UNIVERSITY OF CALGARY
FACULTY OF VETERINARY MEDICINE

UCVM Trainee Research Days 2021

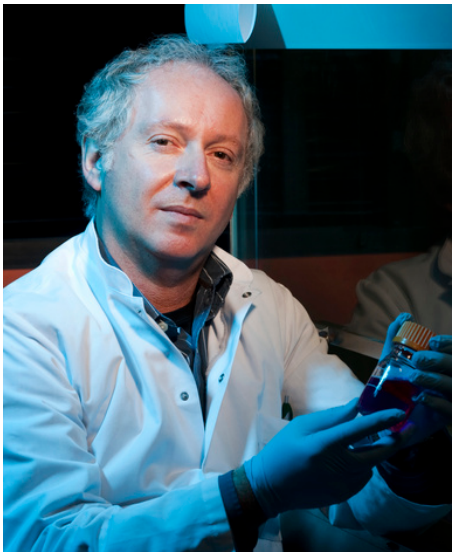
May 06 and 07th, 2021

08:30AM-1:00PM

ABSTRACT BOOKLET

Message from Dr. Hermann Schaetzl, Associate Dean, Research

Dear graduate students, Dear postdoctoral fellows, Dear colleagues,



A warm welcome from the Associate Dean, Research. The basic idea for this meeting is to bring together the UCVM research community and provide our trainees with a platform for showcasing the wide range of research activities they are performing.

This year we wanted to do the meeting differently. First, we wanted to involve our postdoctoral colleagues at the organization and presentation level and have renamed the meeting to “UCVM Trainee Research Day” to reflect this. Second, we wanted this conference organized and run by trainees for trainees, with very little help from faculty, to provide a hands-on and real-time experience of peer-review and conference organization for peers. Accordingly, we struck a committee consisting of five graduate students, five postdoctoral fellows and four faculty members to oversee meeting organization, abstract adjudication, session chairing/moderating and prize selection. Third, we replaced the traditional posters, challenging to do during COVID19 times, with short oral presentations so that every trainee participating has the experience of giving an oral presentation. Fourth, we have a series of awards for best long and short presentations for graduate students and postdoctoral fellows streams.

We hope that this meeting, from early planning to realization and appraisal, will bring students, postdocs and faculty closer together and help us to value the high quality and breadth of research activities our faculty can be proud of.

I would like to use this opportunity to thank my co-organizers Kayla and Waqas; they really did an outstanding job. Many thanks to all members of the organization committee for their help and efforts. Finally, many thanks to our invited guest speaker, Dr. Kathy McCoy, for sharing her research with us, Collene and Rahil for preparing the website, Mary A., Mary G., Lori, and the One Health team (Michele, Golsa and Andrea) for help with organization.

Sincerely,

Hermann M. Schaetzl

Associate Dean Research

Message from Dr. Jacob Thundathil, Associate Dean, Graduate Education and Internationalization

Dear UCVM,

Welcome to UCVM Trainee Research Day-2021! The Trainee Research Day includes DVM students, faculty, staff, graduate students, and postdoctoral trainees. During this event, our trainees will showcase their rigorous laboratory and field research through oral presentations. Thank you, all, for your excellent contributions during the challenging Covid-19 times. Thanks to UCVM supervisors for supporting your trainees with their presentations and oversee this meeting organization. I take this opportunity to thank Dr. Hermann Schaetzl and the Trainee Research Day Organizing Committee for their exceptional effort in developing this inclusive event, which provides a rich educational experience to our trainees.

I hope you will effectively utilize this opportunity to better understand the diverse research programs of our faculty.

Sincerely,

Jacob Thundathil, BVSc& AH, MVSc, PhD

Associate Dean Graduate Education and Internationalization

UCVM Trainee Research Days: 2021

Conference Agenda

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<tr><td rowspan="6">08:35</td><td rowspan="6">Session 1 (Long Presentations)</td><td>Presenters</td><td>Session Chairs</td></tr>
<tr><td>Cristobal Winkens (H. Schätzl)
Hsp110 modifies prion infection in vitro and in vivo</td><td rowspan="5">Dr. Steven Hersch and Summer Hunter</td></tr>
<tr><td>Rebecca Chen (J. Gilleard)
Modular molecular epidemiological toolkit for studying anthelmintic resistance using next-generation sequencing technology</td></tr>
<tr><td>Haley Silas (E. Pajor)
Assessing the impacts of age and duration of maternal separation during spring processing on behavioural indicators of stress in beef calves</td></tr>
<tr><td>Nynke Hoogen, PDF (T. Trang)
Identifying the neurodevelopmental differences of opioid withdrawal</td></tr>
<tr><td>Kat Smith (Y Niu)
Efficacy of bacteriophage mediated colibacillosis control in laying hens</td></tr>
<tr><td>09:45</td><td colspan="3">Break</td></tr>
<tr><td rowspan="6">09:55</td><td rowspan="6">Session 2 (Long Presentations)</td><td>Steven Hersch, PDF (T. Dong)
Engineered type six secretion systems deliver active exogenous effectors and Cre recombinase</td><td rowspan="5">Dr. Johnathan Canton and Dr. Charlie Kwok</td></tr>
<tr><td>Sadman Sakib (I. Dobrinski)
Expansion of porcine germ cells in stirred suspension bioreactors is partially mediated by the Wnt/β-catenin pathway</td></tr>
<tr><td>Sarah Erickson (E. Janzen)
The Epidemiology of Hoof-Related Lameness in Western Canadian Feedlot Cattle</td></tr>
<tr><td>Tahir Ali, PDF (S. Gilch)
Identifying the brain-specific cholesterol metabolizing enzyme (Cyp46A1) as a potential therapeutic target in prion diseases</td></tr>
<tr><td>Priyoshi Lahiri (E. Cobo)
The pathophysiology of impaired skin wound healing in bovine digital dermatitis</td></tr>
<tr><td>11:05</td><td colspan="3">Break</td></tr>
<tr><td>11:10</td><td colspan="3">Short Oral Presentations (Four Breakout Rooms: Agenda Below)</td></tr>
<tr><td>12:10</td><td colspan="3">Break</td></tr>
<tr><td>12:15</td><td colspan="2">Keynote lecture: Microbiome and Metabolites in Regulating Host Immunity</td><td>Prof. Dr. Kathy McCoy</td></tr>
<tr><td>12:50</td><td colspan="2">Closing Remarks</td><td>Dr. Waqas Tahir</td></tr>
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May 6th Breakout Room One

Moderator: Dr. Charlie Kwok

11:10	**Kabita Baral** (J. Biernaskie) *Human skin and nerve derived Schwann cells exhibit subtle transcriptomic and functional differences*
11:20	**Anice Thomas** (E. Pajor) *Impact of digital dermatitis on the behaviour and pain threshold of beef cattle*
11:30	**Maria Arifin** (S. Gilch) *A single amino acid polymorphism within the cervid prion protein alters chronic wasting disease pathogenesis*
11:40	**Chris Chang** (S. Gilch) *PrPSc aggregation state dictates biochemical properties of prions and disease pathogenesis*
11:50	**Eléonore Charrier** (J. Gilleard) *Creating curated databases for different rDNA cistron markers of nematodes to support more flexible and customized nemabiome metabarcoding*
12:00	**Alyssa Butters** (S Checkley) *Examining the relatedness and antimicrobial resistance elements of E. coli isolates within a One Health continuum using whole-genome sequencing*

May 6th Breakout Room Two

Moderator: Ruina Bao

11:10	**Josefien Hommes** (P. Kubes) *ArlRS Two-Component System of Staphylococcus aureus is required for Intracellular Bacterial Replication in Kupffer Cells*
11:20	**Kevin Manera** (T Dong) *Sensing of intracellular Hcp Levels controls T6SS expression in Vibrio cholerae*
11:30	**Micky Ahn** (J. Poissant) *Host age and environment describes variation in gastrointestinal parasite communities of Sable Island horses*
11:40	**Nilesh Sharma** (J. Biernaskie) *Impact of ependymal cell metabolic perturbation on brain function*
11:50	**Larissa Martins** (H. Barkema) *Effective Johne's disease control using phage technology*
12:00	**Annie Nguyen** (Y. Niu) *Identification and characterization of phage – host receptor apparatus of seven bacteriophages against Shiga toxin-producing Escherichia coli*

