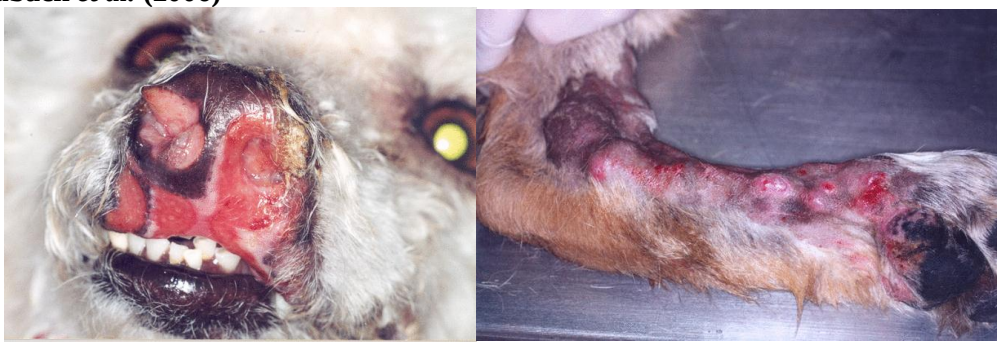
Goad and Goad (1986) diagnosed a case of **osteoarticular sporotrichosis** in a dog referred for evaluation of hind limb lameness. There was radiographic evidence of osteopenia of the fourth tarsal and proximal aspects of the metatarsal bones. The diagnosis was based on histologic findings and results of physical examination, radiography, fungal culturing, and serologic tests. The dog was treated successfully with **Goad and Goad (1986)** diagnosed a case of **osteoarticular sporotrichosis** for 3 1/2 month

Shany (2000) diagnosed an unusual ulcerated masses protruding from **both nostrils** of a 3-year-old terrier histologically as sporotrichosis, and regressed with iodide therapy. Cryptococcus neoformans was recovered from new lesions that appeared near the dog's eye and on the extremities. All lesions regressed with itraconazole therapy.

Sykes *et al.* (2001) diagnosed sporotrichosis in a 2-year-old male Golden Retriever that was allowed to roam free on the owner's Christmas tree farm in Minnesota. Clinical signs had been evident for 1 month and included swelling of the claw bed of the third digit on the left forelimb and a fluctuant **nodular lesion** in the area of the left carpus. Few organisms were seen in affected tissues, and diagnosis was confirmed on the basis of results of fungal culture. The condition responded to treatment with itraconazole.

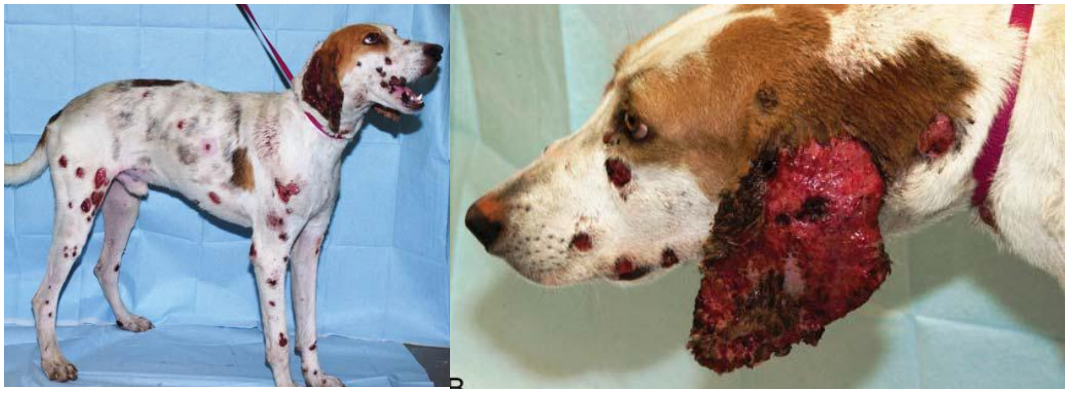
Schubach *et al.* (2006) described a sporotichosis epidemic involving forty-four dogs in the Metropolitan area of Rio de Janeiro. **Solitary skin lesions** were noted in 18 dogs(40.9%), 2-4 such lesions were observed in 17 animals (38.6%), and nine (20.5%) animals had five or more lesions. Twenty-five (56.8%) animals had single ulcerated skin lesions on the nose and nine (20.5%) showed nasal mucosal involvement (three of which also has a skin lesion). Respiratory symptoms were observed in 17 (38.6%) dogs and were found to be the most common extracutaneous signs of infection. Anemia, leukocytosis with neutrophilia, hypoalbuminemia and hyperglobulinemia were the most frequent hematological abnormalities. Histopathological analysis of skin biopsies in most cases revealed granulomatous reactions characterized by histiocytic hyperplasia and neutrophil infiltration. Yeast-like cells were observed in seven (16.7%) of 42 dogs examined histologically. During the study, eight (18.2%) animals were lost to follow-up and three (6.8%) were submitted to euthanasia. Of the remaining 33 dogs, five (15.2%) presented spontaneous regression of the lesions, 26 (78.8%) were cured after treatment, and two (6%) continue to be treated. The present cases indicate that many dogs with sporotrichosis respond well to treatment and in a few dogs, the disease may be self-limiting.

Photograph of a dog with multiple skin lesions on the hind limbs, on the flank and on the head, **Schubach *et al.* (2006)**

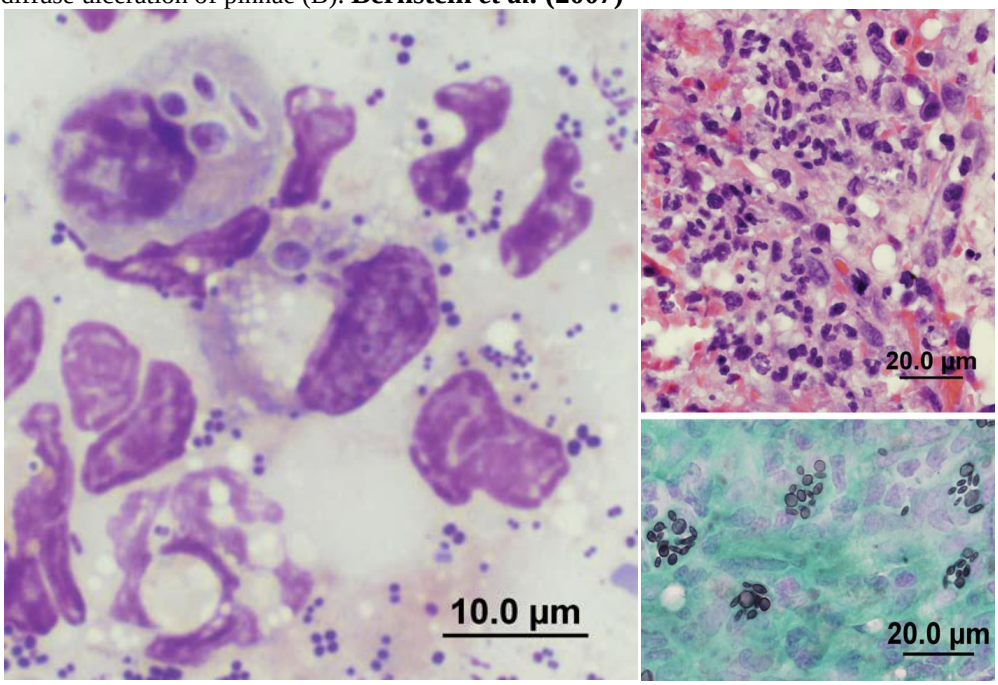


Photograph of a dog with skin ulcers on the nose and destruction of the nostrils, Photograph of a dog with nodular lymphangitis on the hind limbs. **Schubach *et al.* (2006)**

Bernstein *et al.* (2007) reported a 1-year-old male Foxhound/Walker Hound mix with a 6-week history of progressive, multifocal, ulcerative and draining, well-circumscribed lesions in a generalized distribution. Prior to referral, a presumptive diagnosis was made of sterile pyogranulomatous disease; immunosuppressive therapy was instituted but resulted in clinical deterioration. At presentation, the dog had marked neutropenia (1100 neutrophils/microL), and a mild toxic left shift (400 bands/microL). Cytologic findings in the exudates from a draining **skin lesion** included high numbers of markedly degenerate neutrophils (about 95% of nucleated cells) as well as low numbers of macrophages, small mature lymphocytes, and eosinophils. Low numbers of intracellular (within neutrophils and macrophages) and extracellular, pleomorphic, cigar-to-ovoid shaped organisms (approximately 3x9 microm) consistent with Sporothrix were observed. Histopathologic examination of a skin biopsy showed marked, chronic, active, ulcerative, pyogranulomatous dermatitis and panniculitis, with intralesional yeast consistent with Sporothrix sp. The etiologic agent was confirmed as ***Sporothrix schenckii*** by macerated tissue fungal culture. The patient was treated with itraconazole, enrofloxacin, and clindamycin, with clinical resolution occurring over a 3-month period.



The patient at initial presentation with multifocal ulcerative lesions on face, flank, and limbs (A) and with diffuse ulceration of pinnae (B). **Bernstein *et al.* (2007)**



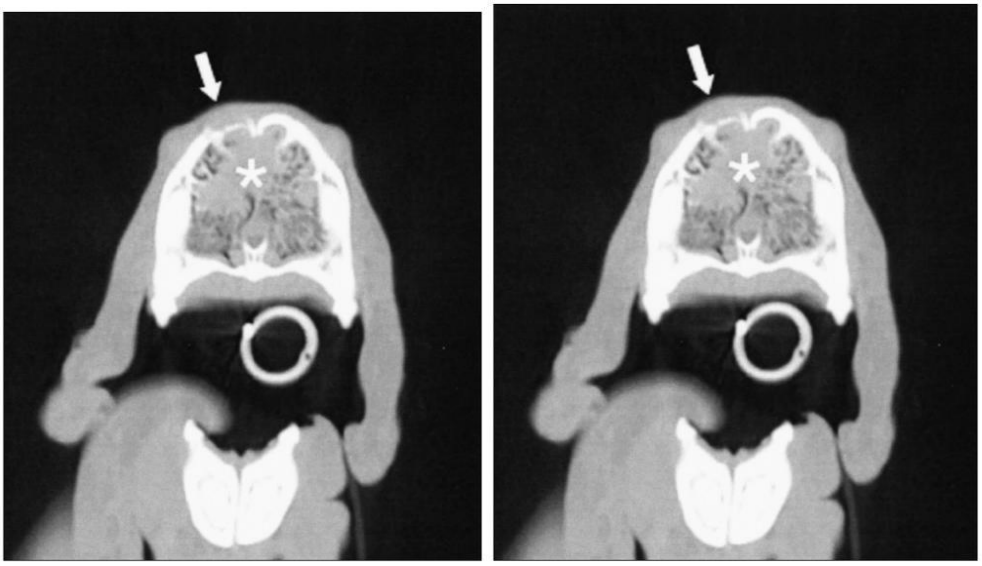
Cytologic preparation of draining exudate from a cutaneous lesion, with marked pyogranulomatous inflammation, Sporothrix organisms, and bacterial cocci. Wright's**,** Histopathologic section of a skin biopsy with pyogranulomatous dermatitis and Sporothrix organisms. (Upper) H&E; (Lower) Gomori methenamine silver. **Bernstein *et al.* (2007)**

Cafarchia *et al*. (2007) reported a case of **lymphocutaneous** and **nasal sporotrichosis** in a hunting dog with a three month history of non-healing skin lesions. Cytological examination of nasal discharge and of the material collected from ulcerated skin surfaces showed a few cigar-shaped organisms within macrophages. Fungal cultures of nasal and ulcerated skin swabs yielded colonies of S. schenckii. The dog received oral itraconazole but died of unrelated causes. Necropsic examination was not performed.

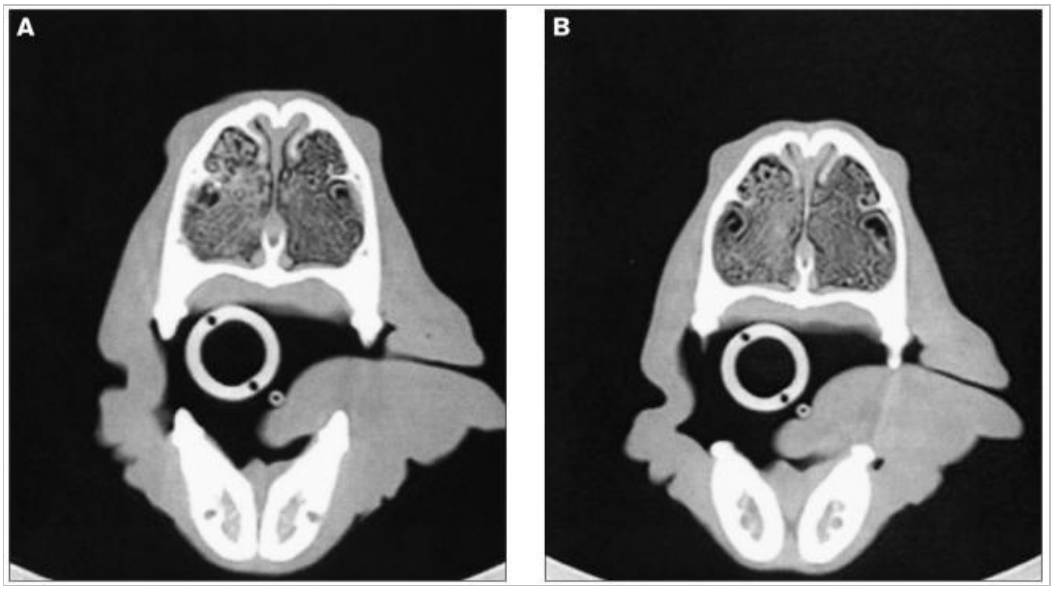
dos Santos et al. (2007) reported 74 dogs from the State of Rio de Janeiro with ulcerated cutaneous lesions. ***Sporothrix schenckii*** was isolated from 41 dogs and Leishmania (Viannia) braziliensis was isolated from 33 animals. Most dogs with sporotrichosis were from the municipality of Rio de Janeiro (53.7%) and presented ulcerated **cutaneous** lesions on the head (68.3%). Laboratory alterations in these animals included anemia (58.5%), hypoalbuminemia (83%) and hyperglobulinemia (75.6%). Histopathology revealed the predominance of a chronic

granulomatous inflammatory infiltrate (70.7%), and yeast-like structures were detected in 17% of the histopathological exams and in 32% of the cytological exams. Three of 41 dogs with sporotrichosis were seropositive by IIF for leishmaniosis and 2 of 20 animals tested within this group had a positive leishmanin skin test. Similarly, most of the 33 dogs with leishmaniosis were from the municipality of Rio de Janeiro (69.7%) and had ulcerated cutaneous lesions on the head (84.8%). Laboratory alterations in these animals included anemia (66.7%), hypoalbuminemia (100%) and hyperglobulinemia (91%). Histopathology showed the predominance of a chronic granulomatous inflammatory infiltrate (63.6%) and amastigote forms were detected in 30.3% of the histopathological exams and in 31.8% of the 22 cytological exams performed. About 72.7% of the dogs were seropositive by IIF and five of seven animals had a positive skin test. Due to the clinical similarities, histopathological and nonspecific laboratory results similarities, the serological and skin tests for leishmaniosis positive in dogs with sporotrichosis, and the overlapping endemic areas in Rio de Janeiro, the differential diagnosis between the two diseases requires the demonstration of their respective etiological agents.

Whittemore *et al*. (2007) reported the diagnosis and treatment of **intranasal** sporotrichosis in a dog presented for a loss of smell, sneezing, and nasal congestion. Following 6 months of itraconazole treatment, a computed tomography scan showed a complete resolution of previously identified abnormalities.



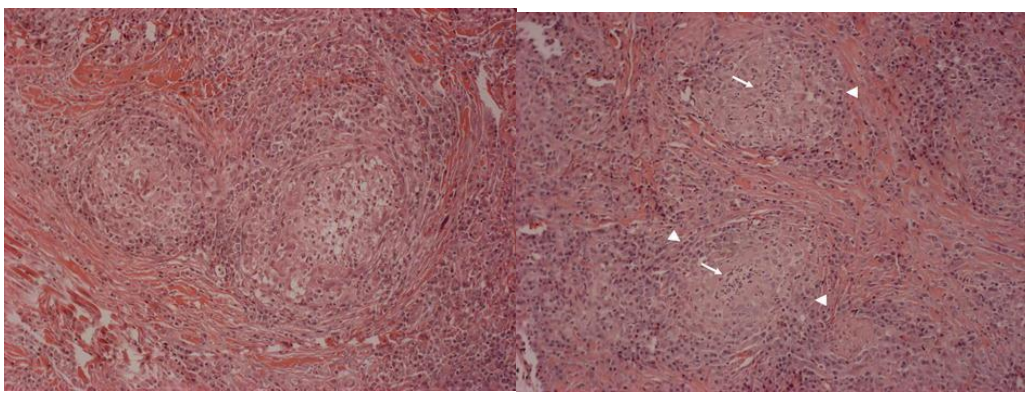
Sequential transverse CT section of the rostral nasal cavity of a dog with chronic nasal obstruction upon initial presentation. A large soft tissue density mass (asterisk*) can be seen filling the dorsal meatus bilaterally. Focal osteolysis of the right side of the nasal bone is indicated by the white arrow. Endotracheal tube diameter = 10 mm, Sequential transverse CT section of the rostral nasal cavity of a dog with chronic nasal obstruction upon initial presentation. A large soft tissue density mass (asterisk*) can be seen filling the dorsal meatus bilaterally. Focal osteolysis of the right side of the nasal bone is indicated by the white arrow. Endotracheal tube diameter = 10 mm, **Whittemore *et al*. (2007)**

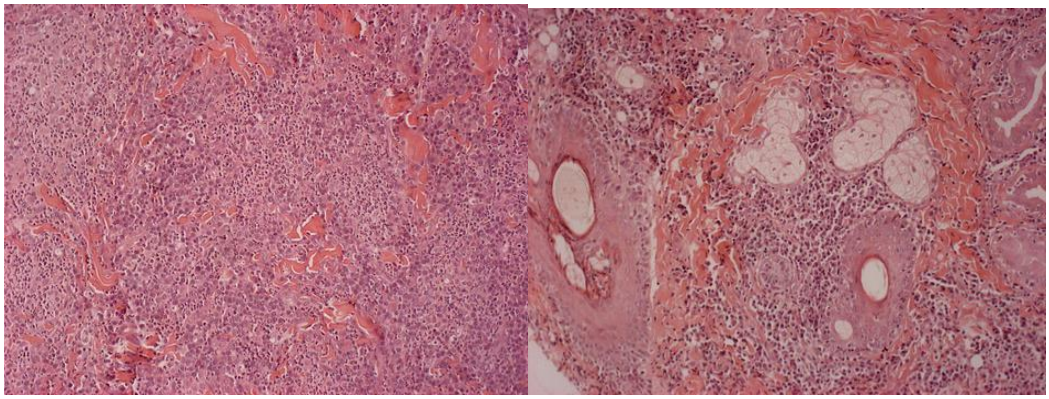


Repeat sequential transverse CT sections of the rostral nasal cavity of a dog with chronic nasal obstruction after 8 mo of itraconazole therapy (9 mo after initial presentation). The previously identified turbinate thickening and osteolysis of the right side of the nasal bone have completely resolved. Endotracheal tube diameter = 10 mm, **Whittemore *et al*. (2007)**

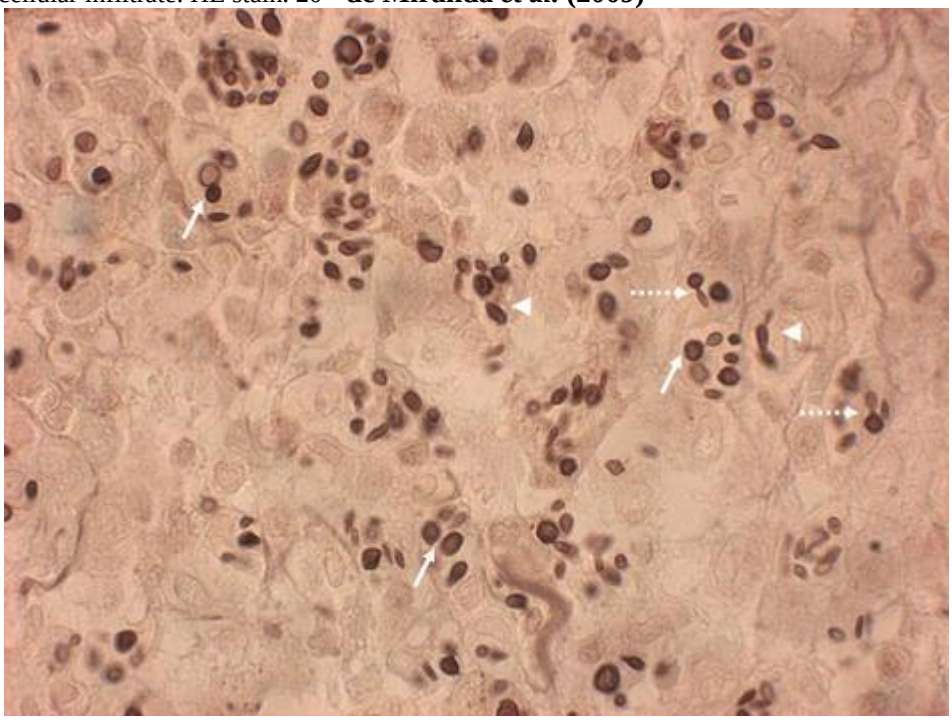
Schubach *et al.* (2008) diagnosed sporotrichosis in64 dogs, in the period 1998 to 2004 in the Evandro Chagas Clinical Research Institute. Of them, 85% were reported to have had contact with cats with sporotrichosis. Canine sporotrichosis presented as a self-limited mycosis.

de Miranda *et al.* (2009) reported the histopathological findings of 86 **skin lesions** of dogs with sporotrichosis from Rio de Janeiro. Suppurative granulomatous inflammation was the predominant finding and was observed in 76 (88.37%) cases. Plasma cells surrounding the suppurative granulomas were detected in 68 (89.5%) cases and an inflammatory infiltrate at the periphery of these granulomatous lesions was observed in 63 (82.9%). Fungus-specific staining revealed yeast cells compatible with ***Sporothrix schenckii*** in 36 cases. These fungal elements were only detected in lesions characterized by suppurative granulomatous inflammation. Thus, specific staining of serial sections is recommended in the case of dogs with skin lesions whose histopathological presentation is consistent with sporotrichosis. However, due to the generally small number of yeast cells in lesions, the hypothesis of sporotrichosis should not be ruled out even if the result is negative, especially in epidemic areas where correlation with epidemiological data is particularly useful.


Well organized granulomas. HE stain. 20× , Granuloma presenting neutrophils inside (*arrows*) and an outer zone of plasma cells (*arrow heads*). HE stain. 20× **de Miranda *et al.* (2009)**

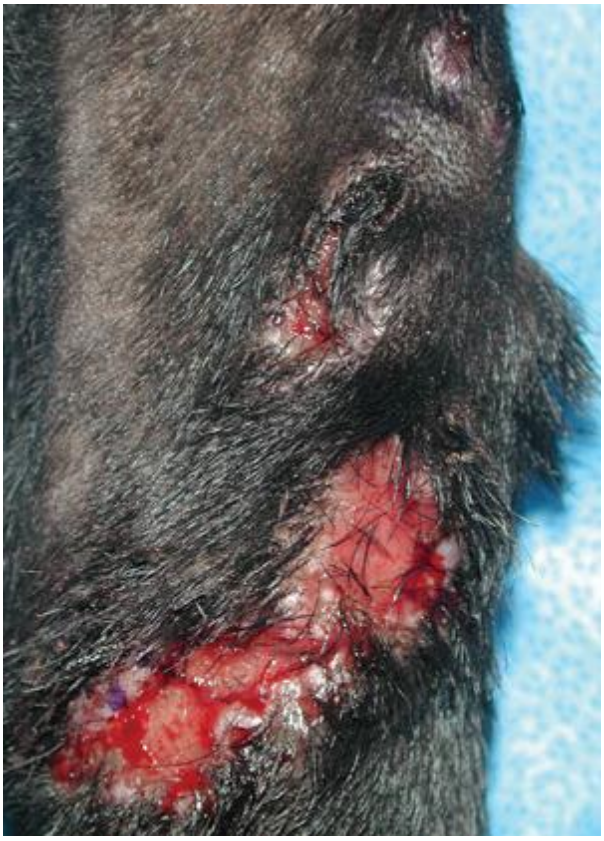


Poorly delimited granuloma with predominance of macrophages. HE stain. 20×, Perifollicular plasmacellular infiltrate. HE stain. 20× **de Miranda *et al.* (2009)**



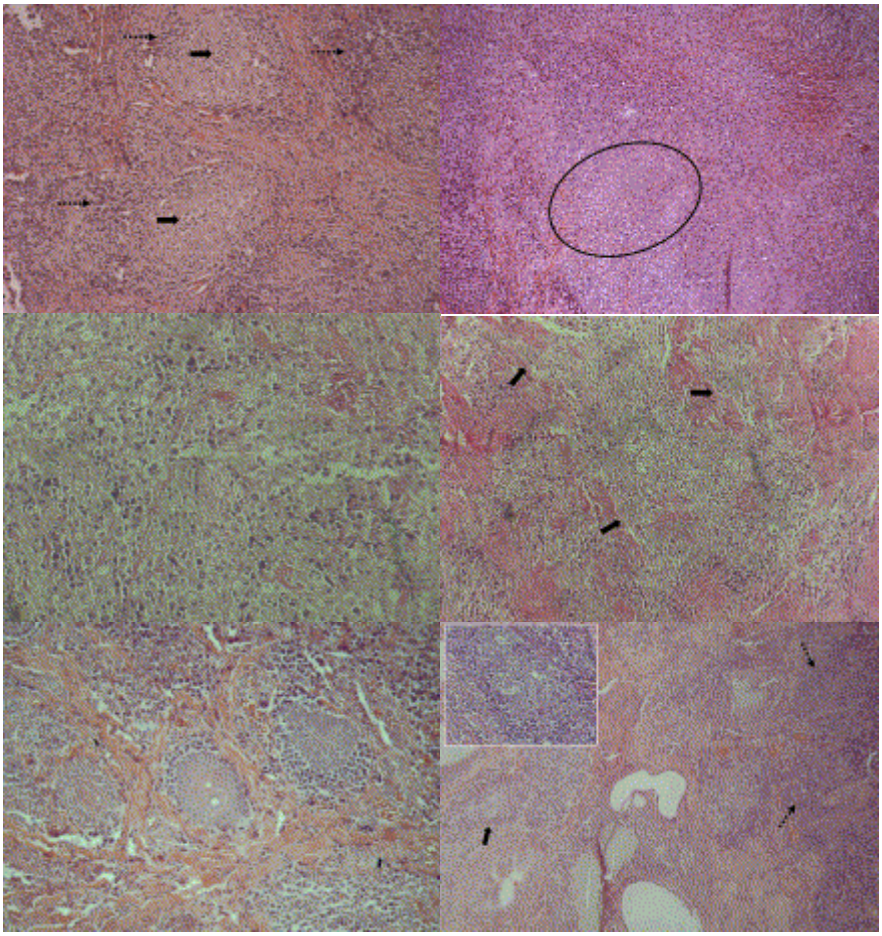
Round (*Arrows*) and cigar-shaped yeast cells (*Arrows heads*) presenting single buds with a narrow base (*Dashed arrows*). Grocott. 100× **de Miranda *et al.* (2009)**

Crothers *et al.* (2009) examined cases of sporotrichosis in 4 dogs between 1987 and 2007 at the University of California, Davis - Veterinary Medical Teaching Hospital, retrospectively evaluated with regard to the historical, clinical, diagnostic and treatment findings. One dog was diagnosed with the localized cutaneous form of sporotrichosis, 2 with the **cutaneous-lymphatic form**, and one with the **disseminated**. One dog did not have skin lesions at the time of diagnosis. The most common mode of diagnosis was demonstration of S. schenckii on histopathological evaluation of tissue. Treatments received included itraconazole (one dog), ketoconazole (three dogs). The prognosis for successful treatment was good in all cases.



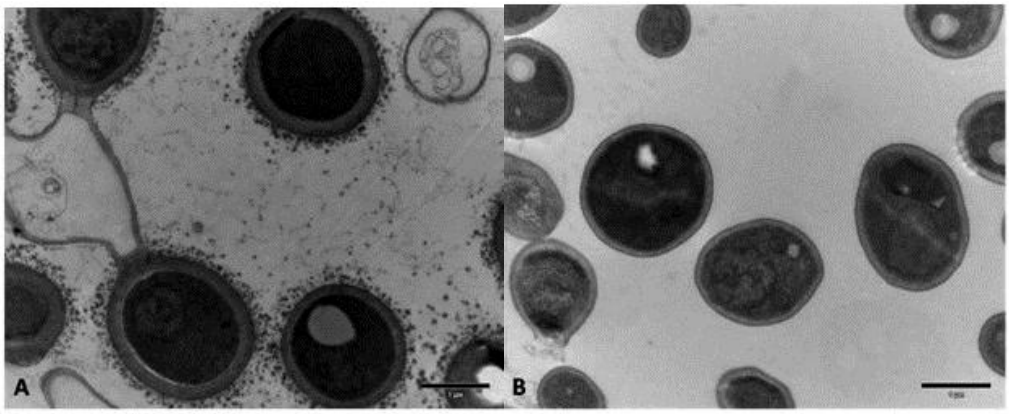
Fore leg of dog with sporotrichosis, case 17. Multifocal areas of ulceration and exudation are visible on the skin over the dorso-lateral surface of the right carpus. Picture courtesy of **Drs Denerolle, White, Taylor and Vandenabeele**

Miranda *et al.* (2010) compares pyogranulomatous lesions from 80 dogs with sporotrichosis and 26 dogs with American tegumentary leishmaniasis (ATL) microscopically in order to identify features that would support the diagnostic suspicion and direct the subsequent search for the aetiological agent of either infection. Odds ratios and their respective 95% confidence intervals were calculated in order to evaluate the impact of the microscopical findings on the diagnosis of either disease. Lesions with well-formed granulomata were 14 times more likely to be due to sporotrichosis than ATL. Marked neutrophil infiltration into granulomata was 12.26 times more likely to be associated with sporotrichosis when compared with lesions having mild neutrophilic infiltration. Absence of lymphocytes and macrophages in the peripheral infiltrate was associated with a 9.71 and 4.93 higher chance, respectively, of being sporotrichosis rather than ATL compared with lesions where these cells were present. Lesions with a perivascular, perifollicular and interstitial peripheral inflammatory infiltrate were 5.48 times more likely to be due to sporotrichosis than ATL when compared with lesions with a diffuse peripheral infiltrate. Histopathological analysis may therefore contribute to the diagnosis of sporotrichosis or ATL **skin lesions** in dogs since this method permits the identification of features that direct the diagnostic suspicion, thus facilitating the search for the aetiological agent in histological sections, permitting the precise request of subsequent tests and thereby reducing costs and time taken to achieve a definitive diagnosis and the initiation of appropriate therapy.



Miranda *et al*. (2010)

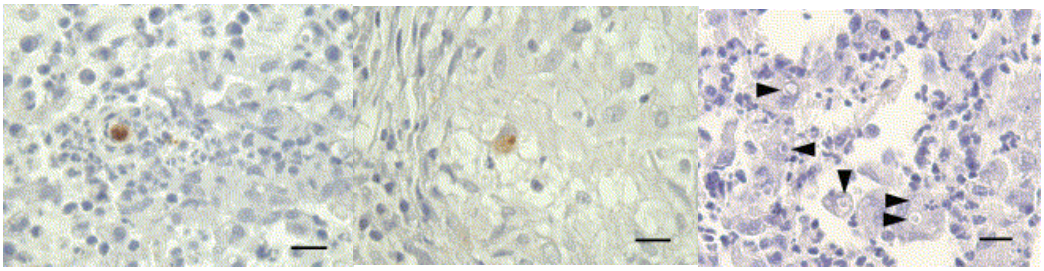
Madrid *et al.* (2011) studied the presence of melanin and cell wall thickness of clinical isolates of ***Sporothrix schenckii*** obtained from cats, dogs and humans as compared to reference strains using transmission electron microscopy. They detected differences regarding presence of the melanin among the clinical isolates of S. schenckii and a correlation between presence of melanin and cell wall thickness.



TEM images of *S. schenckii* cells with (a) and without (b) melanin granules. Scale bar: 1µm **Madrid *et al.* (2011)**

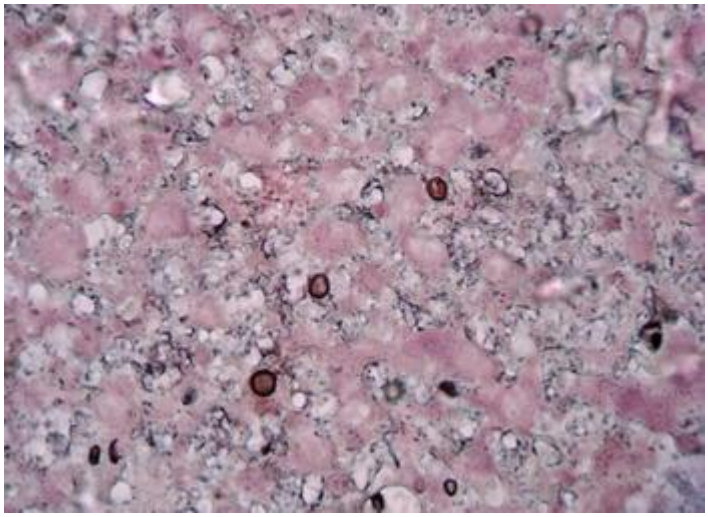
Miranda *et al.* (2011) performed a study with the aim to apply immunohistochemistry (IHC) for the diagnosis of canine sporotrichosis and to compare this method with the Grocott's silver stain (GSS) and periodic acid Schiff

(PAS) techniques. Eighty-seven dogs with sporotrichosis (group 1) and 35 with American tegumentary leishmaniosis (ATL) (group 2) were studied. The fungus was detected in group 1 by GSS, PAS and IHC. IHC was also applied to group 2 to evaluate the occurrence of cross-reactions. PAS, GSS and IHC detected yeast cells in 19.5%, 43.7% and 65.5% of the group 1 cases, respectively. The detection of intracellular antigens of ***Sporothrix schenckii*** by IHC increased the sensitivity of the histological diagnosis to 80.5%. No positive reaction was observed in ATL lesions. The results suggest that IHC may be indicated for the diagnosis of sporotrichosis because of its higher diagnostic sensitivity.