May 6th Breakout Room Three

Moderator: Dr. Catalina Barboza

11:10	**Sara Kim** (T. Dong) *Understanding the mechanisms of new inhibitors against Vibrio cholerae*
11:20	**Jane Fletcher** (Y.D. Niu) *Control of Escherichia coli O157:H7 through the use of lytic bacteriophage cocktails*
11:30	**Anshu Babbar, PDF** (E. Cobo) *Cathelicidin recruits neutrophil during infectious colitis as an early innate gut defense*
11:40	**Fabien Mavrot, PDF** (S. Kutz) *Studying rare occurrences: long-term phylogenetic monitoring of the emerging pathogen Erysipelothrix rhusiopathiae during mortality events*
11:50	**Anna Voigt** (I. Dobrinski) *Gonocytes present a distinct metabolic phenotype*
12:00	**Nadine Whyte** (Y.D. Niu) *Biofilm Formation of O157 And Non-O157 Shiga-Toxigenic Escherichia coli Isolates on Microplates and Stainless-steel Coupons*

May 6th Breakout Room Four

Moderator: Dr. Anne-Marieke Smid

11:10	**Jae Eun Hyun** (Y.D. Niu) *Occurrence of Virulence Genes and Antibiotic-Resistant Escherichia coli in Stormwater from Urban Retention Ponds*
11:20	**Sulav Shrestha** (F. van der Meer) *Effect of selective removal of high proviral load cattle on the herd prevalence of bovine leukosis*
11:30	**Tong Wang, PDF** (J Gillard) *Seasonal epidemiology of major gastrointestinal nematode species in the northern semi-arid climatic zone of western Canada using the ITS-2 nemabiome metabarcoding approach*
11:40	**Zhuohan Miao** (J. De Buck) *Development of a Method for Detection of Bovine Mastitis Pathogens by Combining Isothermal Amplification and Split Trehalase Technologies*
11:50	**Kayley McCubbin** (H. Barkema) *Knowledge gaps in the understanding of antimicrobial resistance in Canada*
12:00	

Program May 7th, 2021			
08:30	**Welcome**		**Kayla Strong**
08:35	Session 3 (Long Presentations)	**Presenters**	**Session Chairs**
		Eren Kutluberk (J. Biernaskie) *Investigating the Effects of CSF1R Inhibition on Skin Regeneration*	Dr. Anne-Marieke and Dr. Catalina Barboza
		Kyle Plotsky, PDF (D. Hall) *Valuing wood bison, their health, and their landscape: Integrating values and valuation*	
		Stefan Gavriliuc (J. Poissant) *Molecular and computational tools for cost-effective, non-invasive genomic analyses in feral horses*	
		Kayla Strong (S. Checkley) *Factors influencing the prevalence of Antimicrobial Resistant Enterococcus spp. in the Canadian Beef Cattle System: A Scoping Review to inform an Integrated Assessment Model*	
		Summer Hunter (J. Rothenburger) *Zoonotic bacteria in free-ranging urban white-tailed jackrabbits (Lepus townsendii) and feral European rabbits (Oryctolagus cuniculus) in Calgary, Alberta*	
09:45	Break		
09:55	Session 4 (Long Presentations)	**J. Morgan Louden** (F. van der Meer) *Comparison of Antibody Presence Between Preconditioned and Traditionally Raised Cattle During the First 40 Days on a Feedlot*	Dr. Julia Pons and Ruina Bao
		Ellen de Jong (H. Barkema) *Mastitis-related antimicrobial use: Current practices on Canadian dairy farms*	
		Bruna David, PDF (P. Kubes) *Extravascular location of Kupffer cells leads to a higher susceptibility to bacterial dissemination in newborns*	
		Jacob Varghese (J. Thundathil) *Effect of Oxidative Stress on Developmental Competence of In Vitro-Produced Embryos*	
		Mohamed Hassan (M. Faizal Abdul-Careem) *Pathogenicity of the Canadian Delmarva (DMV/1639) Infectious Bronchitis Virus (IBV) Infection in Chickens Leading to Reproductive Tract Abnormalities*	
11:05	Break		
11:10	Short Oral Presentations (**Four Breakout Rooms: Agenda Below**)		
12:10	Break		
12:30	Prize distribution and closing		Kayla Strong and Dr. Waqas Tahir

May 7th Breakout Room One

Moderator: Summer Hunter

11:10	**Linda Dorrestein** (H. Barkema) *Herd health and production management visits on Canadian dairy cattle farms: structure, goals and topics discussed*
11:20	**Anne-Marieke Smid, PDF** (H. Barkema) *Perspectives of Western Canadian dairy farmers on providing outdoor access for dairy cows*
11:30	**Maryam Ahmadi** (I. Dobrinski) *Exploring PDGFRα Signaling in Sertoli Cell Primary Cilia and Seminiferous Tubule Morphogenesis*
11:40	**Waqas Tahir, PDF** (Dr. H. Schätzl) *The role of astrocytes in the propagation of prions*
11:50	**Shiama Thiageswaran** (I. Dobrinski) *Proliferation of porcine undifferentiated spermatogonia in co-culture conditions*

May 7th Breakout Room Two

Moderator: Dr. Jennifer Pearson

11:10	**Julia Canet-Pons, PDF** (T. Trang) *Effects of chronic morphine exposure on Oligodendrocytes*
11:20	**Sarthak Sinha** (J. Biernaskie) *Distinct wound microenvironments remodel the regulatory landscape of dermal fibroblasts to unmask either a regenerative or fibrotic response*
11:30	**Charlie Kwok, PDF** (T. Trang) *Role of Primary Afferents in Arthritis Induced Spinal Microglial Reactivity*
11:40	**Ruina Bao** (Y.D. Niu) *Engineered endolysins against multidrug resistant bacteria causing bovine respiratory disease*
11:50	**Rachel Kratofil** (P. Kubes) *Monocytes regulate leptin-driven vascular dysfunction to promote healing during infection*

May 7th Breakout Room Three

Moderator: Dr. Steven Hersch

11:10	**Abby Hodder** (K. Orsel) *Impact of early feed bunk exposure on the behaviour and activity of preconditioned beef calves at the feedlot*
11:20	**Catalina Barboza** (M. Faizal Abdul-Careem) *Efficacy of recombinant viral vector vaccine against a Canadian infectious laryngotracheitis virus infection in chicken*
11:30	**Karma Phuntsho** (D. Hall) *Economic impact of infectious bronchitis virus on Canadian poultry industry*
11:40	**Tatiana Barcenas** (M. Musiani) *Dogs contribution to Echinococcus multilocularis lifecycle in the urban centers of Alberta*
11:50	**Elodie Labit, PDF** (J. Biernaskie) *Hic1 inactivation improves skin regeneration*

May 7th Breakout Room Four

Moderator: Dr. Johnathan Canton

11:10	**Filip Rakic** (S. Kutz) *Hair Trace Minerals as an Emerging Health Indicator within the Bluenose-East Herd and Other Caribou Designatable Units*
11:20	**Abdallah Shahat** (J. Kastelic) *Melatonin and l-arginine mitigate heat stress-induced reductions in quality of frozen-thawed ram sperm*
11:30	**Marit Biesheuvel** (H. Barkema) *Understanding farmer behavior and their decision-making process in the context of cattle diseases: A review of theories and approaches*
11:40	**Pearl Cherry** (S. Gilch) *Prion infection is associated with reduced Rab7 activation and impaired vesicular trafficking*
11:50	**Arpan Neupane** (P. Kubes) *Patrolling alveolar macrophages conceal bacteria from the immune system to maintain homeostasis*

UCVM Trainee Research Days: 2021

Research Abstracts

Listed by First Name Alphabetically

1. Impact of early feed bunk exposure on the behaviour and activity of preconditioned beef calves at the feedlot

Abigail Hodder[1] (Presenting author, MSc student) Ed Pajor[1], Morgan Louden[1], Frank van der Meer[1] & Karin Orsel[1]

[1]Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada

Immune suppression in young beef calves due to stress associated with transitioning from ranch to feedlot, can increase the risk of Bovine Respiratory Disease (BRD). Exposing calves to a feed bunk prior to transport can help reduce the stress when adjusting to unfamiliar environments.