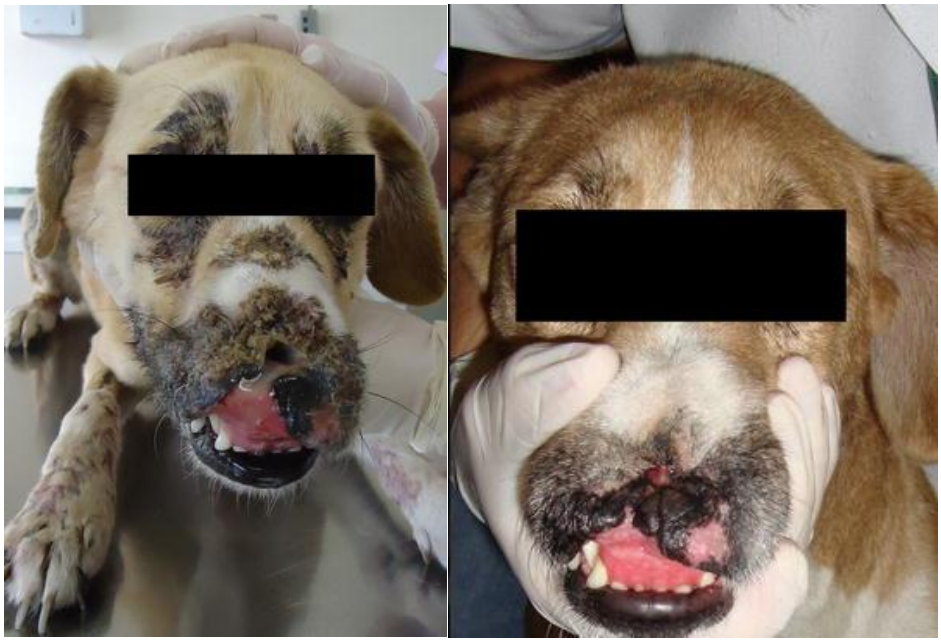
Miranda *et al.* (2011)

Madrid *et al.* (2012) described the epidemiological and laboratory characteristics of 103 clinical cases of sporotrichosis diagnosed over a 10-year period in southern Brazil. The 92 cats and 11 dogs from eight municipalities in Rio Grande do Sul State developed especially the **disseminated cutaneous** and fixed cutaneous forms of the disease. Respiratory signs such as sneezing, **serous nasal** discharge and dyspnea were found in about 57% of the animals. The detection of ***Sporothrix schenckii*** in different clinical samples showed highest isolation in testicles (46.6%), oral cavity (45.2%) and conjunctival mucosa (38.1%). A differentiated histological pattern was found between the fixed cutaneous and disseminated cutaneous (DC) manifestations of the disease; well-organized granulomas of nodular distribution and various fungal structures prevailed in the DC form in cats. Melanin detection in S. schenckii cells by the Fontana-Masson technique was positive in 45.4% of the samples. The study revealed that the State of Rio Grande do Sul is an endemic sporotrichosis area and demonstrated the possibility of involvement of other pathways in the infection and spread of the disease. In addition, it emphasized the importance of laboratory tests for mycosis confirmation, especially in dogs that develop clinical manifestations without the presence of cutaneous lesions.



Sporothrix schenckii positively reactive yeasts for the presence of melanin by Fontana–Masson stain (400×) **Madrid *et al.* (2012)**

Guterres *et al*. (2014) reported, for the first time, the use of (1-3) β-glucan along with itraconazole in the treatment of a canine with sporotrichosis caused by ***Sporothrix brasiliensis***. The animal had ulcerated and crusted lesions, especially on the **nasal** planum. Clinical samples were collected for a complete blood count, cytological analysis of the lesion, and fungal culture. Based on the results of the laboratory examination, and after the fungal culture, antibiotic therapy and treatment with itraconazole were initiated. Two additional fungal cultures were performed, which were positive. After 7 months of the animal treatment with itraconazole, the S. brasiliensis culture was still positive, so that the itraconazole was associated with (1-3) β-glucan. After four weekly applications of glucan, the complete elimination of the fungus was observed based on the fungal culture negative results. The results show, therefore, that (1-3) β-glucan with itraconazole promoted the case resolution, and it may be considered a promising alternative for the treatment of sporotrichosis in cases of resistance to conventional therapy.



Patient upon arrival for clinical examination, showing destruction of the nasal planum with secondary contamination, Patient after the treatment with itraconazole along with (1–3) β-glucan,
Guterres *et al*. (2014)

Jessica *et al*.(2015) The present study included 244 cats from the metropolitan region of Rio de Janeiro, mostly males in reproductive age with three or more lesions in non-adjacent anatomical places. To evaluate the inter-observer reliability, two different observers performed the microscopic examination of the slides blindly. Test sensitivity was 84.9%. The values of positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and accuracy were 86.0, 24.4, 2.02, 0.26 and 82.8%, respectively. The reliability between the two observers was considered substantial. They concluded that the cytopathological examination is a sensitive, rapid and practical method to be used in feline sporotrichosis diagnosis in outbreaks of this mycosis.

5.5. Reports of sporotrichosis in cats

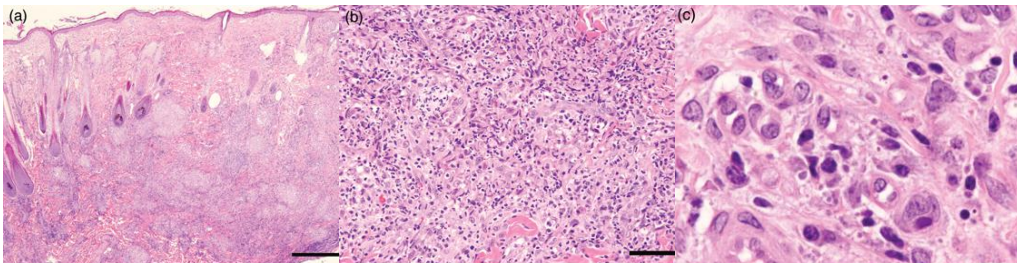
Hirano *et al.* (2006) excised surgically a feline granulomatous lesion and performed histopathological, mycological and molecular examinations. As a result, it was diagnosed as sporotrichosis, which was the second recorded case of a cat so afflicted in Japan. After the operation, they recognized another nodule on the lymph node. Histopathological examination was therefore performed, but no fungi were detected. To prevent recurrence, the cat was administered an antimycotic drug, itraconazole. As a result, no recurrence was found.

Kovarik *et al.* (2008) conducted a study to identify various aspects of sporotrichosis in the endemic area of Abancay, Peru, namely (i) the overall prevalence of sporotrichosis in the cat population, (ii) the most common site where the fungus can be isolated from these cats, and (iii) whether cats without identifiable skin lesions may be carriers of the fungus in the oral mucosa, nasal mucosa, or nails. One household cat in each of 85 neighborhoods within the endemic area of Abancay, Peru was randomly selected. Oral and nasal swabs, as well as nail clippings were taken from 84 of the cats. In addition, samples from skin lesions that were suspected to be due to sporotrichosis were collected from cats or members of families that owned the pets. Cultures inoculated with two nasal swabs and one set of nail clippings from two different cats yielded Sporothrix schenckii, the identity of which were confirmed by rDNA sequencing. The overall prevalence of Sporothrix schenckii colonization was 2.38% (95% CI 0.41-9.14) in this cat population. None of the skin lesion samples from the cats and only one such sample from a family member were positive for Sporothrix schenckii in culture. These results suggest a role for domestic cats as a possible reservoir for sporotrichosis infection in Abancay.

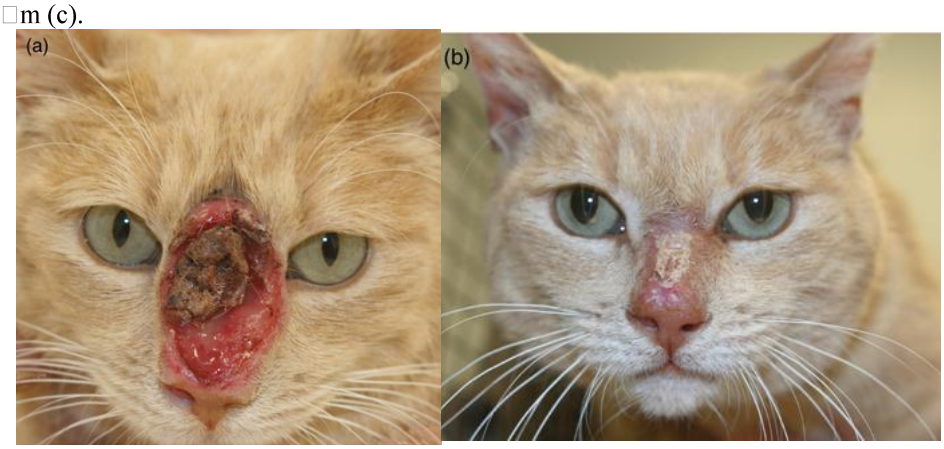
Schubach *et al.* (2008) diagnosed sporotrichosis in 1503 cats in the period 1998 to 2004, in the Evandro Chagas Clinical Research Institute. Feline sporotrichosis varied from subclinical infection to severe systemic disease with haematogenous dissemination of Sporothrix schenckii. The zoonotic potential of cats was demonstrated by the isolation of S. schenckii from skin lesion fragments, and from material collected from their nasal and oral cavities.

Crothers *et al.* (2009) examined 14 cases of sporotrichosis in **cats** between 1987 and 2007 at the University of California, Davis - Veterinary Medical Teaching Hospital, retrospectively evaluated with regard to the historical, clinical, diagnostic and treatment findings. Four cases were diagnosed with the localized **cutaneous** form of sporotrichosis, 4 cases with the **cutaneous-lymphatic** form, and 6 with the disseminated form. One cat did not have skin lesions at the time of diagnosis. The most common mode of diagnosis was demonstration of S. schenckii on histopathological evaluation of tissue. In contrast with most previously described sporotrichosis infections in cats, few to no fungal organisms were seen in histopathological samples (haematoxylin and eosin and special stains) in five of the 14 cats. Treatments received included itraconazole (12 cats), ketoconazole, fluconazole (one cat), sodium iodide (one cat) and potassium iodide (one cat). The prognosis for successful treatment was good in all cases. Fluconazole was successful

in inducing resolution of the cutaneous lesions and controlling the infection in one cat with **disseminated sporotrichosis**.



Skin sections of cat (Case 14) with *Sporothrix schenckii*. Multifocal to coalescing nodular inflammation predominantly within the deep dermis with haematoxylin and eosin stain (H&E), bar 600 □m (a). Pyogranulomatous inflammation present with variable numbers of neutrophils, histiocytes, lymphocytes, multinucleated giant cells, with fewer numbers of mast cells, eosinophils and plasma cells with H&E, bar 130 □m (b). Intracellular, sometimes budding, oval to spherical *S. schenckii* organisms with an outer rim, a clear halo and an inner dense core seen in a horse (case 22) with H&E, bar 25 □m (c).



Face of cat with sporotrichosis, case 14. Focal ulcerative lesion with a central crust is present on the dorsal muzzle. Day 0 (a). The lesions have a reduced to mild adherent crust with scar tissue on the dorsal muzzle. Day 60 (b).

Reis *et al*. (2009) conducted a study to demonstrate the zoonotic character of an epidemic of sporotrichosis in Rio de Janeiro, Brazil, in which cases of human infection were related to exposure to cats, using molecular methodology to characterize 19 human and 25 animal S. schenckii isolates from the epidemic, as well as two control strains. To analyse the isolates, the random amplified polymorphic DNA (RAPD) technique was performed using three different primers, together with DNA fingerprinting using the minisatellite derived from the wild-type phage M13 core-sequence. The analyses generated amplicons with considerable polymorphism. Although isolates exhibited high levels of genetic relatedness, they could be clustered into 5-10 genotypes. The RAPD profiles of epidemic ***S. schenckii*** isolates could be distinguished from that of the United States isolate, displaying 20% similarity to each primer and 60% when amplified with the M13 primer. DNA fingerprinting of S. schenckii isolated from the nails (42.8%) and the oral cavities (66%) of cats were identical to related human samples, suggesting that there is a common infection source for animals and humans in this epidemic. It is clear that cats acted as a vehicle for dissemination of S. schenckii.

Madrid *et al*. (2010) studied clinical cases of feline sporotrichosis, originating in the Pelotas region and diagnosed at the Laboratory of Infectious Diseases (UFPel), in the period from 2002 to 2006. The animals were evaluated according to the clinical forms

of the mycosis, time of lesion appearance, severity of the clinical diagnosis and evolution of cutaneous lesions throughout the treatment period. Mycological analyses, carried out through direct examination, cultivation of tissue samples and exudates of feline lesions all confirmed the diagnosis of sporotrichosis in the 15 animals under study. The **cutaneous dissemination form** was observed in 10 animals, of which three showed prostration, anorexia and dehydration. The zoonosis occurred in 20% of case studies, and the pet owners and one attendant at a veterinary clinic were infected, developing the fixed and disseminated cutaneous forms. The treatment of mycosis was carried out with itraconazole, 10 mg kg(-1), once a day, on 12 animals. The cure of the clinical symptoms was observed on 50% of the felines. This study demonstrates a good clinical response of felines with sporotrichosis, when they were treated itraconazole and calls the attention for the incidence of human sporotrichosis on people related to the veterinary activity as well as for pet owners.

Ulcers regression in the first twenty days of treatment with itraconazole. **Madrid *et al*. (2010)**

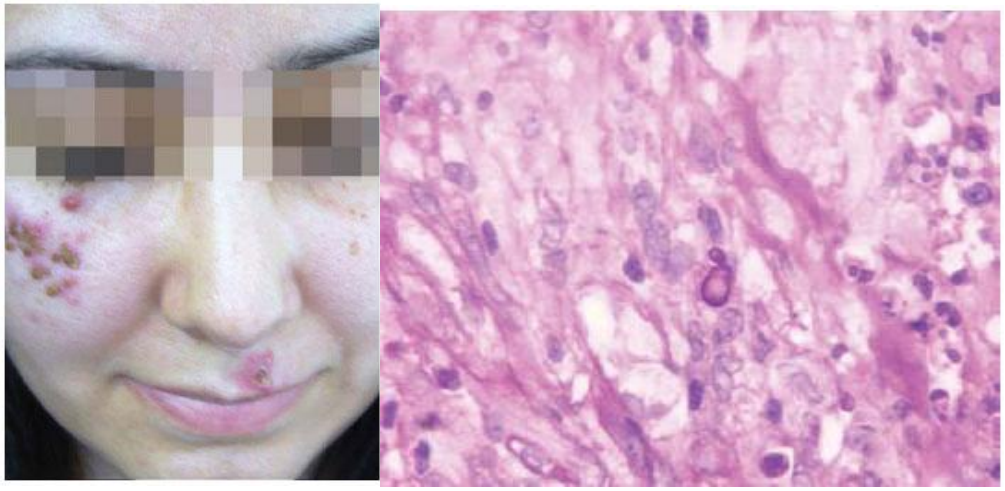
Evolution of the cutaneous lesions in three months of the antifungal therapy. **Madrid *et al*. (2010)**

Pereira *et al.* (2010) compared the effectiveness and safety of treatment with ketoconazole and itraconazole in 773 sporotrichosis-infected cats over a four-year period (2002 to 2005). Five hundred and ninety-eight cats received oral ketoconazole and 175 received oral itraconazole. Treatment was successful in 238 (30.8 per cent) cats, of which 171 (28.6 per cent) of 598 received 13.5 to 27.0 mg/kg/day ketoconazole and 67 (38.3 per cent) of 175 received 8.3 to 27.7 mg/kg/day itraconazole. Adverse effects were reported in 306 (39.6 per cent) of the cats, 105 (13.6 per cent) died and 430 (55.6 per cent) dropped out of treatment or were still under treatment at the time of data analysis.

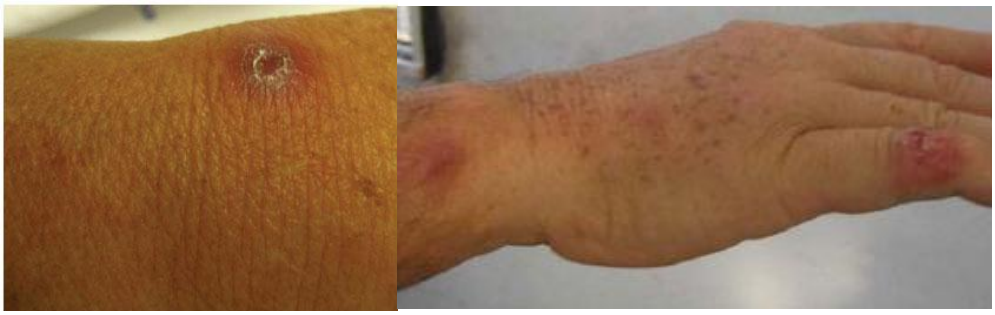
Weingart *et al.* (2010) reported a four-year-old male castrated Domestic Shorthair Cat imported from North America with a chronic parapreputial wound. Cytological

and mycological examination revealed a sporotrichosis caused by ***Sporothrix schenckii*** Clinical signs, diagnostics, as well as therapy and course of the disease were described. Treatment with itraconazole was successful.

Cordeiro *et al.* (2011) presented simultaneous occurrence of sporotrichosis in three members of the same family by scratches from an infected domestic cat. Two patients developed the lymphocutaneous form and one only developed the fixed cutaneous form. Two patients were successfully treated with saturated solution of potassium iodide; however, the third case reported side effects and had his therapy substituted for itraconazole, with resolution of his lesions.



Papules and pustules above the lip and the molar area, presence of round yeasts with a pale centre and reinforced colouration on the periphery showing budding surrounded by lymphohystiocitic infiltrate **Cordeiro *et al.* (2011)**

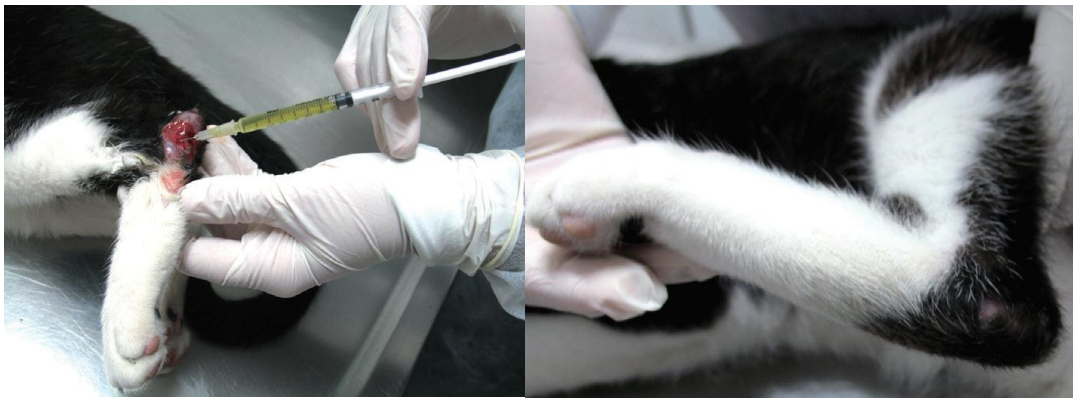


Mother of the index case with erythematous plaque on the right forearm, Father of the index case with nodular ulcerated lesions along the lymphatic channels of the forearm, **Cordeiro *et al.* (2011)**

Fernandes *et al.* (2011) performed a study to standardize an ELISA for the diagnosis of feline sporotrichosis. They proposed an ELISA test for the diagnosis of cat sporotrichosis , which detected ***S. schenckii***-specific antibodies in feline sera. Two different kinds of antigens were used: "SsCBF", a specific molecule from S. schenckii that consists of a Con A-binding fraction derived from a peptido-rhamnomannan component of the cell wall, and a S. schenckii crude exoantigen preparation. The ELISA was developed, optimized, and evaluated using sera from 30 cats with proven sporotrichosis (by culture isolation); 22 sera from healthy feral cats from a zoonosis center were used as negative controls. SsCBF showed 90% sensitivity and 96% specificity in ELISA; while crude exoantigens demonstrated 96% sensitivity and 98% specificity. The ELISA assay described here would be a valuable screening tool for the detection of specific S. schenckii antibodies in cats with sporotrichosis. The

assay is inexpensive, quick to perform, easy to interpret, and permits the diagnosis of feline sporotrichosis.

Gremião *et al.* (2011) performed a study to describe the use of intralesional amphotericin B in localised lesions for the treatment of 26 cats from Rio de Janeiro, Brazil, with sporotrichosis refractory to oral itraconazole. The 26 cats in this study were diagnosed with sporotrichosis, confirmed by isolation of ***Sporothrix schenckii***, and presented residual localised skin lesions refractory to treatment with oral **itraconazole** for a minimum period of 8 weeks. The animals received weekly applications of intralesional amphotericin B in conjunction with oral itraconazole. In cases of owner unavailability, a maximum of 2 weeks between the infiltrations was accepted. Twenty-two (84.6%) of the 26 treated cats achieved clinical remission, 16 (72.7%) of which were cured, and in the remaining six (27.3%) the lesions recurred at the same site. Lack of clinical response was observed in one animal and three owners abandoned treatment.



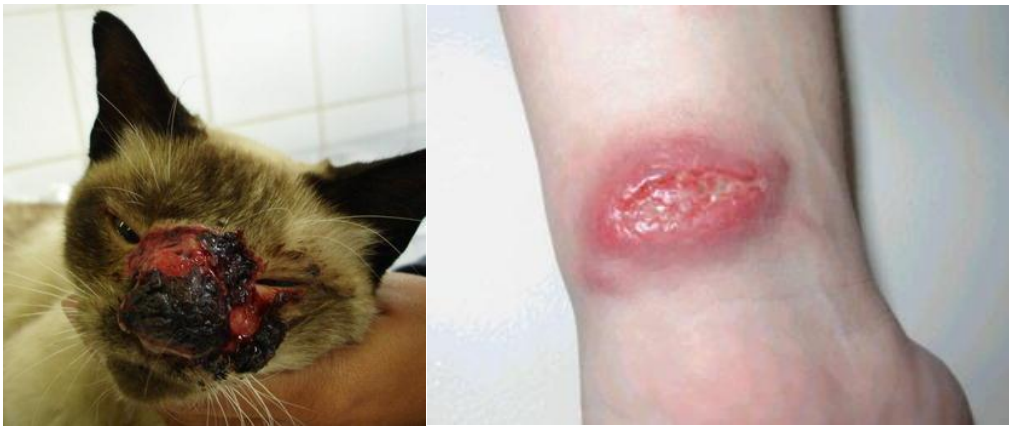
Administration of intralesional amphotericin B in an ulcer on the left tarsal region. Scar tissue on the left tarsal region after intralesional amphotericin B therapy and oral itraconazole. **Gremião *et al.* (2011)**

Ulcer on the bridge of the nose after six months of itraconazole therapy. Scar tissue on bridge of the nose at 2 months after clinical cure using intralesional amphotericin B therapy and oral itraconazole. **Gremião *et al.* (2011)**

Rees and Swartzberg (2011) reported a case of cat-associated sporotrichosis in an adult female in California. A retrospectively diagnosed cutaneous sporotrichosis infection in the patient's cat and the unusual site of the primary lesion in the patient contributed to delayed diagnosis and treatment.

Borges *et al.* (2013) conducted a study was to determine the occurrence of sporotrichosis in domestic cats and in wild or exotic felines in captivity through the isolation of Sporothrix spp. from claw impressions in a culture medium. The samples

included 132 felines, of which 120 (91.0 %) were domestic cats, 11 (8.3 %) were wild felines, and one (0.7 %) was an exotic felid. Twenty-one (17.5 %) were outdoor cats. Of the total, 89 (67.4 %) had contact with other animals of the same species. It was possible to isolate ***Sporothrix schenckii*** from the **claws** of one (0.7 %) of the felids probed; this animal exhibited generalised sporotrichosis and had infected a female veterinarian.



Siamese cat, female, 4 years old, positive to sporotrichosis with a large ulcer, haemorragic crusts and alopecia in the head—Female veterinarian positive to sporotrichosis with an ulcerated nodular lesion presenting purulent secretion on her *right* forearm—Dermatology Service (FMVZ-USP). GRAPHIC PROGRAM: ADOBE PHOTOSHOP CS6), **Borges *et al.* (2013)**

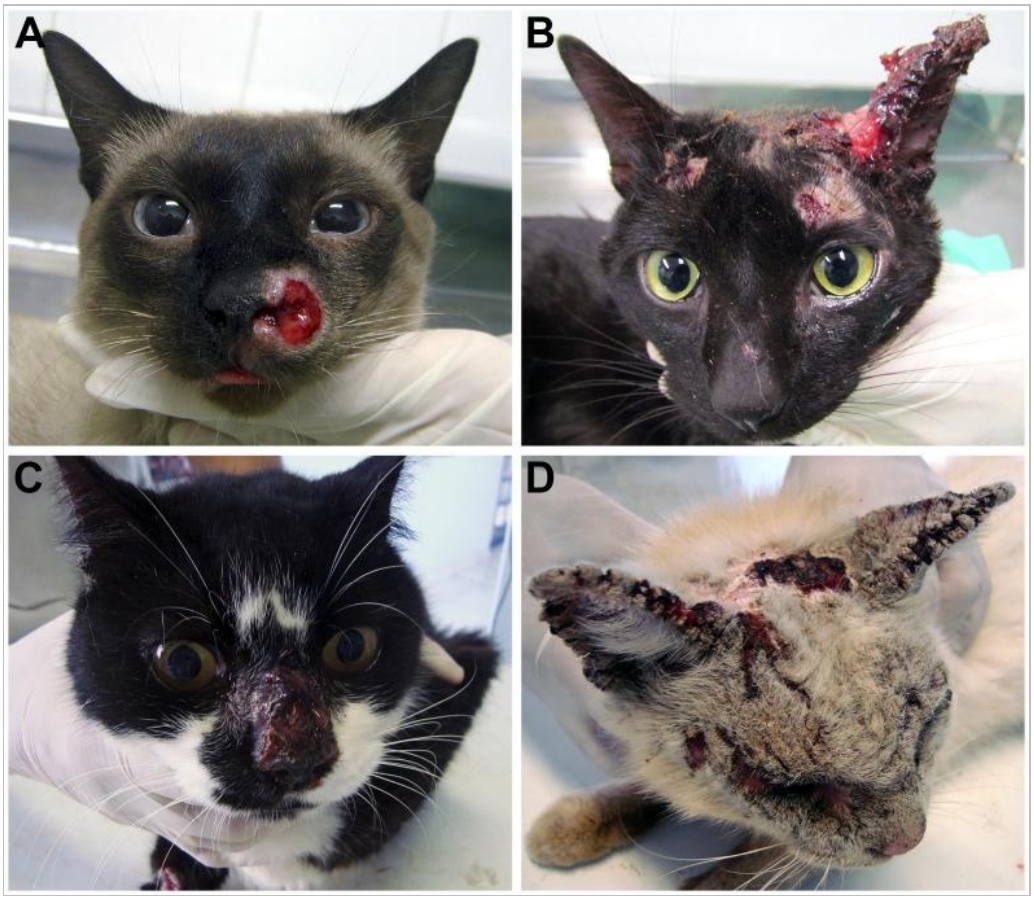
Chaves *et al.* (2013) described the epidemiological, clinical and mycological aspects of feline sporotrichosis cases attending the Laboratory of Clinical Research on Dermatozoonosis in Domestic Animals - Evandro Chagas Clinical Research Institute (LAPCLIN-DERMZOO/IPEC/FIOCRUZ), from 1998 to 2005. It was possible to get in contact with 147 (19.2%) cat owners. One hundred and thirteen (76.9%) cats were male, 117 (79.6%) had no defined race and 87 (59.2%) were sexually intact. The age ranged from 72 to 216 months (median = 108 months). Nineteen cats were reassessed: eleven (57.8%) were male, thirteen (36.8%) were breed and fifteen (47.3%) castrated. Fourteen (52.6%) animals lived at home and did not roamed the streets. Seven (36.8%) had normal clinical findings and negative mycological examination. Twelve (63.1%) cats had **skin lesions** compatible with sporotrichosis. Thirty-one (21%, n = 147) cats disappeared after abandoning treatment, 36 (24.5%, n = 147) were alive and 80 (54.4%, n = 147) had died. Causes of death informed by the owners were: sporotrichosis in 35 (43.7%, n = 80), accidental death in 27 (33.7%, n = 80) and other diseases in 18 (22.5%, n = 80). Withdrawal of treatment occurred mainly at the time of clinical improvement and may represent a serious obstacle to the control of sporotrichosis.

dos Santos *et al.* (2013) reported a 7-year-old Siamese cat with three ulcerated **cutaneous nodules** in the lumbosacral region in Rio de Janeiro, Brazil. **Histopathological** analysis showed that the lesions consisted of polyhedral and spindle-shaped voluminous mononuclear cells with loose chromatin and clearly visible nucleoli, few giant cells, and foci of coagulative and caseous necrosis -- findings suggestive of a vaccine-induced sarcoma. No significant mitotic rate, cytological atypias or asteroid bodies were observed. Special histopathological staining with periodic acid-Schiff and Grocott's silver stain demonstrated the presence

of small yeast cells characterized by simple and narrow-base budding compatible with ***Sporothrix schenckii***. Mycological culture grew S schenckii. Cytopathology was negative for yeast cells. These atypical clinical and histopathological signs support the importance of histopathological analysis with special staining techniques, in addition to mycological culture in the diagnosis of feline sporotrichosis.

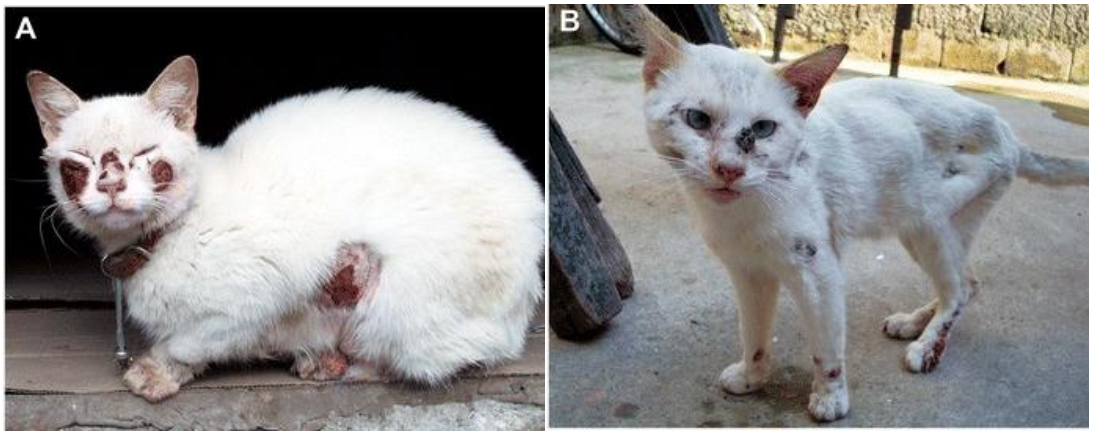
Miranda *et al.* (2013) described the histopathology and fungal load of the lesions in different clinical presentations of feline sporotrichosis. Cats with sporotrichosis were separated into groups L1, L2 and L3 (lesions in one, two and three or more locations, respectively) and subjected to skin biopsies for histopathology. Eighty-six cats were included in the study. Lesions were suppurative granulomatous in 84 cases and poorly formed granulomas were predominant. The well-formed granulomas were associated with group L1. The high fungal load was predominant in group L3 and in poorly formed granuloma cases and did not occur in well-formed granulomas cases. The good general condition was associated with low fungal load. These findings suggested that the fungal load control in animals with more localized lesions and well-organized response was linked with the improvement in the outcome of infected cats.

Rodrigues *et al.* (2013) conducted a survey among symptomatic cats in order to understand the eco-epidemiology of feline sporotrichosis and its role in human sporotrichosis. Prevalence and **phylogenetic** relationships among feline Sporothrix species were investigated by reconstructing their phylogenetic origin using the calmodulin (CAL) and the translation elongation factor-1 alpha (EF1α) loci in strains originated from Rio de Janeiro (RJ, n = 15), Rio Grande do Sul (RS, n = 10), Paraná (PR, n = 4), São Paulo (SP, n =3) and Minas Gerais (MG, n = 1). The results showed that ***S. brasiliensis*** is highly prevalent among cats (96.9%) with sporotrichosis, while ***S. schenckii*** was identified only once. The genotype of Sporothrix from cats was found identical to S. brasiliensis from human sources confirming that the disease is transmitted by cats. Sporothrix brasiliensis presented low genetic diversity compared to its sister taxon S. schenckii. No evidence of recombination in S. brasiliensis was found by split decomposition or PHI-test analysis, suggesting that S. brasiliensis is a clonal species. Strains recovered in states SP, MG and PR share the genotype of the RJ outbreak, different from the RS clone. The occurrence of separate genotypes among strains indicated that the Brazilian S. brasiliensis epidemic has at least two distinct sources. We suggest that cats represent a major host and the main source of cat and human S. brasiliensis infections in Brazil.