This study aims to understand the impact of early feed bunk exposure of preconditioned calves at the ranch on feed bunk attendance during feed delivery and time spent eating on arrival at the feedlot, in comparison to non-preconditioned calves. Preconditioned (n=100) and non-preconditioned (n=20) calves were enrolled, and feed bunk attendance was measured through behavioural and video observations. Calves were observed using scan sampling technique every 5 minutes in 2-hour intervals, 3 times per day for a total of 7 days to measure the number of calves present with their head in the feed bunk. On average 31% of preconditioned calves attended the feed bunk on day 1 on arrival in comparison to 5% of non-preconditioned calves. By day 7, preconditioned calves had reached a bunk attendance of 55% during feed delivery, in comparison to non-preconditioned at 33% bunk attendance. Results from the current study will provide further insight on the effectiveness of early exposure to a feed bunk at the ranch on reducing stress through increased feed intake and preparing calves for the feedlot environment in order to improve the health and welfare of beef cattle.

Keywords: Feeding Behaviour, Weaning, Animal Welfare, Calf Health

2. Melatonin and l-arginine mitigate heat stress-induced reductions in quality of frozen-thawed ram sperm

Abdallah Shahat,[a,b] Jacob Thundathil,[a] John Kastelic[a]

[a]Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada; [b]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Melatonin or l-arginine enhanced post-thaw sperm quality and protected against cryopreservation-induced oxidative stress; our objective was to determine their protective effects on frozen-thawed sperm from rams subjected to heat stress (HS). Ten Dorset rams were group-housed indoors (~18 ℃), allocated into 2 equal groups: scrotal-neck insulation for 96 h or whole-body warming (28 ℃, 30-34% RH) for 8 h/d for 4 d). Semen was collected once weekly from 1 wk before to 5 wk after HS, extended and divided into 5 aliquots: no additives or 0.5 or 1 mM of melatonin or l-arginine. Semen was loaded into 0.5-ml straws and cryopreserved. Straws were thawed at 37 ℃ for 35 s and evaluated for post-thaw motility (CASA; Sperm Vision®), morphology (eosin-nigrosin) and acrosome integrity (FITC-PSA). Data were analyzed using repeated measures and a Bonferroni test. For total and progressive motility, there were effects of group (P=0.023 and P=0.0008, respectively); for total abnormalities, effects of group (P=0.001) and a group*week interaction (P=0.003); and for acrosome integrity, effects of group (P=0.046) and week (P=0.0001). On all days, all end points were significantly better for all treatments compared to the Control. All treatments improved motility, whereas improvements in total abnormalities and acrosomal integrity were dose-dependent. Total and progressive motility were improved by ~5 to 10 percentage points, whereas total abnormalities and intact acrosomes were improved by ~7 and 12 percentage points, respectively. Results supported the hypothesis that exogenous melatonin or l-arginine in semen extender mitigates HS-induced reductions in quality of frozen-thawed ram sperm.

Keywords: Ram, sperm, melatonin, l-arginine, HS

3. Examining the relatedness and antimicrobial resistance elements of *E. coli* isolates within a One Health continuum using whole-genome sequencing

Alyssa Butters[1,2], Karen Liljebjelke[1,2], Sheryl Gow[3,4], Cheryl Waldner[4], Sylvia Checkley[1,2]

[1]University of Calgary, Faculty of Veterinary Medicine, Department of Ecosystem and Public Health, [2]AMR One Health Consortium, [3]Public Health Agency of Canada (CIPARS/FoodNet), [4]University of Saskatchewan, Western College of Veterinary Medicine

Other Collaborators: Agriculture and Agri-Food Canada, Alberta Agriculture and Forestry, Alberta Precision Laboratories

Presenting Author: Alyssa Butters, Graduate student, Faculty of Veterinary Medicine, Department of Ecosystem and Public Health

Introduction: Previous dogma maintained that isolation of *E. coli* from environmental samples represents recent fecal contamination. The identification of “naturalized” *E. coli* capable of persistence and reproduction in the environment necessitates reconsideration of this paradigm, but the contribution of naturalized strains to antimicrobial resistance (AMR) transmission is not well understood. Because of the genetic mobility provided by horizontal gene transfer, antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) are relevant units of study for AMR transmission, however the genetic discrimination in many previous studies is insufficient to establish relatedness of AMR elements within a One Health continuum.

Objectives: This study will identify genetic elements conferring AMR in *E. coli* isolated from routine surveillance (2018-2019) of Alberta feedlot beef and broiler chickens production, retail beef and chicken meats, wastewater and well water. It will assess the genetic relatedness of *E. coli* from these sources and define epidemiological patterns of resistance determinants.

Methods: Short- and long-read whole-genome sequences will be combined bioinformatically into hybrid assemblies with resolution of MGEs. ARGs will be identified by BLAST search of AMR databases, and phylogenetic trees will be constructed to assess isolate relatedness. Regression analysis with adjustment for clustering will identify associations in the pattern and frequency of resistance elements.

Anticipated Results/Conclusions: The resolution of MGEs will address a significant deficit in the literature. Temporal and geographical restriction will allow a robust comparison of relatedness and AMR carriage of the isolates, and epidemiological factors identified will inform AMR mitigation strategies and risk assessment models.

Keywords: AMR, One Health, Whole-genome sequencing

4. Inuit Knowledge of Dolphin and Union Caribou: Partnerships, Ecology, and Co-Management

Andrea Hanke[1*], Amanda Dumond[2*], Lisa-Marie Leclerc[3] & Dr. Susan Kutz[1]

[1]Department of Ecology and Public Health, Faculty of Veterinary Medicine, University of Calgary; [2]Kugluktuk Angoniatit Association, [3]Department of Environment, Government of Nunavut

Roles: *Andrea Hanke: PhD student, lead presenter; *Amanda Dumond: Research partner and thesis committee advisor, co-presenter

Emails: andrea.hanke1@ucalgary.ca; kugluktuk@krwb.ca; lisa.leclerc@hotmail.com; skutz@ucalgary.ca

Abstract: Dolphin and Union (DU) caribou (*Rangifer tarandus groenlandicus x pearyi*, locally referred to as Island tuktu) were reassessed as endangered by the COSEWIC in 2017. We started a collaboration amongst the Kugluktuk Angoniatit Association, Government of Nunavut, and the University of Calgary in 2017 to take a closer look at what Inuit knowledge has to say about this herd. We used a mix of structured interviews, semi-structured individual interviews, focus groups, feedback sessions, and participatory mapping to purposefully engage 30 harvesters in 2003 and 33 knowledge keepers from 2018-2021.

The results from these studies provided critical insights into the abundance and distribution trends of DU caribou and brought forward Kugluktukmiut co-management recommendations for the herd. Key findings demonstrate that the cumulative DU caribou distribution is much broader than it is today, and that seasonal distribution and migration is perhaps more variable than previously thought. Also, we highlight the importance of involving knowledge keepers from across the DU caribou range to understand the full life-history of DU caribou and to develop effective herd-level conservation approaches. This work has resulted in 3 presentations and 1 report for the co-management partners and 2 presentations for the community.