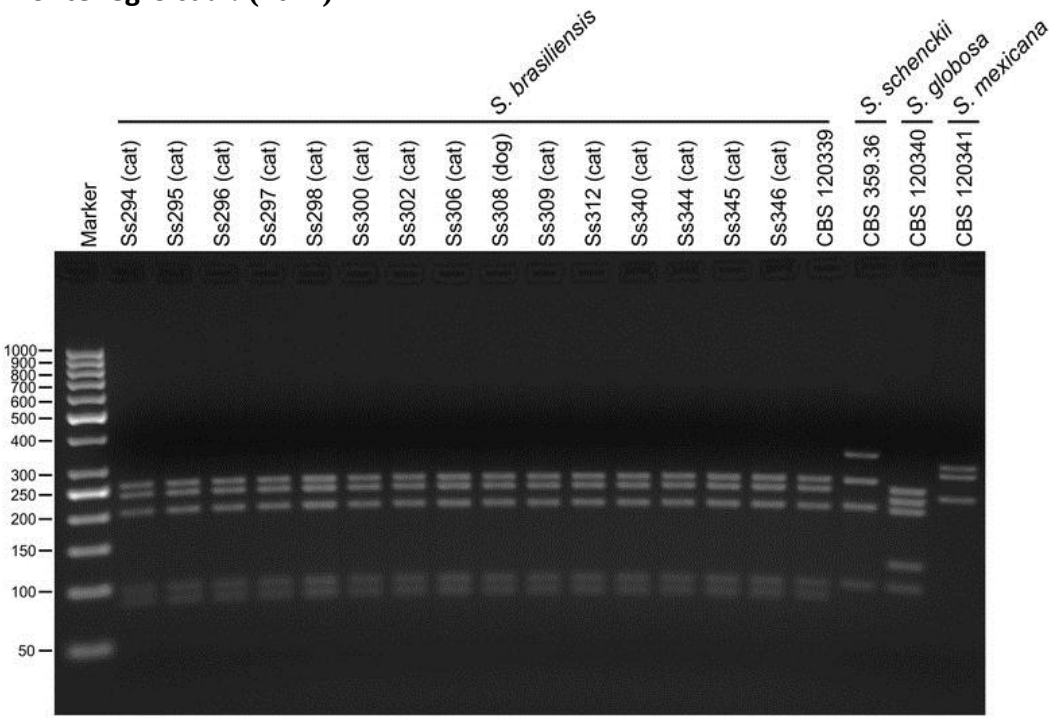


Clinical aspects of feline sporotrichosis in Brazil. Cats presenting ulcerated cutaneous lesions in the cephalic region. (A) and (B) felines from Rio de Janeiro; (C) and (D) felines from Paraná, **Rodrigues *et al.* (2013)**

Montenegro *et al.* (2014) described the recent emergence of feline sporotrichosis in the metropolitan region of São Paulo, Brazil, with an overwhelming occurrence of ***S. brasiliensis*** as the etiological agent. A phylogenetic and a haplotype approach were used to investigate the origin of this epidemic and the impact of feline transmission on genetic diversity. During the last 3-year period, 163 cases of feline sporotrichosis were reported in São Paulo with proven S. brasiliensis culture. The haplotype diversity of feline S. brasiliensis isolates revealed the expansion of a clonal population with low genetic diversity. Haplotype analysis confirmed that isolates from São Paulo shared the haplotype originated in the long-lasting outbreak of cat-transmitted sporotrichosis in Rio de Janeiro, which differed from the haplotype circulating in the Rio Grande do Sul epidemic. The fast spread of sporotrichosis in a short period of time highlights the potential for outbreaks and suggests that the mycosis may affect an urban population with a high concentration of susceptible felines. The feline sporotrichosis epidemic shows no signs of slowing, and this epidemiological pattern may require specific public health strategies to control future outbreaks.



Clinical aspects of feline sporotrichosis. (A) Wet, ulcerated skin lesions, often particularly concentrated in the cephalic region. **(B)** Weight loss during the evolution of the disease. **Montenegro *et al.* (2014)**



Genotyping of feline sporotrichosis isolates by PCR-RFLP. Representative profiles of 16 samples are shown. Positive controls: *Sporothrix brasiliensis* (CBS 120339), *S. schenckii* (CBS 359.36), *S. globosa* (CBS 120340). The amplicons were sized by comparison with bands of known size in the 100-bp DNA Step Ladder (Promega). **Montenegro *et al.* (2014)**

Pereira *et al.* (2014) reviewed the medical records of 2,301 feline sporotrichosis cases. The numbers reported in this study represent only those feline cases diagnosed at IPEC/FIOCRUZ, a reference center for the diagnosis and treatment of fungal diseases, thus accounting for the probable majority of cases. However, other public and private institutions located in the same region also perform sporotrichosis diagnosis but were not included in this study. All feline isolates were morphologically identified as ***S. schenckii*** at the time of diagnosis. For 15 of the isolates, it was possible to characterize the species by reconstructing their phylogenetic origin using the calmodulin locus, which resulted in ***Sporothrix brasiliensis*** in all cases. Currently, determining the scale of feline

epizootic sporotrichosis is very difficult because reporting of this disease is not required. It is believed that sporotrichosis control can be achieved through basic educational measures that emphasize the responsible ownership of animals, programs to limit feline reproduction and effective action on the part of governmental institutions responsible for public health. Furthermore, updating the number of feline cases diagnosed at Lapclin-Dermzoo/IPEC/FIOCRUZ alerts health professionals, researchers and sanitary authorities to the difficulties related to sporotrichosis control.

Pohlman *et al.* (2014) reported case of disseminated pyogranulomatous inflammation with intralesional Sporothrix organisms in a 4.5-year-old spayed female domestic shorthair cat with numerous crusted, ulcerated and fistulated lesions on the dorsum. Ventrum. Head, tail and limbs.

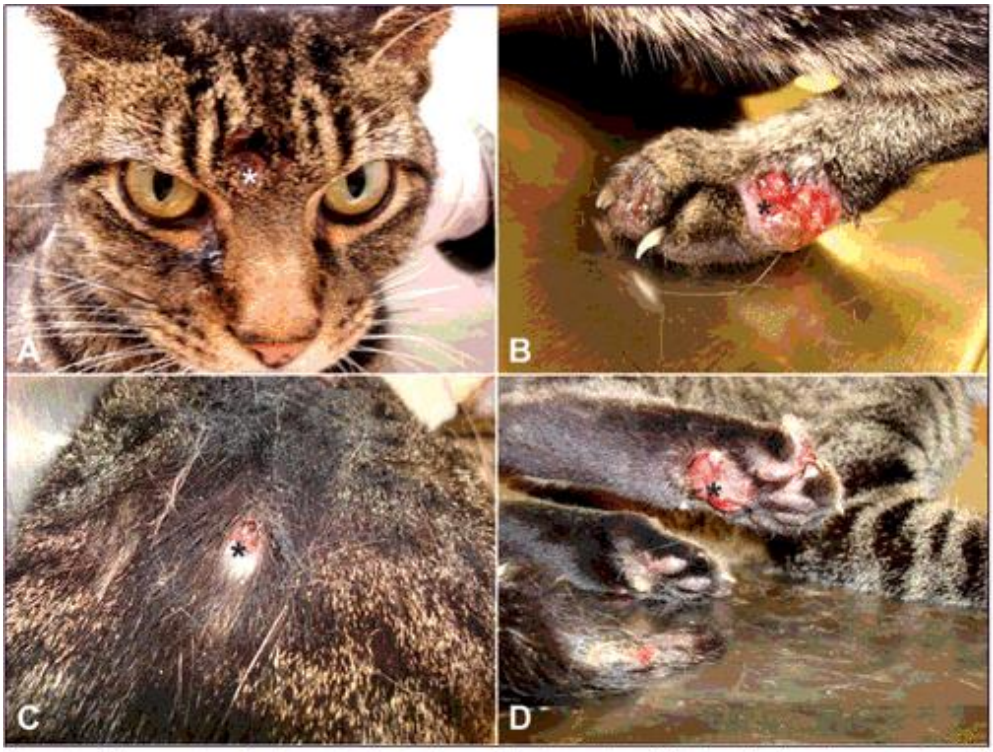


Figure 1—Photographs of a domestic shorthair cat with ulcerated and fistulated lesions (asterisks) on the forehead (A), left hind paw (B), dorsum (C), and tail and right hind paw (D). The initial lesion had developed on the dorsal aspect of the left hind paw; however, despite treatment during a 2-month period, the lesion continued to worsen and spread to other areas of the body.

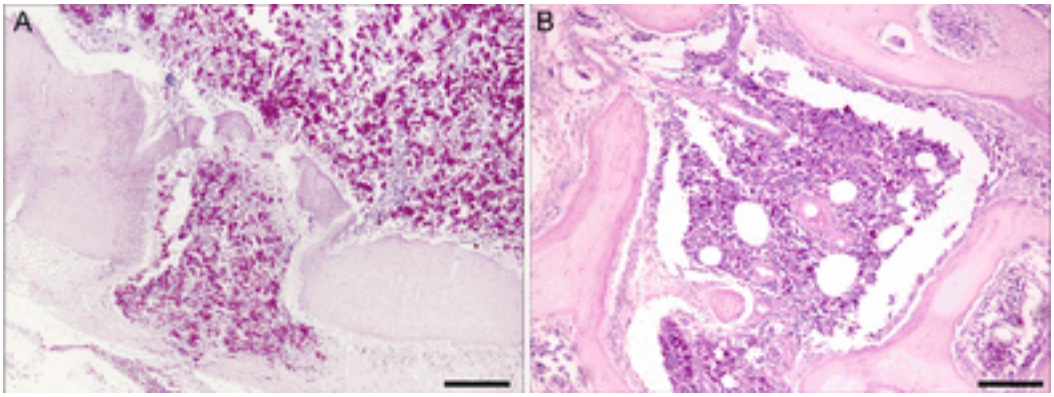
Pohlman *et al.* (2014)

Teixeira *et al.* (2014) conducted a comparative **genomic study** to explore the presence of virulence factors in ***S. schenckii*** and ***S. brasiliensis***; to compare S. brasiliensis, which is cat-transmitted and infects both humans and cats with S. schenckii, mainly a human pathogen and to compare these two species to other human pathogens (Onygenales) with similar thermo-dimorphic behavior and to other plant-associated Sordariomycetes. The genomes of S. schenckii and S. brasiliensis were pyrosequenced to 17x and 20x coverage comprising a total of 32.3 Mb and 33.2 Mb, respectively. Pair-wise genome alignments revealed that the two species are highly syntenic showing 97.5% average sequence identity. Phylogenomic analysis revealed that both species diverged about 3.8-4.9 MYA suggesting a recent event of speciation. Transposable elements comprise respectively 0.34% and 0.62% of the S. schenckii

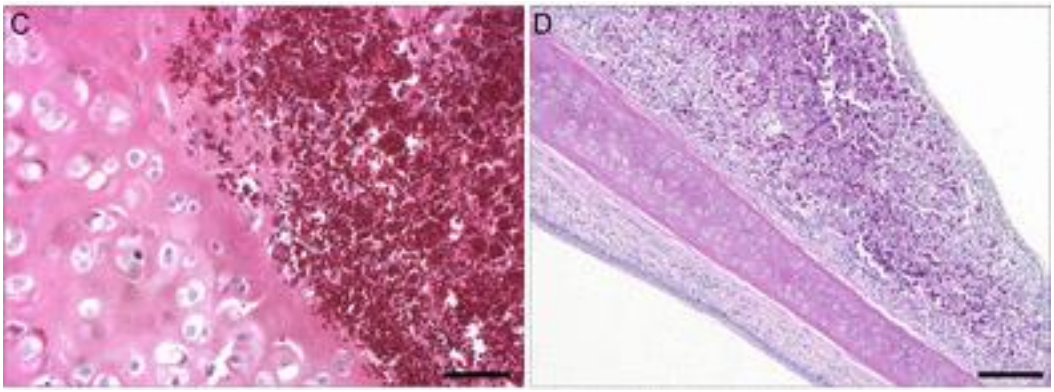
and S. brasiliensis genomes and expansions of Gypsy-like elements was observed reflecting the accumulation of repetitive elements in the S. brasiliensis genome. Mitochondrial genomic comparisons showed the presence of group-I intron encoding homing endonucleases (HE's) exclusively in S. brasiliensis. Analysis of protein family expansions and contractions in the Sporothrix lineage revealed expansion of LysM domain-containing proteins, small GTPases, PKS type1 and leucin-rich proteins. In contrast, a lack of polysaccharide lyase genes that are associated with decay of plants was observed when compared to other Sordariomycetes and dimorphic fungal pathogens, suggesting evolutionary adaptations from a plant pathogenic or saprobic to an animal pathogenic life style. Comparative genomic data suggested a unique ecological shift in the Sporothrix lineage from plant-association to mammalian parasitism, which contributes to the understanding of how environmental interactions may shape fungal virulence. Moreover, the striking differences found in comparison with other dimorphic fungi revealed that dimorphism in these close relatives of plant-associated Sordariomycetes is a case of convergent evolution, stressing the importance of this morphogenetic change in fungal pathogenesis.

Gremião *et al.* (2015) mentioned that feline sporotrichosis, which is caused by species of the Sporothrix schenckii complex, is endemic to Rio de Janeiro, Brazil. More than 4000 cases of the disease were diagnosed at Fundação Oswaldo Cruz, Brazil, between 1998 and 2012. Sporotrichosis in cats has been reported in several countries, but nowhere has an outbreak of animal sporotrichosis been as large as that seen in Brazil. The clinical manifestations of the disease range from an isolated skin lesion that can progress to multiple skin lesions and even fatal systemic involvement. Nodules and ulcers are the most common types of lesions, and respiratory signs and mucosa involvement are frequent. The definitive diagnosis depends on isolation of the etiologic agent in culture. Cytology, histopathology, and serology are useful tools for preliminary diagnosis. Severe pyogranulomatous inflammatory infiltrate, high fungal load, and extension of lesions to mucosa, cartilage, and bone in the nose of cats are indicative of an agent of high virulence in this endemic region. Itraconazole is the drug of choice, while, in refractory cases, amphotericin B or potassium iodide might be alternative treatments; however, recurrence after discharge may occur. Sporotrichosis persists as a neglected disease in Rio de Janeiro, and the treatment of cats remains a challenging and long-term endeavor.

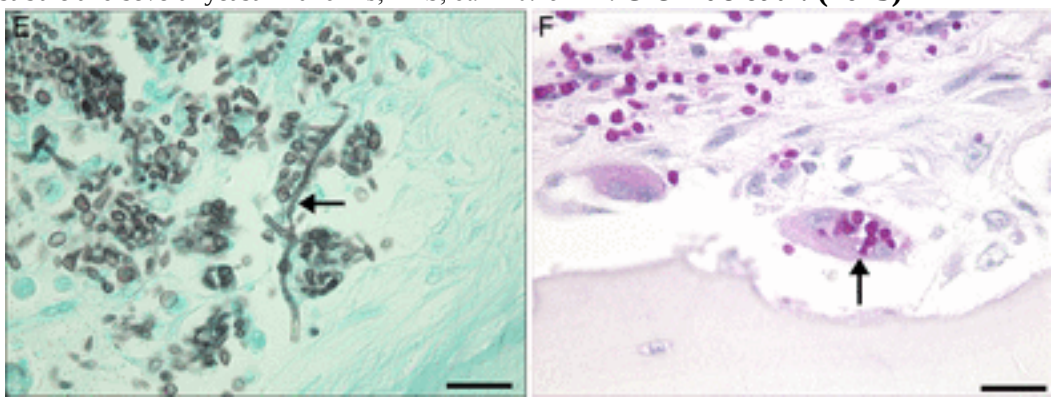
Feline sporotrichosis: lesions located on the face. Feline sporotrichosis: ulcer on the bridge of the nose.
Gremião *et al.* (2015)



Histological alterations in the nose of cats with sporotrichosis. Cats receiving no treatment. (A) Severe pyogranulomatous rhinitis showing lyses of osseous tissue and several yeast-like forms within macrophages; periodic acid Schiff (PAS), bar = 0.15 mm. (B) Severe pyogranulomatous osteomyelitis showing several yeast-like forms of *Sporothrix* within macrophages in the bone marrow; PAS, bar = 0.15 mm. **Gremião *et al.* (2015)**



(C) Mucosa of vestibule showing several yeast-like forms within macrophages or extracellular invasion and causing necrosis of hyaline cartilage; PAS, bar = 0.04 mm. (D, E) Cats refractory to treatment with itraconazole. (D) Severe pyogranulomatous rhinitis showing unilateral thickening of the mucosa of the vestibule and several yeast-like forms; PAS, bar = 0.15 mm. **Gremião *et al.* (2015)**



(E) Several cigar-shaped or round to oval yeast-like forms and a hypha (arrow) in the mucosa of the vestibule; Grocott methenamine silver, bar = 0.01 mm. (F) Cat with no previous history of treatment for sporotrichosis. Osteomyelitis showing cigar-shaped or round to oval yeast-like forms that were located extracellularly or within an osteoclast (arrow) and macrophages; PAS, bar = 0.01 mm. **Gremião *et al.* (2015)**

Jessica *et al.* (2015) conducted a study to evaluate the accuracy and reliability of **cytopathological examination** in the diagnosis of feline sporotrichosis. The present study included 244 cats from the metropolitan region of Rio de Janeiro, mostly males in reproductive age with three or more lesions in non-adjacent anatomical places. To evaluate the inter-observer reliability, two different observers performed the

microscopic examination of the slides blindly. Test sensitivity was 84.9%. The values of positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and accuracy were 86.0, 24.4, 2.02, 0.26 and 82.8%, respectively. The reliability between the two observers was considered substantial. \it was concluded that the cytopathological examination is a sensitive, rapid and practical method to be used in feline sporotrichosis diagnosis.

Kano *et al.* (2015) reported **molecular** epidemiological data on the aetiologic agents of feline sporotrichosis in Malaysia epidemiology of Sporothrix schenckii isolates from cats with sporotrichosis in Malaysia. They characterised 18 clinical isolates from cats in Malaysia based on molecular properties, including sequence analyses of the calmodulin gene and the rDNA ITS region and selective PCR of mating type (MAT) loci. In this study, isolates from feline sporotrichosis were identified as a ***S. schenckii*** sensu stricto by sequence analyses of the calmodulin gene and the internal transcribed spacer (ITS) region. Notably, phylogenetic analysis of the ITS confirmed assignment to clinical clade D (and not C) of S. schenckii sensu stricto. Therefore, clinical clade D of S. schenckii sensu stricto appeared to be the prevailing source of feline sporotrichosis in Malaysia. The ratio of MAT1-1-1:MAT1-2-1 in these Malaysian isolates was found to be 1 : 0. This result suggested that a clonal strain of S. schenckii is the prevailing causative agent of feline sporotrichosis in Malaysia.