Our collaborative research process has created opportunities to have more Inuit knowledge available at co-management meetings. Moving forward, Inuit knowledge research should continue to have a sustained focus on community partnerships, and it would be valuable to widen the research scope to include other communities within the DU caribou range.

Keywords: caribou, Inuit knowledge, conservation, community-based research, interviews

5. Impact of digital dermatitis on the behaviour and pain threshold of beef cattle

Anice Thomas[1], Karin Orsel[1] and Edmond Pajor[1]

[1]University of Calgary, Faculty of Veterinary Medicine, Department of Production Animal Health, Calgary, Canada

Presenting author: Anice Thomas, graduate student

Digital dermatitis (DD) is an infectious disease of the foot causing ulcerative and necrotic lesions. Most lesions are painful when palpated, some cause lameness and in general significantly impact animal welfare and productivity. Our objectives were to determine if changes in behaviour were associated with DD in cattle, and to quantify pain associated with DD lesions and lameness. In total, 255 animals across three feedlots were enrolled. A subset of 120 animals were fitted with accelerometers to record behaviour. Cattle were scored for lameness, both hind feet scored for DD (M-stage scoring system) and the pressure pain threshold of lesions determined using pressure algometry. Animals and feet were classified as DD absent or present. Further, DD present was split into active or chronic. Behaviour 2 to 5 days before DD identification was analyzed. Animals without DD spent more time ruminating compared to DD present animals that ruminated 3% less daily (P<0.05). Further, animals with active lesions ruminated 5% less daily (P<0.05). Daily inactivity time was greater in animals with DD and the effect of day depended on type of lesion (P<0.05). Feet without DD lesions withstood 5.6N more pressure (P<0.001). Active lesions were most sensitive withstanding 8.1N less pressure and chronic lesions 4.1N less pressure than DD absent feet (P<0.05). Moderate to severely lame animals withstood 3.1N less pressure than sound animals (P<0.001). In conclusion, rumination is depressed, and inactivity increased in DD affected animals. Both active and chronic lesions are painful, and lame animals have a lower pain threshold.

Keywords: beef cattle, rumination, inactivity, pain, lameness

6. Gonocytes present a distinct metabolic phenotype

Anna Laura Voigt, Douglas Andrew Kondro, Diana Powell, Hanna Valli-Pulaski, Mark Ungrin, Jan-Bernd Stukenborg, Claudia Klein, Ian A. Lewis, Kyle E. Orwig, Ina Dobrinski

Presenting Author: Anna Laura Voigt, PhD Candidate

Spermatogonial stem cells (SSCs) originate from gonocytes that developed from primordial germ cells (PGCs). While adult SSCs rely on glycolysis, PGCs enact mainly OXPHOS. It is unclear, when the metabolic transition occurs and the metabolism of gonocytes has not been described. Higher mammal and humans have a long prepubertal phase that is accompanied by the presence of gonocytes for an extended period.

Our results show that prepubertal human spermatogonia have distinct round perinuclear accumulated mitochondria, a phenotype shared with pig gonocytes. The metabolism of porcine gonocytes is characterized by the reliance on pyruvate consumption and OXPHOS. Gonocytes consumed 1.23 ± 0.16 mM of pyruvate and only 0.147 ± 0.09 mM glucose over 48 h of culture yet produced 0.681 ± 0.1 mM lactate. Therefore, pyruvate is not only oxidized but also reduced and excreted as lactate. Intriguingly, addition of lactate increased glucose consumption and mTOR activity. Stem cell maturation was accompanied with a decrease in mitochondrial activity (mean intensity MitoTracker Deep Red 44664 ± 2479 vs 31176 ± 919, p=0.012, n=3: basal respiration 159.8 ± 9.54 pmol/min/cells vs 114.1 ± 9.93 pmol/min/cells, p=0.0293, n=3), upregulation of anaerobic metabolism associated uncoupling protein 2 (UCP2) by 1.7 fold, and increased glycolytic flux. Simultaneously, stem cell specific promyelocytic leukaemia zinc finger protein (PLZF) protein expression, glial cell-derived neurotropic factor (GDNF) pathway activation, and enhanced mTOR activity could be detected.

The obtained results will help us to create an age-related metabolic fingerprint of prepubertal human germ cells to establish appropriate culture conditions.

Supported by NIH/ORIP 9 R01 OD016575-17A1

Keywords: SSC maturation, OXPHOS, ROS, mTOR

7. Perspectives of Western Canadian dairy farmers on providing outdoor access for dairy cows

Anne-Marieke C. Smid[*,1], Pauline H.J. Inberg[*], Saskia de Jong[*], Shane Sinclair[†], Marina A.G. von Keyserlingk[‡], Daniel M. Weary[‡], and Herman W. Barkema[*]

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[†] Faculty of Nursing and Department of Oncology, University of Calgary, 2500 University Drive NW, Calgary, AB T2N 1N4
[‡] Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, BC. V6T 1Z6, Canada
[1]Presenting author, PDF

Free-stall housed dairy cows are highly motivated to access pasture, especially at night. In 2015, however, a survey reported that only 29 and 57% of Canadian dairy farms provided lactating and dry cows pasture access, respectively. To better understand reasons why dairy farmers choose to provide or not provide outdoor access, we conducted 11 focus group discussions with a total of 50 Western Canadian dairy farmers to understand their perspectives on outdoor access for dairy cows. Transcripts were analyzed using template analysis. Reasons to not provide outdoor access fell into 5 main themes: 1) adverse climate conditions, 2) negative implications of outdoor access for cow welfare including concerns about udder health, 3) concerns regarding decreases in profitability, 4) farm infrastructure not set up for outdoor access, and 5) higher ability to manage animals kept indoors. Reasons to provide outdoor access fell into the 5 main themes: 1) local climate conditions conducive for outdoor access, 2) beneficial effects of outdoor access on cow welfare including lower lameness prevalence, 3) increased profitability due to a premium milk price provided to farmers that allow outdoor access to their cows, 4) farm infrastructure set up for outdoor access, and 5) easier management of animals outdoors. We conclude that the decision to provide outdoor access depends on how farmers weigh these factors given the specific requirements of their farm. Our results may help guide debate on outdoor access for dairy cattle in the dairy industry and beyond.

Keywords: barrier, motivator, dairy farming, outdoor access

8. Identification and characterization of phage – host receptor apparatus of seven bacteriophages against Shiga toxin-producing *Escherichia coli*

Nghi Nguyen[1], Jared Schlechte[2], Matthew Walker[3], Kim Stanford[4], Tim A. McAllister[5], Alexei Savchenko[1], Jeroen De Buck[1], Yan D. Niu[1*]

[1]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary
[2]Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary
[3]Canadian Science Centre for Human and Animal Health, Public Health Agency of Canada, Winnipeg, Manitoba, R3E 3R2, Canada.
[4]University of Lethbridge, Lethbridge, AB T1K 1M4, Canada.
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Bacteriophages (phages) have been considered as a potential alternative to antibiotics for the treatment of bacterial pathogens, including Shiga Toxin-producing *Escherichia coli* (STEC) – a diverse group of foodborne pathogens causing life threatening disease in human worldwide, especially in North America. This study investigates seven environmental phages from *Tequintavirus*, that can infect diverse STEC serogroups. As phage tails are molecular machines responsible for bacterial recognition and attachment, we predict that extensive gene rearrangements may occur in phage tail regions to respond to distinct STEC hosts. Comparative genomic analysis suggested that all newly founded phages share high similarity with the *Tequintavirus* reference phage AKFV33 (>92% nucleotide identity). However, the tail genes encoding for L-shaped tail fiber proteins (TFPs) and receptor binding proteins (RBPs) are highly diverse compared to other known host attachment proteins. Bioinformatic analysis on protein sequences of the newly found proteins suggests that low similarity in TFPs and RBPs of our phages (30 – 65%) may lead to the differences in host range recognition and attachment. Furthermore, microplate phage virulence index assay is employed to screen their host range and examine their lytic capability. Tail proteins of two out of seven interesting phages – AKFV33 and AXO103A which were published with host range and lytic activity, will be overexpressed and examined their binding activities with surface receptors of bacterial cells to verify their function in initial host attachment. This study will provide novel insights regarding gene variance and generate new knowledge regarding the molecular mechanisms underlying host recognition and infection of STEC phages.