Sanchotene *et al.* (2015) conducted an epidemiological surveillance in endemic areas of feline sporotrichosis in the southern region of Rio Grande do Sul state, Brazil. Over the last 5-year period the number of feline sporotrichosis in Rio Grande increased from 0.75 new cases per month in 2010 to 3.33 cases per month in 2014. The wide geographic distribution of diagnosed cases highlights the dynamics of Sporothrix transmission across urban areas with high population density. Molecular identification down to species level by PCR-RFLP of cat-transmitted Sporothrix revealed the emergence of the clonal offshoot ***S. brasiliensis*** during feline outbreaks; this scenario is similar to the epidemics taking place in the metropolitan areas of Rio de Janeiro and São Paulo.

Brilhante *et al.* (2016) evaluated the in vitro activity of amphotericin B, caspofungin, itraconazole, voriconazole, fluconazole, and ketoconazole against cat isolates of ***S. brasiliensis***. The susceptibility tests were performed through broth microdilution (M38-A2). The results showed the relevant activity of itraconazole, amphotericin B, and ketoconazole against S. brasiliensis, with the following MIC ranges: 0.125-2, 0.125-4 and 0.0312-2 μg/ml, respectively. Caspofungin was moderately effective, displaying higher variation in MIC values (0.25-64 μg/ml). Voriconazole (2-64 μg/ml) and fluconazole (62.5-500 μg/ml) showed low activity against S. brasiliensis strains. This study contributed to the characterization of the in vitro antifungal susceptibility of strains of S. brasiliensis recovered from cats withsporotrichosis, which have recently been considered the main source of human infections.

de Souza *et al*. (2016) evaluated the efficacy of **cryosurgery** in association with **itraconazole** for the treatment of feline sporotrichosis and compared the length of treatment protocol with others reported in the literature. Cats naturally infected with fungi of the Sporothrix schenckii complex were evaluated. Diagnosis was confirmed by **cytology** and fungal **culture**. Prior to the cryosurgical procedure, every animal was receiving itraconazole 10 mg/kg/day PO, for different time periods. The same protocol was maintained until 4 weeks after complete healing of the lesions. Eleven of 13 cats were considered clinically cured. The treatment duration ranged from 14-64

weeks (median 32 weeks). The combination of cryosurgery and itraconazole was effective in treating cases of feline sporotrichosis and decreased the treatment length compared with protocols using only medication.

Miranda *et al.* (2016) described the **leukocytes profile** in blood of cats with sporotrichosis by flow cytometry and its correlation with histopathology and fungal load. The cats with sporotrichosis were separated into groups L1, L2, and L3 (lesions at one, two, and three or more noncontiguous skin locations, respectively) and were classified as good, fair, or poor general conditions. The highest percentage of CD4+ cells was associated to L1 (P = .04) and to good general condition (P = .03). The percentage of CD8+ cells was greater in L2 and L3 (P = .01). CD8(low) expression occurred in 20 animals with sporotrichosis, mainly in L3 (P = .01) and was not observed in healthy controls. This expression was related to macrophage granulomas (P = .01) and predominated in cases with high fungal load. Altogether, the results indicated that control over feline sporotrichosis, with maintenance of a good general condition, fixed lesions, well-organized response and lower fungal load, is associated with increased CD4+ cells percentages. In contrast, a poor general condition, disseminated lesions and high fungal load were related to increased CD8+ cell percentages and increased expression of CD8(low). As conclusion these results point to an important role of the CD4:CD8 balance in determining the clinical outcome in feline sporotrichosis.

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E. Diseases in cats and dogs caused by algae

1. Protothecosis in cats and dogs

The genus *Prototheca* entails species of achlorophyllous, unicellular, saprophytic, aerobic algae closely related to *Chlorella* spp. These algae are ubiquitous in the environment and may be isolated from fresh and marine water, soil, mud, tree sap, and sewage. Five species of *Prototheca* are currently recognized, including *P. blaschkeae, P. stagnora, P. ulmea, P. wickerhami,* and *P. zopfii* ; a sixth species, *P. cutis,* was recently proposed based on genetically distinct isolates from a human patient with skin disease. Of these, *P. wickerhami* and *P. zopfii* are recognized as pathogenic to humans, cattle, and dogs. Human cases have largely been reported from

Canine cases of protothecosis are uncommon but are increasingly recognized worldwide.

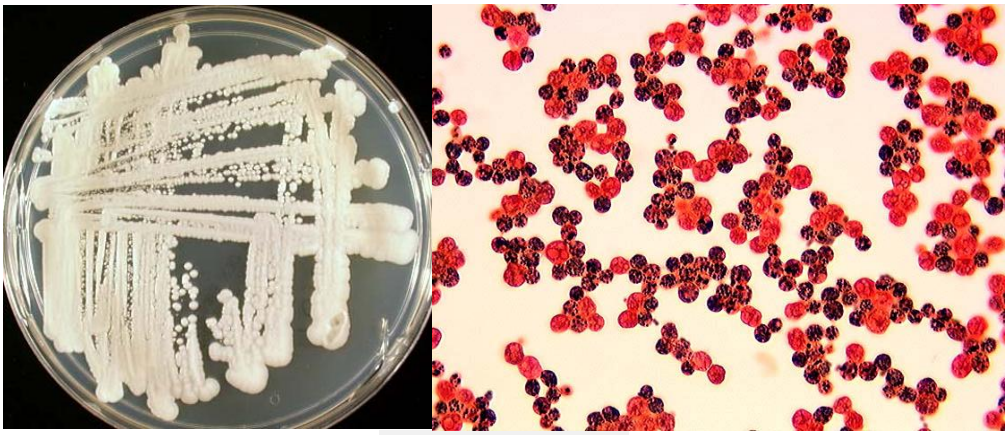
In contrast to the human disease, canine protothecosis typically involves a broadly disseminated infection, particularly involving the colon, nervous system, and eyes, as well as the heart, kidneys, skeletal muscle, and liver. Frequent involvement of the colon makes colitis (with or without hematochezia) a common presenting complaint; other common presenting complaints include neurologic disease, blindness, and less frequently, polyuria and polydipsia.

The clinical form of the protothecosis in animals is most commonly observed in countries with a warm and moist climate, only a few reports describing cases of this infection in cooler areas of the word exist. In the case of large bowel infection in dogs, organisms colonise the lamina propria and submucosa causing severe necrotizing ulcerative or haemorrhagic enterocolitis.

Aetiology

Prototheca zopfii W. Krüger, Hedwigia 33: 264 (1894)

Prototheca grows easily on glucose containing media and is typically grown on Sabouraud's agar, but it will not grow on media that contain cycloheximide. The colonies appear yeast-like and are smooth, moist, and white to cream in colour. Prototheca are unicellular organisms that are typically round to oval and 8-16 um in diameter, though they can range from 3 to 30 um depending on the species and the degree of maturation. The organisms reproduce by a mother cell undergoing internal cleavage. This results in the internal accumulation of smaller endospores surrounded by the wall (or theca) of the mother cell. The mother cell then bursts, releasing the endospores and the cycle is repeated. The endospores are 4 to 5 um in diameter and can number from two to 50. These endospores give Prototheca its characteristic "cart wheel" appearance of a round structure with internal septations. The larger forms can have very thick walls. They are basophilic and have been reported to be Gram positive, though in this case the theca are Gram negative and the endospores Gram. PAS staining highlights the starch granules which are occasionally present.



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Reports

Buyukmihci *et al*. (1975) reported an 8 1/2-year-old Collie dog with chronic diarrhea as well as sudden blindness and leukokoria of the right eye. An organism morphologically similar to Prototheca sp was recovered from the subretinal fluid and was found at necropsy in the eyes, gastrointestinal tract, lungs, lymph nodes, kidneys, heart, abdominal fat, and omentum.

Imes *et al.* (1977) reported on the clinical history and pathological lesions of a dog suffering from **disseminated protothecosis** due to ***Prototheca zopfi***. Clinically, the dog was presented with bilateral conjunctivitis followed by blindness, deafness and posterior paresis. Pathological lesions were most severe in the eyes and consisted of subacute panophthalmitis with secondary posterior subcapsular cataract, posterior synechia, retinal detachment and microscopic evidence of glaucoma. The kidney, liver, brain, spleen and lungs were also affected. This is believed to be the first published account ofprotothecosis in mammals other than man in Africa. A review of the literature is included.

Tyler *et al.* (1980) identified a case of progressive neurologic disease in a 4-year-old mixed-breed spayed bitch. Prototheca organisms were identified by histopathology, culture, and electron microscopy. Specific fluorescent antibody procedures revealed two species--***Prototheca wickerhamii*** and ***Prototheca zopfii***. Organisms and

pyogranulomatous lesions were found in the brain, spinal cord, right eye, kidneys, and heart.

Cook *et al.* (1984) euthanatized a 3-year-old Collie bitch, suffering from **disseminated protothecosis**, two weeks after the onset of blindness and deafness. The hearing deficit had been localized by clinical signs, brain stem auditory evoked responses, and impedance audiometry. Protothecosis was diagnosed by cytologic and histologic examinations. The organism was identified as ***Prototheca zopfii*** . Organisms and granulomatous lesions were found in kidney, heart, liver, skeletal muscle, thyroid gland, colon, bronchial lymph node, brain, and cochlea.

Font and Hook (1984) described a case of **disseminated protothecosis**, due to **Prototheca wickerhamii** in a two-year-old female dog with a nine-month history of hemorrhagic colitis and diarrhea. Shortly thereafter, the dog developed "acute blindness" of the left eye. Euthanasia was done after medical therapy failed to control the disease. Histologically, the eye had multiple microabscesses and necrotic foci containing myriad protothecal organisms under the detached retina. Numerous organisms also were present in the mucosa and walls of the colon. The identification of P. wickerhamii was confirmed by the histologic appearance and immunofluorescent studies. The ultrastructural features of P. wickerhamii also were studied.

Gaunt *et al.* (1984) reported a **disseminated protothecosis** in a dog with forelimb lameness, bilateral retinal detachment, and hemorrhagic diarrhea. Necropsy demonstrated multifocal lesions in the skeletal musculature, myocardium, liver, thyroid glands, kidneys, eyes, and brain. Microscopically, the lesions contained numerous organisms and minimal cellular infiltrates. The morphologic and cultural characteristics of the organisms were similar to prototheca. The organisms in tissue sections reacted positively for ***Prototheca zopfii,*** using an indirect fluorescent antibody technique.

Moore *et al.* (1985) diagnosed **systemic protothecosis** in a 7-year-old dog that had only **ocular manifestations**. During the 3-month course of disease, a variety of drugs was administered, including amphotericin B, gentamicin, and ketoconazole. The ocular signs initially abated, but subsequently worsened during this period. The dog was found dead 3 months after initial examination, and systemic protothecosis was confirmed at necropsy.

Thomas and Preston (1990) described a case of **generalised protothecosis** in a Collie dog. A long-standing history of severe colitis was the major clinical sign. Dissemination to many organs was confirmed histologically. Possible pathogenesis is discussed along with a review of the literature. The possibility of a breed disposition in Collie dogs is discussed. The organisms are ubiquitous in the environment and generalised disease suggests the possibility of immune competence.

Ginel *et al.* (1997) infected a dog systemically with ***Prototheca wickerhamii*** but showed only **cutaneous protothecosis**. The lesions appeared progressively and consisted of non-pruritic scrotal swelling and ulceration, cutaneous nodules, crusty ulcerative lesions over the trunk and serous rhinitis. The diagnosis was based on skin biopsy findings and specific culture. Microscopic examination revealed a diffuse pyogranulomatous dermatitis and numerous protothecal organisms of different sizes within the cytoplasm of phagocytic cells. Treatment with oral ketoconazole for six months resolved all the clinical signs except the scrotal granuloma which, although it

was significantly reduced, had to be removed surgically. However, after five months the condition returned.

Pérez *et al*. (1997) evaluated the distribution of the cellular inflammatory infiltrate associated with **cutaneous canine protothecosis** (***Prototheca wickerhamii***) by consecutive biopsies taken before, during and after treatment. Antibodies specific to canine immunoglobulins (IgG, IgM, IgA), human CD3 antigen (pan T-lymphocyte marker) and human myeloid/ histiocyte antigen (macrophage/neutrophil marker) were used. Before treatment, cellular infiltrate was very scanty in the inflamed areas, but it increased during the treatment, whereas the number of protothecal organisms decreased. Statistical analysis revealed an inverse relation between the number of protothecal organisms and the number of infiltrating macrophage/neutrophils ($P < 0.004$), T lymphocytes ($P < 0.001$), and cells containing immunoglobulin G ($P < 0.001$), M ($P < 0.001$) and A ($P < 0.001$) at different stages of the disease. These findings suggest either that protothecal organisms inhibit the migration or proliferation of cellular inflammatory infiltrate or that only dead protothecal organisms induce an effective local immune response.

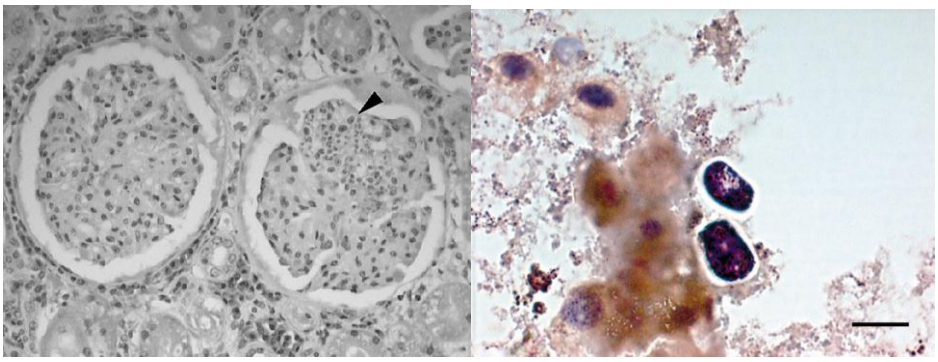
Schultze *et al*. (1998) reported 2 cases of sudden onset of blindness associated with **ocular protothecosis** in dogs . Both were adult, spayed female, mixed-breeddogs that lacked the usual clinical signs of systemic infection with Prototheca species. Physical abnormalities at the time of presentation were limited to the affected eyes which had serous discharge, hyperemic conjunctiva, and aqueous flare. The pupillary light reflexes were slow, and the menace reflexes were absent. Both dogs had glaucoma. Results of complete blood counts and serologic titres for antibodies to Blastomyces dermatitidis and Histoplasma capsulatum were within reference intervals. Protothecosis was diagnosed by cytologic analysis of vitreous humor and was confirmed at necropsy. These two cases were unusual because of their presenting signs and prolonged course of disease progression.

Hollingsworth (2000) mentioned that canine protothecosis remains a difficult condition to manage. The paucity of clinical cases hinders the development of successful treatment strategies. The clinical signs associated with the disease are nonspecific, and the course is so insidious that, by the time a definitive diagnosis is reached, the organism has often disseminated throughout the body. At this point, the condition is beyond treatment, and death occurs owing to failure of any number of organ systems, including the gastrointestinal, cardiovascular, renal, and central nervous systems. It is of some encouragement that the few patients that have undergone aggressive early treatment have survived longer than patients presenting late in the disease course. Nevertheless, the outlook for any dog with protothecosis is grave, and it remains to be determined whether early diagnosis can truly provide a better long-term prognosis. By including protothecosis as a consideration for dogs initially brought in with a history of chronic diarrhea or acute blindness and with a subsequent finding of exudative retinal separation, early diagnosis is possible. This recognition potentially affords the opportunity for an immune status work-up and intervention with increasingly better treatment options.

Hosaka and Hosaka (2004) reported a 10-year-old spayed mongrel dog with repeated intercurrent hematochezia and anal bleeding. The dog was vigorous and had a normal appetite, and the fecal test showed no abnormal signs. Despite treatment primarily with sulfasalazine, the condition did not improve and unilateral blindness developed. A Prototheca zopfii infection was identified by further examination with

bowel culture on Sabouraud's agar without cyclohexane and antibiotics. Subsequent to a vision loss in the other eye, the dog died showing signs of neurological disorder.

Pressler *et al*. (2005) reviewed records of 13 dogs with **systemic infection** with Prototheca sp. from 3 veterinary teaching hospitals. Acute renal failure secondary to disseminated infection with Prototheca zopfii was diagnosed in 2 dogs. In 1 dog, acute renal failure developed during administration of immunosuppressive drugs for treatment of anterior uveitis. During diagnostic evaluation of this dog, Prototheca sp. organisms were noted in urine sediment and renal biopsy specimens. In the 2nd dog, acute renal failure was diagnosed after treatment for bacterial cystitis. After diagnosis of protothecosis, organisms were successfully isolated by aerobic urine culture. Both dogs with acute renal failure did not respond to conventional medical therapy. In total, Prototheca sp. was noted in urine sediment in 4 of 8 dogs and successfully cultured from urine in 5 of 7 dogs. Four of 5dogs had organisms noted in the kidneys on histopathologic examination. In all dogs, the species identified was P zopfii. Sensitivity testing of 3 isolates revealed wide differences in in vitro drug resistance. Examination and culture of urine is recommended as a practical method for diagnosis of systemic infection with Prototheca sp.