Keywords: bacteriophages, phage – host interaction, whole genome analysis, protein overexpression, phage host range.

9. Cathelicidin recruits neutrophil during infectious colitis as an early innate gut defense

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Neutrophils are central in the colon mucosa during diarrheic infectious colitis caused by *Citrobacter rodentium*, a natural murine pathogen that resembles attaching effacing enteropathogens. Colonic epithelial cells initiate the recruitment of neutrophils by sensing bacterial LPS via TLR4 in association with endogenous cathelicidins and secreting chemokines. Infiltration of neutrophils is controversial as they eliminate microbial pathogens but have deleterious tissue effects.

To test if cathelicidin influences early waves of neutrophils into the colon that control infection before damaging persistent inflammation, we challenged wild type (*Camp*$^{+/+}$) and cathelicidin deficient (*Camp*$^{-/-}$) mice with *C. rodentium* to determine the neutrophil influx in colons during colonization, peak, and resolution phases (0, 2, 5, 7, 10, 14, and 21-days post challenge). We showed infected *Camp*$^{-/-}$ mice have lesser Ly6G^{+}CD11b^{+} neutrophils in the colonic epithelial fraction and higher faecal bacterial load during early time points. *Camp*$^{-/-}$ neutrophils did not show any functional deficiency compared with normal counterparts and similarly expressed formyl receptor 2 and purinergic P2X7, receptors that interacted with cathelicidin peptides during chemoattraction.

We conclude cathelicidin regulates neutrophil recruitment during infectious colitis, key to control infection. The peptide effect seems mostly as chemoattractant and not related with neutrophil performance. Intervention by cathelicidin and analogues during early infection might aid as immunomodulator in recruiting neutrophils and limiting the infection.

10. Patrolling alveolar macrophages conceal bacteria from the immune system to maintain homeostasis

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During respiration humans breathe in more than 10,000 liters of non-sterile air daily, allowing some pathogens access into the alveolar spaces. The immune response to this basal degree of pathogen invasion at the alveolar level of the lung remains poorly characterized. Alveolar macrophages (AMs), the resident phagocytic cells in the alveolar spaces, are crucial responders against inhaled pathogens. Recently, we developed a method for *in vivo* real-time intravital imaging (IVM) of the alveoli and revealed that AMs crawled and patrolled in and between the alveolar spaces. Importantly, crawling AMs sensed, chemotaxed and with high efficiency, phagocytosed inhaled bacterial pathogens such as *P. aeruginosa* and *S. aureus*, cloaking them from neutrophils. Impairing AM chemotaxis towards bacteria, induced superfluous neutrophil recruitment leading to inappropriate inflammation and injury. Considering these observations, we investigated whether AM behavioural alteration underpin the high incidence of bacterial co-infections (or super infection) that are observed during influenza A viral infection. Using IVM, here we demonstrate that AMs in flu infected mice have a significant impairment in their ability to crawl in the alveolar spaces. Moreover, these AMs could no longer migrate towards and capture inhaled secondary bacterial pathogens. Mechanistically, we show that type II interferon signaling pathway contributed to the impairment in AM behaviour. Overall, our results highlight the importance AM crawling and chemotaxis behaviour in maintaining the alveolar spaces free of pathogens.

Keyword: Alveolar macrophage; Intravital microscopy; Bacterial infection; Neutrophils; Lungs

11. Extravascular location of Kupffer cells leads to a higher susceptibility to bacterial dissemination in newborns

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Despite worldwide efforts, neonatal mortality remains unacceptably high. Due to their immature immune system, newborns present a dysregulated immune response after bacterial infections which commonly culminates into sepsis. The main cells responsible for bacterial clearance from the bloodstream are the Kupffer cells (KCs) – the largest population of tissue resident macrophages – located within the liver sinusoids, in direct contact with the bloodstream. Our hypothesis was that newborn KCs are not mature enough to recognize, capture and kill bacteria. To evaluate the role of newborn KCs after a bacterial infection, we first developed a unique protocol to image the liver of 1-day old mice. Using in vivo imaging, we accessed and measured the bacteria catching by adult and newborn KCs, using fluorescent (GFP) *Escherichia coli*. Our data showed significant impairment in the ability of bacteria capture by newborn KCs. Therefore, more bacteria disseminated to other organs, where they proliferate in the first hours, leading to death 24 hours after infection. Evaluating our in vivo images, we noticed that, in newborns, the KCs are located outside the blood vessels, dramatically reducing their access to the blood flow. This contrasts with adults, where KCs are located inside the blood vessel. So far, we believe that the limited contact within the bloodstream is responsible for the impaired bacterial capturing by newborn KCs. Now, the main goal is to identify the molecules responsible for driving the KCs to the intravascular compartment, and then test them as a treatment to accelerate the process of controlling bacterial infections.

Keywords: newborn, bacterial infection, liver, Kupffer cells.

12. Efficacy of recombinant viral vector vaccine against a Canadian infectious laryngotracheitis virus infection in chicken

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Presenter: Catalina Barboza Solis, MSc Student

Canadians rely on high quality meat and eggs originated from Canadian poultry industry. Infectious laryngotracheitis (ILT) is a respiratory disease caused by a herpesvirus commonly referred as Infectious laryngotracheitis virus (ILTV). ILTV impacts egg production and decreases growth leading to economic losses. A potential source of transmission is the latently infected carriers in non-commercial flocks, where wild-type ILTV strains are the second common cause of ILT outbreaks in Alberta. In Alberta, the commercial chickens are not vaccinated leaving them susceptible to infection. The objective of this study was to determine the efficacy of one of the safer options of commercially available vaccines, a recombinant viral vector, against a Canadian wild-type ILTV. At 1 day of age, we separated 44 chickens into four groups. Two of those groups were vaccinated while the remaining two were mock vaccinated. At 3 weeks of age, one of the vaccinated groups and one of the mock vaccinated groups were infected. For 2 weeks, the chickens were observed twice a day for clinical signs. At 3, 7, 10 and 14 days post-infection (dpi), body weights, feather tips, cloacal and oropharyngeal swabs were collected. At 5 and 12 dpi, blood was collected to quantify CD8+ and CD4+ T cells. At 14 dpi, the chickens were euthanized, and tissue samples were collected. Results showed that the vaccine prevented weight loss, reduced viral shedding, and increased CD8+ T cells in early infection in the vaccinated chickens. Nonetheless, it failed to mitigate clinical signs at the peak of the disease.

Keywords: infectious laryngotracheitis virus, rHVT-LT recombinant vaccine, chicken, immune response, vaccine

13. Role of Primary Afferents in Arthritis Induced Spinal Microglial Reactivity

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Microglia, the resident immune cells of the central nervous system, are cellular modulators of chronic pain. This study sought to understand how peripheral injury engages spinal microglial responses, using a rat model of monosodium-iodoacetate (MIA)-induced knee joint injury.

In male rats, an increase in joint afferent firing and microglia activation within the spinal dorsal horn were observed within the first week of MIA injections. The functional link between afferent activity and microglial responses was examined via pharmacological silencing of C and A fibres with co-injections of QX-314 (membrane impermeable sodium channel blocker) and bupivacaine (local anaesthetic), capsaicin (C-fibre targeting), or flagellin (A-fibre targeting) into the knee joints. All treatments prevented the development of mechanical allodynia (pain evoked by subthreshold mechanical stimulation) and spinal microglial activity after MIA injections.

We next hypothesized that ATP is a key signalling molecule for neuroimmune communications. Elevated levels of ATP in the cerebrospinal fluid (CSF) and increased expression of the ATP transporter vesicular nucleotide transporter (VNUT) in the spinal dorsal horn were observed 2 weeks after MIA injections. Selective silencing of primary joint afferents subsequently inhibited ATP release into the CSF. In addition, the increase in spinal microglial reactivity, and alleviation of MIA-induced arthralgia with co-administration of QX-314 and bupivacaine were recapitulated in female rats.

Our results demonstrate that peripheral joint injury activates joint nociceptors, which triggers a central spinal microglial response. Elevation of ATP in the CSF, and spinal expression of VNUT suggest ATP signalling may modulate communication between sensory neurons and spinal microglia.

Keywords: pain, arthritis, joint afferents, QX-314, ATP

14. PrP^{Sc} aggregation state dictates biochemical properties of prions and disease pathogenesis

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Prion diseases are devastating neurodegenerative diseases caused by the conversion of the host-encoded cellular prion protein (PrP^{C}) into the pathological misfolded isoform called PrP^{Sc}. Cervids, cattle, sheep, and humans are among the affected species. Various prion strains exist, each of which consists a unique conformational, biochemical, and biological profile. Our study investigates whether such properties of individual PrP^{Sc} aggregation states are stable upon serial passage in vivo. Prion-positive brain homogenate was fractionated in a sedimentation velocity gradient, and fractions containing prion aggregates of different molecular weight (MW) were intracerebrally inoculated into transgenic mice overexpressing elk PrP^{C} (tgElk). Brains were harvested upon the progression of terminal prion disease, and some were used for second passage into tgElk mice. Animals inoculated with low MW PrP^{Sc} exhibited symptoms of hyper-excitability, while those inoculated with high MW aggregates were lethargic. Mice inoculated with medium MW PrP^{Sc} had a decreased incubation time, while those inoculated with high MW aggregates had PrP^{Sc} that was more resistant to proteolytic digestion; however, these observations were only present in the first passage. The sedimentation profiles of the prions markedly differ between animals inoculated with different molecular aggregates, but these differences were not sustained upon subsequent passaging. Similar vacuolation profiles but variations in PrP^{Sc} deposits were observed across the brain samples inoculated through histopathological analyses. Our study demonstrates that inoculation of different PrP^{Sc} aggregates separated by molecular weight can lead to differing pathogenesis that is retained upon passaging, along with transient changes to the biochemical characteristics of prions.

Keywords: Prion, Strain, Aggregation, Infection, Protein

15. Hsp110 modifies prion infection *in* vitro and *in vivo*

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Prion diseases are fatal transmissible neurodegenerative disorders affecting humans and wild or domesticated animals. Molecularly, they are caused by a misfolding of the cellular prion protein (PrP^{C}) into a highly toxic variant termed PrP^{Sc}. Over time, PrP^{Sc} aggregates into fibrils which are fragmented to recruit and convert further PrP^{C}. Even though this process is essential to the replication of PrP^{Sc}, the molecular players involved remain unknown. Based on reports showing that the molecular chaperone Hsp110 forms part of a mammalian disaggregation machinery, we hypothesize that Hsp110 is necessary for the fragmentation of PrP^{Sc}.

To test this, Hsp110 levels were manipulated in 22L-prion infected mouse neuroblastoma (N2a) cells: Transient knock-down was found to reduce PrP^{Sc} levels, while transient overexpression increased PrP^{Sc} in a dose-dependent manner. Knockout of Hsp110 by CRISPR/Cas9 reduced the susceptibility of N2a cells to prion infection.

Next, mice overexpressing Hsp110 were inoculated with the 22L and Me7 prion strains. Hsp110 overexpression significantly prolonged the survival of Me7- but not 22L-inoculated animals. To test whether the prolongation of survival occurred due to an altered propagation of PrP^{Sc}, the biochemical features of PrP^{Sc} were examined. No difference was found between PrP^{Sc} in wild-type versus overexpressing animals, suggesting that Hsp110 overexpression may have been protective independently of PrP^{Sc}.

Overall, our *in vitro* studies are consistent with a role of Hsp110 in PrP^{Sc} fragmentation, but our *in vivo* work is inconclusive in this regard. We, therefore, propose further research aimed at exploring prion fragmentation and the role of Hsp110 in prion infection.

Keywords: Prion disease, Neurodegeneration, Molecular chaperones

16. Creating curated databases for different rDNA cistron markers of nematodes to support more flexible and customized nemabiome metabarcoding

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Presenting author: Eleonore Charrier (Ph.D. Candidate)

We previously developed short-read nemabiome metabarcoding, using the rDNA internal transcribed spacer 2 (ITS-2) region for large-scale relative quantitation of nematode species diversity. ITS-2 provides excellent phylogenetic resolution for the most important nematode species within the order Strongylida making this a powerful approach for gastrointestinal parasite communities of domestic grazing ruminant (sheep, cattle, horses, bison). However, this marker has limitations for providing comprehensive coverage of all nematode species present, resolving of closely related species and more distantly related taxa. This is important when applying metabarcoding to host groups with limited information of the likely species present, and to free-living nematode communities. Consequently, we are developing a more modular approach by incorporating different markers providing wider and more accurate phylogenetic resolution. The coding rDNA regions -18S, 5.8S and 28S- contain highly conserved domains generally used to resolve deeper phylogenetic relationships whereas the ITS-1/ITS-2 regions, are more rapidly evolving and used to resolve more shallow relationships. rDNA cistron reference sequences present in public databases are often partial, and sometimes misannotated, thus metabarcoding assays need to be supported, with well curated, and regularly updated bespoke databases. We previously developed a nematode ITS-2 rDNA database using the markerDB pipeline supporting ITS-2 rDNA nemabiome metabarcoding (Workentine et al, 2020). We are now producing a set of similar databases for different regions of the rDNA cistron (SSU, LSU, ITS-1-5.8S-ITS-2 regions and the entire rDNA cistron). This modular set of rDNA marker databases will support more flexible and customized metabarcoding including long-read and short-read metabarcoding assays.

Keywords: Nemabiome Metabarcoding, rDNA cistron, Databases

17. Mastitis-related antimicrobial use: Current practices on Canadian dairy farms

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Antimicrobial use is one of the main drivers for antimicrobial resistance. In the dairy industry, the majority of antimicrobials are used to prevent and/or treat clinical mastitis (CM). Reducing antimicrobial use by treating CM cases based on a selection protocol, whereby only cases caused by Gram-positive bacteria are being treated, is not very common. Dairy producers (n = 146) in 5 Canadian provinces (British Colombia, Alberta, Ontario, Quebec and Nova Scotia) were asked to indicate on a scale from 'Very Important' to 'Not Important', which factors guided their decision to treat CM cases. 59% of producers used a selective treatment protocol. Cow production was mentioned as 'Very Important' or 'Important' by 42% of producers with a selective CM treatment protocol. The need to fill quota was only indicated as 'Very Important' or 'Important' by 18% of the producers. Cow age, genetics and cull and replace costs were not often taken into consideration (only mentioned as 'Very Important' or 'Important' by 23, 20 and 7% of producers, respectively). Confirmed or suspected bacteria was listed as 'Very Important' or 'Important' by 66% of producers who selectively treated CM. In conclusion, a large proportion of dairy producers selectively treat CM cases and use criteria to make informed treatment decisions. Determining current practices has been an important step in understanding CM treatment decisions and has highlighted the potential to reduce antimicrobial use on dairy farms.