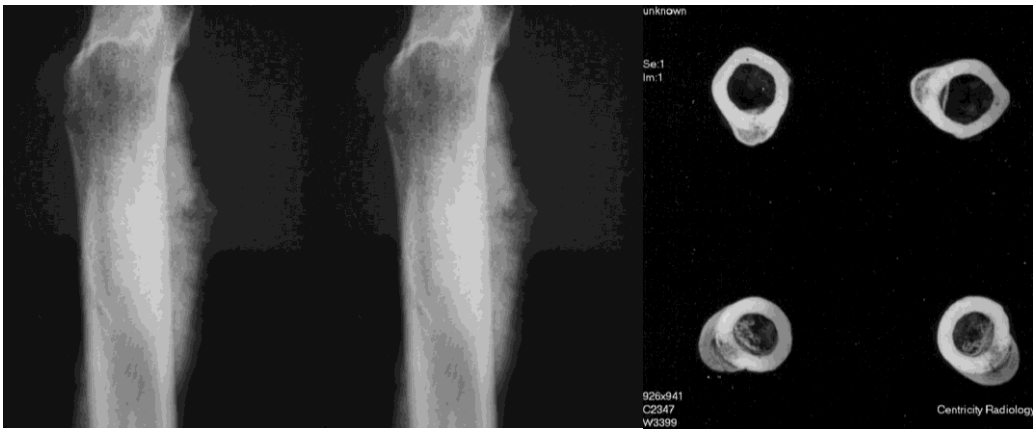


Renal tissue from a dog with acute renal failure secondary to disseminated protothecosis. There is multifocal inflammatory cell infiltration throughout the interstitium, and disruption of a glomerulus by large numbers of organisms (arrowhead). Hematoxylin and eosin stain, 1003. *Prototheca* sp. in a cytospin urine sediment preparation from a dog with systemic protothecosis. Diff-Quik stain, 6003. Bar 5 10 mm.

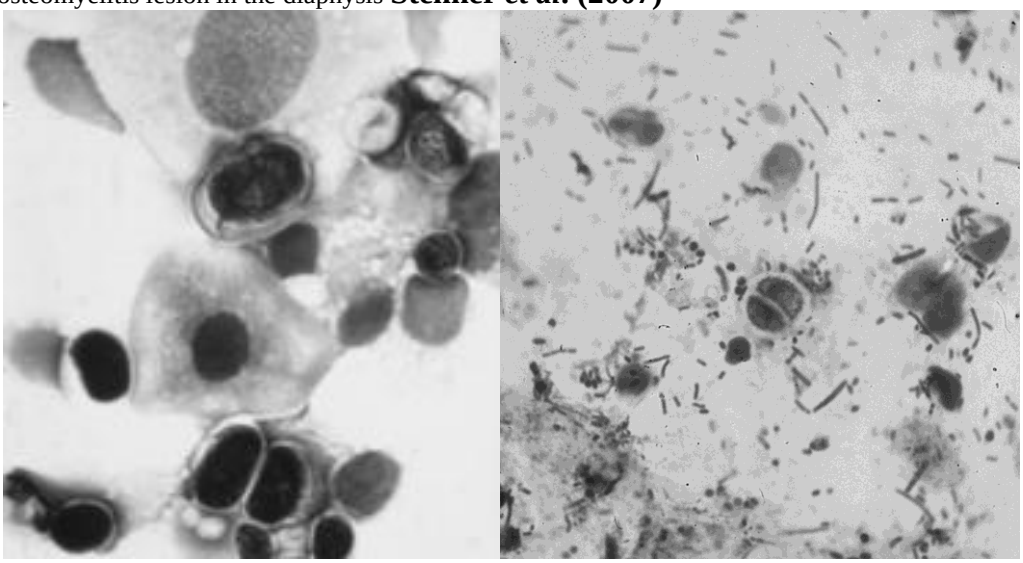
Tsuji *et al.* (2006) identified the isolate from a canine **disseminated protothecosis** to be ***Prototheca wickerhamii*** by its morphological and biochemical characteristics. The isolate was grouped into a cluster identical to the type strain of P. wickerhamii, ATCC 16529(T) in phylogenetic trees based on the small subunit (SSU) and the 5' end of the large subunit (LSU) ribosomal DNA (rDNA); the cluster was close to that including the other Prototheca species. However, the strains of P. wickerhamii, SAG 263-11 and Pore 1283 belonged to a cluster different from the other isolates of Prototheca species and closely related to those of Auxenochorella species. Therefore, P. wickerhamii could be divided into two distinct genetic groups, one group close to the other Prototheca spp. including a standard strain of P. wickerhamii, and another group consisting of isolates previously reported to be close to the Auxenochorella species.

Stenner *et al*. (2007) diagnosed **systemic protothecosis** in 17 Australian dogs . There was a preponderance of young-adult (median 4 years), medium- to large-breed dogs. Females (12/17 cases) and Boxer dogs (7 cases, including 6 purebreds and one Boxer cross) were over-represented. Sixteen of 17 dogs died, with a median survival of four months. A disproportionate number of cases were from coastal Queensland. In most patients, first signs were referable to colitis (11/17 cases), which

varied in severity, and was often present for many months before other symptoms developed. Subsequent to dissemination, signs were mostly ocular (12 cases) and/or neurologic (8 cases). Two dogs had signs due to bony lesions. Once dissemination was evident, death or euthanasia transpired quickly. Prototheca organisms had a tropism for the eye, central nervous system (CNS), bone, kidneys and myocardium, tissues with a good blood supply. Microscopic examination and culture of urine (5 cases), cerebrospinal fluid (CSF;1 case), rectal scrapings (4 cases), aspirates or biopsies of eyes (5 cases) and histology of colonic biopsies (6 cases) as well as skin and lymph nodes (2 cases) helped secure a diagnosis. Of the cases where culture was successful, P wickerhamii was isolated from two patients, while P zopfii was isolated from five. P zopfii infections had a more aggressive course. Treatment was not attempted in most cases. Combination therapy with amphotericin B and itraconazole proved effective in two cases, although in one of these treatment should have been for a longer duration. One surviving dog is currently still receiving itraconazole. Protothecosis should be considered in all dogs with refractory colitis, especially in female Boxers.

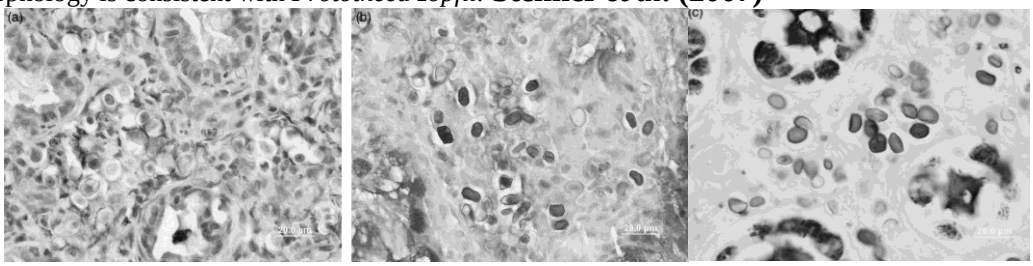


(a) Hematogenous algal osteomyelitis affecting the femoral diaphysis in a dog (case 1) with disseminated protothecosis. Note the irregular periosteal new bone formation. (b) A similar lesion is present in the femur of a different patient (case 5), although the periosteal new bone is much smoother in appearance. (c) Microradiography of the femur (*ex vivo*) obtained from case 5 at necropsy. Note that the higher resolution of this imaging reveals there is lysis as well as periosteal new bone formation in the osteomyelitis lesion in the diaphysis **Stenner *et al*. (2007)**

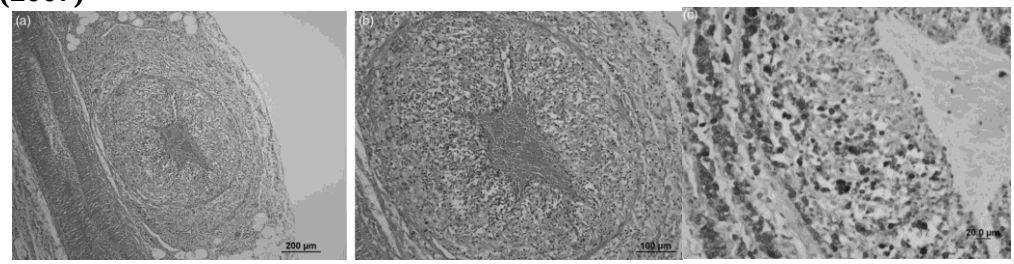


DiffQuik stain of urine sediment showing more detailed morphology of *Prototheca* organisms from case 5. The oval shape of organisms is characteristic of *Prototheca zopfii*. Original

magnification×330, DiffQuik stained smear of a rectal scraping from a dog (case 5) with protothecal proctitis. Note the *Prototheca* organisms against a background of faecal bacteria. Again, the morphology is consistent with *Prototheca zopfii*. **Stenner *et al*. (2007)**

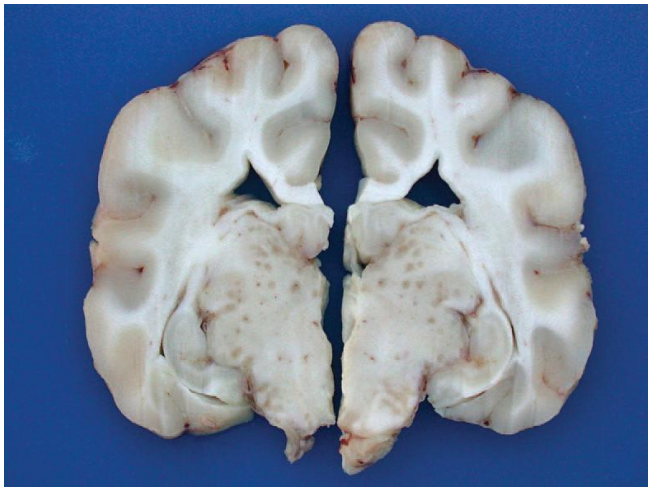


Morphological appearance of *Prototheca* organisms in sections of the colonic wall. The sections show the mild mixed inflammatory infiltrate in the lamina propria consisting of plasma cells, neutrophils and macrophages. Interspersed amongst the inflammation are moderate numbers of large oval organisms. Although organisms are easily discernible in H&E stained sections (a), they are better visualised in PAS (b) and methenamine silver stained (c) sections. The organism morphology in these photomicrographs is consistent with a*Prototheca zopfii* infection **Stenner *et al*. (2007)**

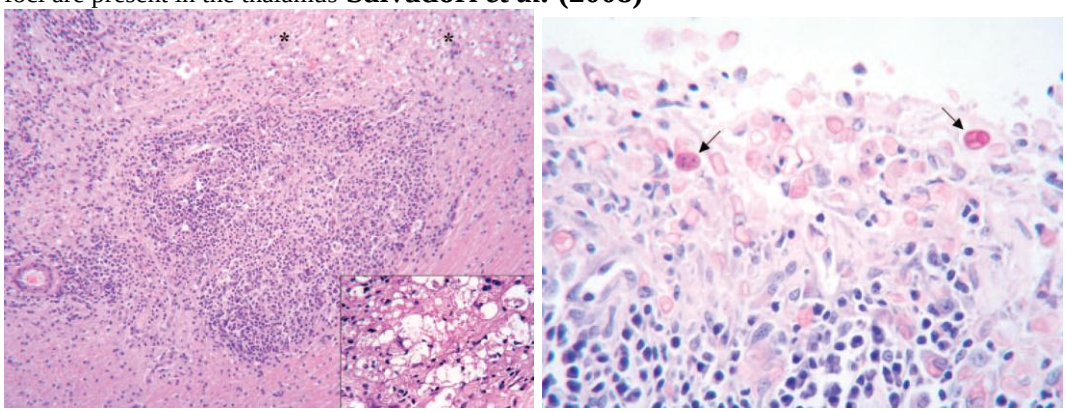


Granulomatous colitis and arteritis due to *Prototheca zopfii*. A thrombus is evident in the lumen of an artery on the serosal portion of the the colonic wall (a). Organisms can be seen invading the artery (b), although they are easier to visualize in (c) because of the higher magnification and the periodic acid Schiff (PAS) stain Appearance of *Prototheca zopfii* organisms in a wet preparation of urine (from case 5) at high power (Original magnification×660). **Stenner *et al*. (2007)**

Salvadori *et al.* (2008) reported a case of central nervous system protothecosis in a three-year-old male Maremma sheepdog with a two month history of diarrhoea associated with progressive tetraparesis, depression and right circling. Stupor, severe proprioceptive deficits, bilateral decreased thoracic limb flexor reflexes and bilateral deficit of the menace reaction were detected on neurological examination and a multi-focal neurological localisation was suspected. Histopathological evaluation revealed multi-focal granulomatous foci in the thalamus, hippocampus and caudal brainstem containing numerous oval-rounded organisms with a thick, periodic acid-Schiff-positive and Gomori's methenamine silver-positive cell wall, a basophilic cytoplasm and one nucleus. Scattered sporangia containing two to four endospores were also observed. Morphological features were consistent with Prototheca species. Ultrastructurally, numerous degenerated algae were present within macrophages mainly lacking nuclei and cytoplasmic organelles. Generally, protothecosis in dogs is characterised by systemic signs because of a multi-organ involvement, and haemorrhagic colitis or ophthalmologic signs are the most frequent presenting signs. However, protothecosis should be added, also in Europe, to the list of the differential diagnoses in adult dogs with a multi-focal neurological localisation even in absence of other clinical signs.

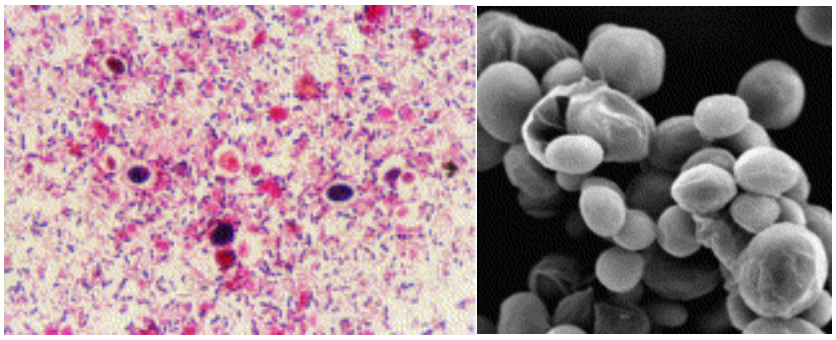


Transverse section through thalamus, hippocampus and temporal cortex: bilateral multifocal malacic foci are present in the thalamus **Salvadori *et al.* (2008)**



Thalamus. Granulomatous foci composed of numerous macrophages, lymphocytes and plasma cells with a perivascular pattern are evident in the grey matter. Spongiosis with axonal degeneration and myelin ballooning is also present (asterisks) (H&E, 3125). Inset: Area of malacic change of the white matter (H&E, 3500) Thalamus. Numerous algae with a periodic acid-Schiff (PAS)-positive, 1 mm thick cell wall are evident. Scattered sporangia containing two to four endospores with well evident nuclei are also present (arrows) (PAS, 3125) **Salvadori *et al.* (2008)**

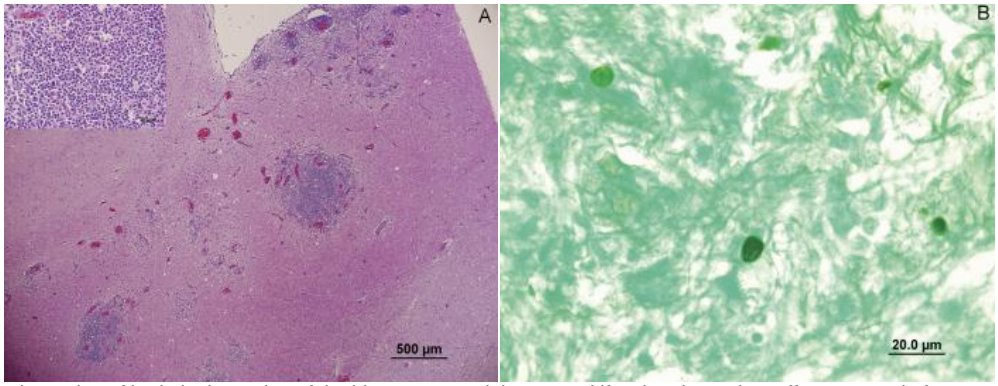
Ribeiro *et al.* (2009) reported enteric protothecosis caused by Prototheca zopfii in an eight-year-old male mixed breed dog with a history of chronic bloody diarrhea, loss of appetite and weight loss. Algae were isolated from rectal scrapings in defibrinated sheep blood agar and dextrose Sabouraud agar. Cytological evaluation showed the presence of globular and cylindrical organisms with a defined capsule and variable number of endospores, characteristic of the genus Prototheca, in the rectum of the animal. Scanning electron microscopy of P. zopfii strains at different development stages confirmed the diagnosis of algal infection. Molecular identification using a conserved 18S rDNA gene sequence determined that the strain belonged to genotype 2. This report describes success on treatment of canine protothecosis, diagnosed based on clinical, cytological, microbiological, scanning electron microscopy and genotypical findings



Ribeiro *et al.* (2009)

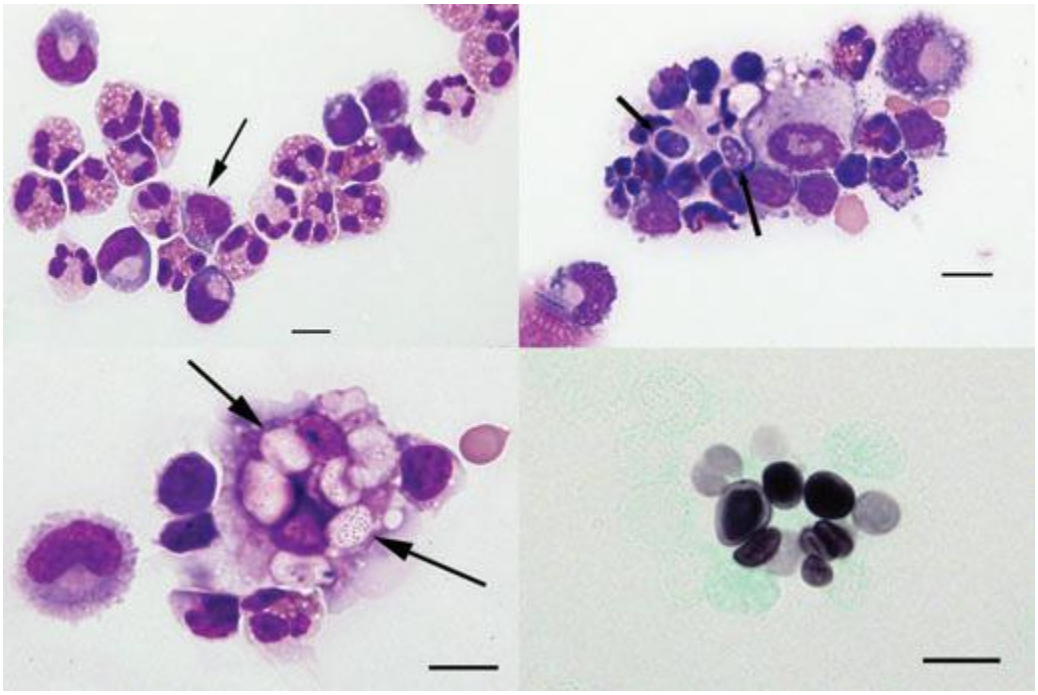
Sapierzyński *et al.* (2009) eported the intestinal form of protothecosis in 1.5-year-old, male, mongrel dog with chronic hemorrhagic diarrhoea. History revealed that the dog spent some time in the countryside and afterwards diarrhoea with fresh blood appeared. The results of morphological and biochemical blood analysis were normal and stool examination did not reveal the presence of parasites. Treatment with anti-inflammatory doses of prednisone, metronidazole and enrofloxacin followed by sulphasalazine resulted in a short period of improvement, but was followed by deep deterioration of animal status. Because of the relapse diagnostic laparotomy was performed and tissue samples of the colon and jejunum were obtained for histopathology. On the basis of the clinical signs, exploratory laparotomy findings and histopathology the diagnosis of canine intestinal prototecosis was made and medical treatment was recommended.

Gupta *et al.* (2011) reported a 6-year-old spayed female Boxer dog with a 3-week history of ataxia, walking sideways, crossing over of limbs, and dragging her hind feet. She had a repair of her left cranial cruciate ligament approximately 4 months previously with uneventful recovery. Neurologic examination revealed severe ataxia, worse on the left side, and a left head tilt. No abnormalities were observed on computed tomographic scan of the brain. Magnetic resonance imaging of the brain revealed a lesion involving approximately 50% of the left side of the pons and medulla and extending dorsally to the 4th ventricle. Histologic section of brain in the region of the hippocampus showed multifocal perivascular cuffs composed of aggregates of numerous lymphocytes, plasma cells, and small numbers of histiocytes. And algal organisms with characteristic radially arranged multiple wedge-shaped endospores.

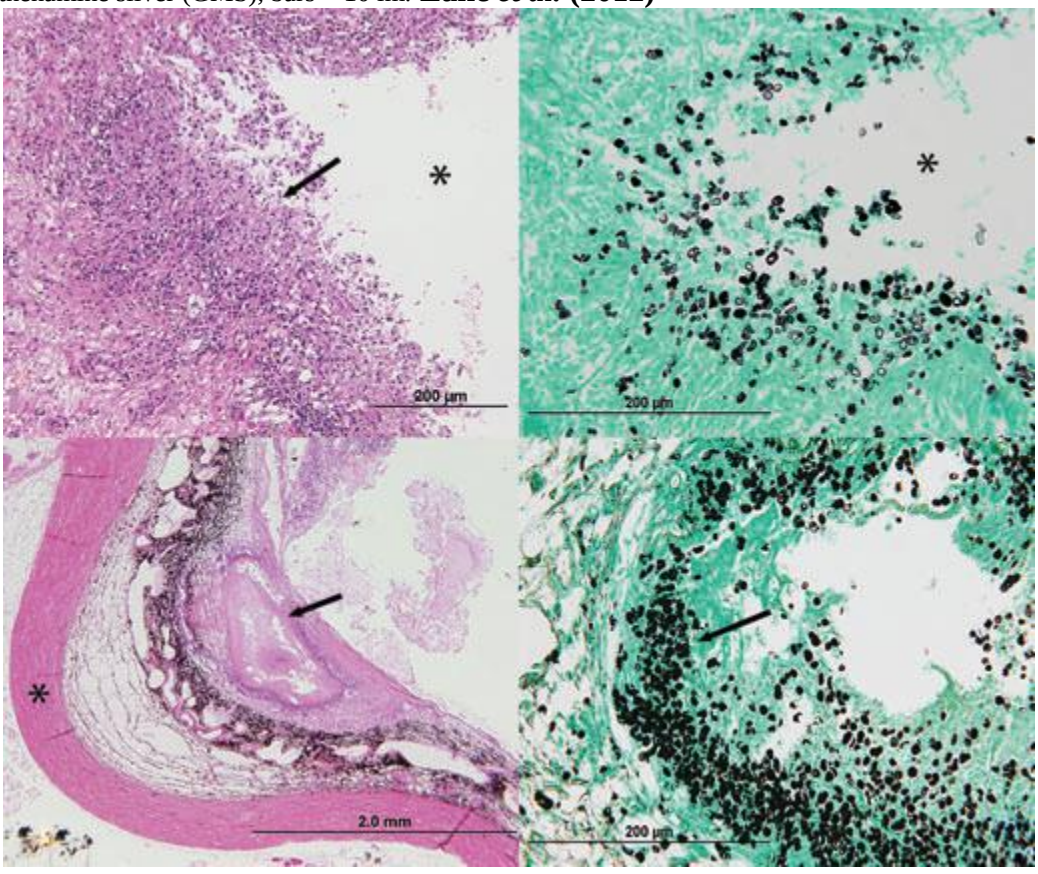


Histologic section of brain in the region of the hippocampus. (A) Note multifocal perivascular cuffs composed of aggregates of numerous lymphocytes, plasma cells, and small numbers of histiocytes. H&E. Inset: Inflammatory cells in a perivascular cuff. H&E, bar = 20 mm. (B) Note algal organisms with characteristic radially arranged multiple wedge-shaped endospores. Gomori's methanamine silver.

Lane *et al.* (2012) evaluated a 5-year-old female spayed Shetland Sheepdog Mix dog for a history of seizure activity, progressive hind limb ataxia, polyuria, and polydipsia and no history of gastrointestinal signs. Physical examination findings included conscious proprioceptive deficits, ataxia, and anterior uveitis along with a hypermature cataract in the right eye. Results of a CBC, serum biochemical profile, urinalysis, and computed tomography scan of the brain were unremarkable. Cerebrospinal fluid (CSF) analysis revealed marked eosinophilic pleocytosis and rare organisms consistent with Prototheca spp within neutrophils and macrophages. On postmortem histologic examination, mononuclear inflammation and numerous intralesional algal organisms, similar to those seen on the cytologic preparation of CSF, were found in the brain, eyes, kidneys, and heart. Abnormalities were not detected on gross and histologic examination of the gastrointestinal tract. Cultures of CSF and subdural/olfactory bulb, but not intestinal tract, yielded growth of Prototheca spp, and PCR analysis and DNA sequencing confirmed the organism as Prototheca zopfii genotype 2. We have reported a rare case of disseminated protothecosis that was diagnosed by evaluation of CSF in a dog presented with neurologic signs and no overt enteric disease. Protothecosis should be considered as a rare cause of seizures, even in the absence of obvious enteric signs, and should be included in the differential diagnosis of eosinophilic pleocytosis.



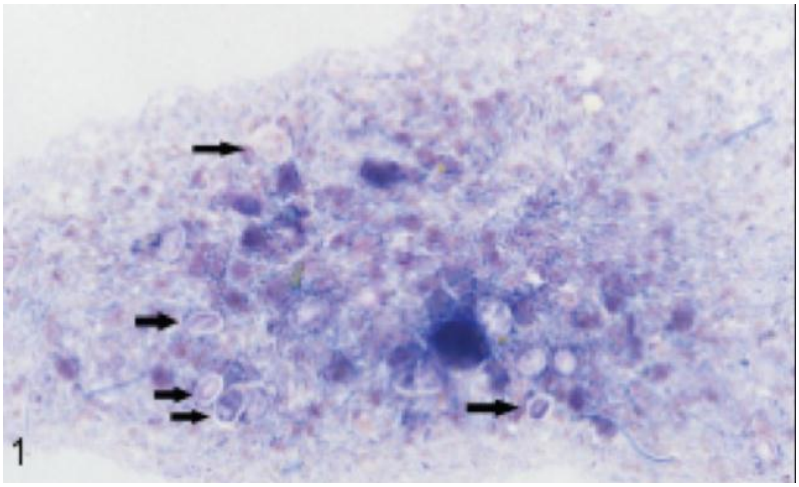
Cytocentrifuged preparation of cerebrospinal fluid from a dog with protothecosis. (A) Marked eosinophilic pleocytosis; note the large granular lymphocyte with numerous azurophilic granules (arrow). (B) Several oval to kidney-bean shaped basophilic and granular Prototheca organisms with thin non-staining casings (arrows). (C) Empty casings (theca) in the cytoplasm of a large mononuclear cell (arrows). (D) Numerous GMS-positive organisms. (A-C) Modified Wright–Giemsa, (D) Gomori's methenamine silver (GMS), bars = 10 lm. **Lane *et al.* (2012)**



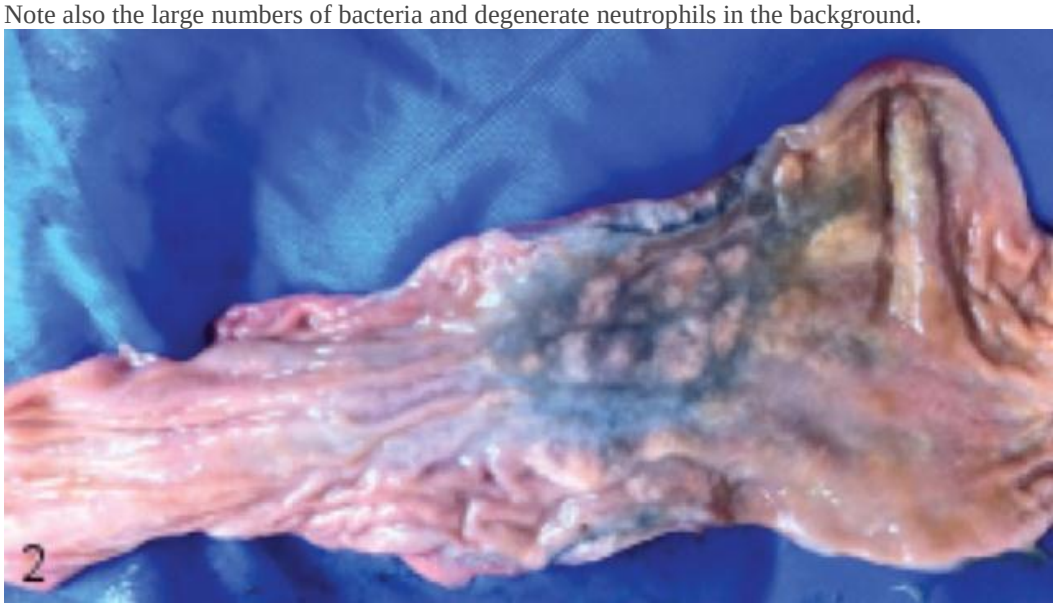
2. (A) Histologic section of brain through lateral ventricle (asterisk). Note increased cellularity of the ependymal lining of the lateral ventricle (arrow). H&E. (B) Same section as (A); note the numerous GMS-positive Prototheca organisms in the ependymal lining of the lateral ventricle (asterisk).

Gomori's methenamine silver (GMS). (C) Histologic section of the right eye. The choroid is markedly expanded with a pocket of inflammation (arrow); the sclera (asterisk) provides orientation. H&E. (D) Same section as (C) showing increased magnification of the inflammatory pocket in the choroid; note numerous GMS-positive organisms. Gomori's methenamine silver. **Lane *et al.* (2012)**

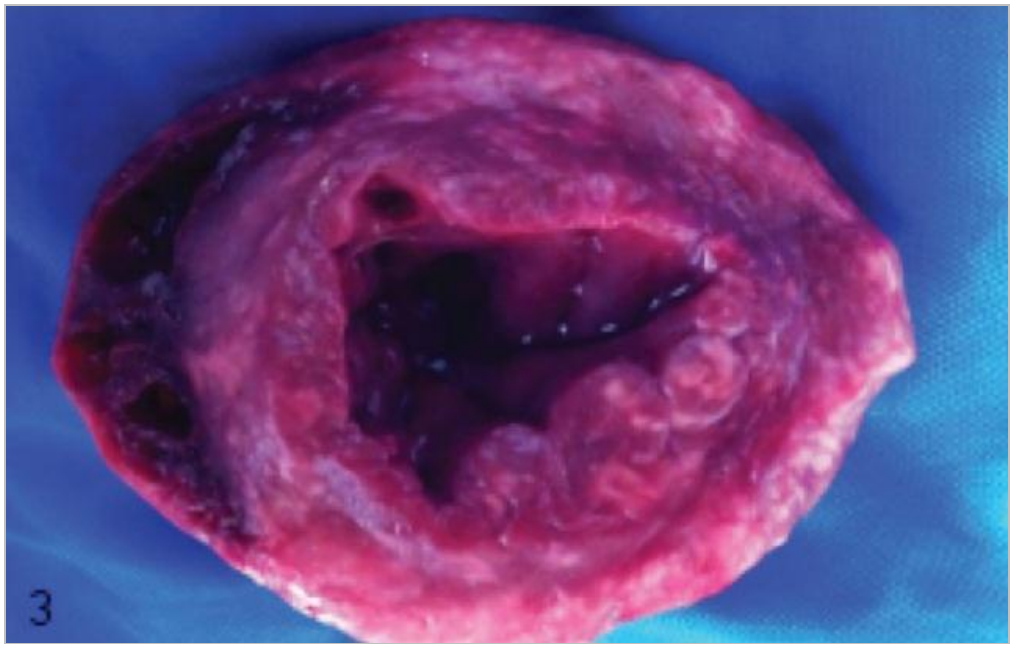
Vince *et al.* (2014) reported a case of a **disseminated** algal infection in a young rough-coated collie dog with progressive neurologic deficits, blindness, and hemorrhagic diarrhea. Prototheca zopfii organisms were cultured from feces, urine, and blood. At necropsy, granulomas containing typical organisms were identified within the proximal colon, heart, kidneys, and eyes.



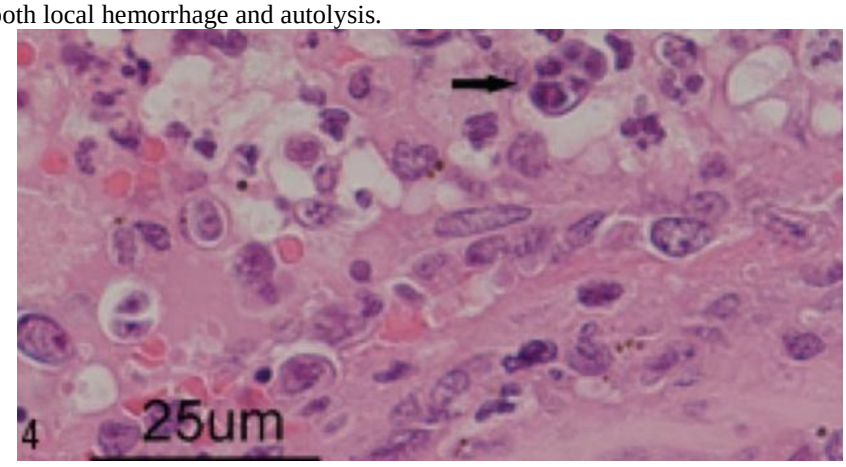
Rectal scraping cytology, modified Wright's stain, 600× magnification. Prototheca organisms (arrows) are round to oval, measuring 2 to 30 μm and have a granular, basophilic cytoplasm with clear cell wall. Note also the large numbers of bacteria and degenerate neutrophils in the background.



Heart, cut section of apex. At all levels of the myocardium there are numerous poorly defined pale tan-white nodules.



Colon and rectum, mucosal surface. There is segmental dark discoloration of the colonic wall, throughout which are multiple poorly defined white-tan nodules. Some green discoloration is present as a result of both local hemorrhage and autolysis.



Posterior chamber of the eye, H&E stain, 600× magnification. Within the posterior uvea and anterior chamber are numerous 8 to 12 µm diameter round-oval algal sporangia, with thick hyaline cell walls and 1, 2 or 4 endospores; 1 of these sporangia has a typical "Maltese cross" appearance for *Prototheca* spp. These sporangia are surrounded by large numbers of neutrophils.

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