Keywords: Dairy, mastitis, antimicrobial use, antimicrobial resistance, behaviour

18. Hic1 inactivation improves skin regeneration

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Presenting author: Elodie Labit, Post doc

After severe injury or burn, adult mammalian wound healing occurs by formation of fibrotic scar, loss of skin appendages and permanent functional impairment. In mice, severe skin injuries exhibit partial regeneration, including formation of new hair follicles (HFs) and adipogenesis in the center of the wound. Hic1 (Hypermythylated in Cancer 1)-lineage cells are actively recruited into wounds where they reconstitute the neodermis, including the inductive mesenchyme of neogenic HFs, and surrounding adipocytes (Abbasi et al, Cell Stem Cell).To understand the functional contribution of Hic1 during skin regeneration, we performed lineage tracing following injury using aSMAcre tdTomato:Hic1$^{flox/flox}$ mice which enables tamoxifen-mediated deletion of Hic1, exclusively in activated, wound-responsive fibroblasts. Contrary to the results observed in muscle tissues, our results show that conditional loss of Hic1 caused a marked improvement in skin regeneration compared to Hic1$^{wt/wt}$ mice (both receiving tamoxifen). Hic1-deficient mice had twice as many neogenic HFs ($p<0.05$). Interestingly, this enhanced regeneration was associated with an increase of aSMA+ fibroblast density within the central regenerative zone, but not within the peripheral fibrotic scarring zone. This is the consequence of the increase of BrdU+ aSMA+ cells, 12 days after wounding, in Hic1 depleted mice in comparison with the control mice. Our work highlights that an early, post injury and transient depletion of Hic1 is sufficient to significantly improve skin regeneration. Ongoing scRNAseq studies are examining how the increase of proliferation in the activated fibroblasts improves their regenerative fate.

Keywords: Skin regeneration, Fibrosis, Fibroblasts, scRNAseq

19. Investigating the Effects of CSF1R Inhibition on Skin Regeneration

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After injury, mammalian skin heals by producing fibrotic non-functional scar devoid of hair follicles (HFs). Interestingly, in mice, after a large open wound (LOW), regeneration of new HFs is observed in the center of the wound. CSF1 is a cytokine that acts via the CSF1R, expressed by monocytes and macrophages and stimulates proliferation and differentiation. The CSF1-CSF1R axis has been suggested to influence regenerative outcomes in the gut and liver. Here we asked whether CSF1 signaling influences skin regeneration. To test this, CSF1R inhibitor or control chow diets were given to mice for two time courses: 7 days before LOW until D0 (time of injury) (early) or 2 days before LOW until D5 post-injury (late).

Our results show that only late CSF1R inhibition changes wound healing outcomes. Indeed, CSF1R inhibition induced more rapid wound closure (D1, CSF1R inhibition: 1.37±0.15 cm^2, vehicle: 2.22±0.217 cm^2, $P<0.05$) and an increase in HF regeneration (CSF1R inhibition: 28.00±7.99, vehicle: 3.20±2.60, $P<0.05$). After validating the CSFR1-inhibitor mediated depletion of $F4/80^+$ macrophages in the skin by immunohistochemistry and FACS, we will perform scRNA-seq on macrophages and fibroblasts (cells responsible for HF regeneration) to evaluate: i) the diversity of macrophage populations and their state changes after CSF1R inhibition, and ii) how the connectome between macrophages and fibroblasts evolves with CSF1R inhibition. In the end, identification of a novel, macrophage-mediated, pathway responsible for activating regenerative skin fibroblasts (or suppressing scarring fibroblasts) could allow for new regenerative therapies to improve wound healing in humans and animals.

20. Studying rare occurrences: long-term phylogenetic monitoring of the emerging pathogen *Erysipelothrix rhusiopathiae* during mortality events

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Presenting: Fabien Mavrot (PDF)

In recent years, *Erysipelothrix rhusiopathiae*, a zoonotic bacterium with a wide range of host species, has emerged as a health concern in Arctic wildlife. It has been associated with abnormal mortalities and disease syndromes in various species. Rare occurrences, such as mortality events or the finding of a fresh carcass from a diseased animal, are difficult to study, more so in remote areas with scarce human presence.

Here, we present the results of an ongoing long-term passive monitoring of *E. rhusiopathiae* which started with an investigation of several muskox mortalities in the Arctic Archipelago in 2009-2013. This monitoring is sustained through collaboration with multiple partners and opportunistic sampling. It has allowed us to assemble a unique library of over 80 *E. rhusiopathiae* bacterial isolates from various free-ranging species ranging from Alaska to the south-eastern coast of Canada.

Using whole genome-sequencing and phylogenetic analyses, we map spatial and temporal relationships between the different stains of *E. rhusiopathiae*. An important finding was the isolation of a single strain on three neighboring islands in the Arctic Archipelago collected during separate muskox mortality events (from 2010 to 2017) and in four different species (muskox, Peary caribou, ringed seal, and Arctic fox). This is in contrast with other areas where strain diversity was higher, even during single mortality events.

Our results bring new insights into the epidemiology of *E. rhusiopathiae*, in particular regarding strain diversity and persistence in different regions, as well as possible differences in pathogenicity between strains and factors associated with infection.

Keywords: wildlife, disease surveillance, *Erysipelothrix rhusiopathiae*, phylogenetics, mortality events

21. Hair Trace Minerals as an Emerging Health Indicator within the Bluenose-East Herd and Other Caribou Designatable Units

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Presenter Status: MSc Student

The Bluenose-East (BNE) caribou herd is a distinct population of barren-ground caribou that has experienced a significant population decline (122,000 to 19,000 individuals) in the last decade. As part of an overall health assessment of this herd, we determined the hair trace mineral and heavy metal status of harvested animals from 2017-2019. Trace minerals are elements occurring in the body at extremely low concentrations, and many are essential for normal physiological function. They may be used as a proxy indicator of nutritional status in wildlife, with sufficient evidence that deficiencies and or excesses have potential negative effects on health, while heavy metals are elements with potentially toxic effects at threshold concentrations. The objective of this research was to determine the trace mineral and heavy metal concentrations in hair of the BNE herd and to compare these results to and among caribou designatable units. Eighty-six hair samples obtained through hunter-collected sampling kits from Kugluktuk, Nunavut, Canada between 2017-2019 were analyzed for trace minerals. Hair trace mineral concentrations were determined through plasma mass spectrometry at the Alberta Centre for Toxicology. Elements assessed were iron, copper, zinc, selenium, molybdenum, cadmium, and lead. We evaluated differences in concentrations between sex, year and age class within the BNE herd and compared our findings to two other caribou designatable unit populations, the Dolphin and Union caribou herd and Northern Mountain caribou. Developing standardized hair trace mineral reference values for caribou is the first step to determining if hair trace minerals are a sensitive health monitoring tool.

Keywords: Bluenose-East Caribou, Health, Trace-minerals, Community-Sampling, Nutrition, Heavy metals

22. Assessing the impacts of age and duration of maternal separation during spring processing on behavioural indicators of stress in beef calves

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Spring processing requires restraining animals and can involve castration, branding, and vaccination, which can cause stress and pain and therefore negatively impact animal welfare. However, evidence-based guidelines regarding age at processing and duration of maternal separation during processing are lacking. This study aims to quantify the impact of age and duration of maternal separation on the welfare of calves at processing. A total of 92 calves were enrolled in a two-by-two factorial study with two age groups (four- and eight-weeks of age), and two separation durations (two- and six-hours of separation). Behaviours were quantified before and after processing to determine the impact of processing on calf welfare. To assess post processing behaviour, ANOVA's and post-hoc analyses were used with age and separation duration as the main effects, an age-separation duration interaction, and pre-processing behaviour, and processing day as covariates. Calves in the short separation group spent significantly more time standing (P=0.006), less time lying (P=0.004), and had a higher foot stomp rate (P=0.002) than calves in the long separation group. This suggests a brief separation from the dam is more stressful than a long separation; however, the decrease in activity level by the long separation group could be attributed to exhaustion rather than decreased stress. This is the first study, to investigate the interaction between the age and duration of maternal separation on calf processing stress. Understanding the impact of these variables can guide better recommendations regarding the timing of spring processing and improve calf welfare.

Keywords; Stress, Maternal Separation, Age, Welfare, Behaviour

23. Effect of Oxidative Stress on Developmental Competence of In Vitro-Produced Embryos

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Recapitulating physiological conditions during *in vitro* culture is critical to the success of assisted reproductive technologies. We previously observed murine embryo culture under reduced (5 or 7%) oxygen concentrations significantly increased percentage blastocyst production and total cell number relative to those cultured under atmospheric O_2. However, the underlying physiological responses contributing to these observations remain poorly understood. In collaboration with the Regional Fertility Program, our objective was to further characterize the molecular and cellular responses of *in vitro*-cultured embryos under 5 versus 2% O_2 relative to *in vivo*-derived blastocysts. We hypothesized that culturing zygotes under higher O_2 imparts increased oxidative stress and compromises viability through eliciting apoptosis, increasing production of reactive oxygen species (ROS), and altering gene expression. Murine and human zygotes were cultured to blastocysts under 2% or 5% O_2. Relative expressions of select oxidative stress-related and imprinted genes were quantified through RT-qPCR, and murine *in vitro*-blastocysts were compared to *in vivo*-derived embryos. ROS production was assessed in human blastocysts through confocal microscopy with a fluorescent probe for hydrogen peroxide (H_2DCFDA). Apoptotic responses were evaluated using an immunofluorescence assay for caspase-3. Preliminary results suggest differential antioxidant, apoptotic, and imprinted gene expression between embryos cultured under 5 versus 2% O_2, and between *in vivo*- and *in vitro*-culture. We also observed a trend toward increased H_2O_2 and decreased caspase-3 in embryos cultured under 5 versus 2% O_2. These initial findings demonstrate the need for optimizing O_2 concentrations to narrow existing transcriptomic and epigenetic disparities between *in vitro*- and *in vivo*-produced embryos.

Keywords: Embryo culture; Oxidative stress, ARTs, Blastocyst

24. Occurrence of Virulence Genes and Antibiotic-Resistant *Escherichia coli* in Stormwater from Urban Retention Ponds

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Stormwater, an alternative water source, is frequently contaminated with fecal waste and may contain human pathogens such as intestinal pathogenic *Escherichia coli* (InPEC) and extraintestinal pathogenic *Escherichia coli* (ExPEC), causative agents of gastroenteritis and urinary tract infection, respectively. This study aims to evaluate the occurrence of InPEC and ExPEC associated virulence genes and antibiotic resistance of *Escherichia coli,* including virulence gene-positive isolates, in stormwater samples (n=83) collected from retention ponds. *Escherichia coli* will be isolated using selective growth media and analyzed for virulence genes - eaeA, ehxA, LT1, ST1, ipaH, bfp, aggR, papC, sfa/foc, fimH, hlyA, cnf1, fuyA, iroN, iutA and ibeA - using a multiplex PCR assay (mPCR). Moreover, all isolates will be screened against several classes of antibiotics, such as aminoglycoside, cephalosporin, and tetracycline, via antimicrobial susceptibility testing using the Sensititre platform. As previously reported from various studies, we believe InPEC and ExPEC virulence genes will be relatively prevalent in stormwater. We expect to detect virulence gene-positive isolates resistant to one or more classes of antibiotics as the virulence and resistance genes are commonly acquired collectively via mobile genetic elements. Furthermore, we expect to observe a population level of tetracycline-resistance *E. coli* as its extensive use in agricultural and clinical settings has driven widespread resistance worldwide. The results obtained from this study will provide an insight into the microbial quality of stormwater, contributing to the development of effective strategies for microbial management and prevent potential public health risks associated with stormwater reuse.

Keywords: public health risks, stormwater, virulence gene, antibiotic-resistance, pathogenic *Escherichia coli*.

25. Control of *Escherichia coli* O157:H7 through the use of lytic bacteriophage cocktails

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E. coli O157:H7 (EC) is a pathogenic bacterium that causes significant public health and economic issues. Interest is increasing into research on developing bacteriophage cocktails against bacteria to reduce development of anti-phage mutants, however, phage interference can compromise the effectiveness of this biocontrol strategy. Previous work has indicated that lytic activity of individual phage does not necessarily reflect their activity in cocktails.

Individual phage (T5, T1, T4, and rV5) and cocktails of phage (Cocktail A: T1+T4+rV5, Cocktail B: T1+T4+rV5+T5) were evaluated for biocontrol against EC EDL933. EC was incubated with phage treatments at a final MOI of 1000, for 24h at 37°C. OD600 was measured over 24h, and culture samples were taken at 7, 19, and 24h PI for bacterial and phage enumeration, using serially diluted plates and soft agar enumeration.

Collected data indicated that T5 alone had the highest lytic activity against this strain of EC, with an initial 7-log reduction in CFU/ml. The phage treatments in decreasing order of efficacy according to CFU/ml bacterial counts at 7, 19, and 24h respectively were T5, Cocktail B, Cocktail A, T4, rV5, and finally T1. Phage concentration did not necessarily reflect high lytic activity.

Our preliminary data supports previous research indicating that the lytic activity of individual phages does not always reflect their activity within phage cocktails. Consequently, phage cocktails are not necessarily more effective at lysis than individual phages. While the use of phage cocktails may reduce the development of host resistance, additional research is required to evaluate phage interference.

Keywords: bacteriophage, *E. coli*, biocontrol, phage therapy, virology

26. ArlRS Two-Component System of *Staphylococcus aureus* is required for Intracellular Bacterial Replication in Kupffer Cells

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Staphylococcus aureus (*S. aureus*) bloodstream infections cause high morbidity and mortality. Out of all organs, the liver possesses the biggest barrier function of bloodstream infections. Liver-resident macrophages, Kupffer cells (KCs), are strategically located within the sinusoids to catch and eliminate bacteria from the bloodstream. However, a small proportion of *S. aureus* is able to survive within KCs and can start replicating. Over time, bacterial numbers are increased, and the bacteria can lyse KCs to disseminate to other organs in the body, leading to sepsis. So far, it is unclear how intracellular replication by *S. aureus* is initiated. In this study, we demonstrated by using spinning-disc confocal intravital microscopy that the *S. aureus* two-component system (TCS) ArlRS was required for intracellular replication inside KCs. When bacteria were genetically deprived of ArlRS, no replication occurred inside KCs. Complemented knockout strains showed a similar phenotype as wildtype bacterial strains. Moreover, in the absence of the direct downstream protein of ArlRS, MgrA, a similar phenotype was observed. Mutant ArlRS bacteria showed increased survival of infected mice, and livers of these mice present fewer lesions. Finally, intracellular replication by *S. aureus* was independent of ROS- or protease-mediated killing. We conclude that ArlRS recognizes a, yet unknown, signal to initiate intracellular replication. These results are the next step into elucidating the mechanism behind intracellular replication of *S. aureus* during bloodstream infections. Understanding this mechanism could lead to better treatment options for bloodstream *S. aureus* infections.

27. Effects of chronic morphine exposure on Oligodendrocytes

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Opioids remain an important analgesic in modern medicine despite their side effects. Alterations in the white matter of chronic opioid users have been linked to myelin pathology and cognitive deficits. Oligodendrocytes, the myelinating cells of the central nervous system (CNS), are a heterogeneous group of cells that exist at different maturation states in the adult CNS and are known to express opioid receptors. However, little is known about the effects of opioids on oligodendroglia. To better understand these effects, we have established Oligodendrocyte progenitor cells (OPCs) primary cultures as an *in vitro* model. OPCs are immature oligodendrocytes that can divide and differentiate into myelinating oligodendrocytes in response to damage or external stimuli. Upon chronic morphine treatment, we have observed an increase of μ opioid receptor and myelin-related factors at the transcriptional level, while viability was not affected in OPC cultures. These data suggest an inducing-maturation effect of morphine on this cell type *in vitro*. Oligodendrocyte maturation is accompanied by extensive membrane changes and lipid synthesis. Further experiments will focus on understanding how morphine modulates these changes and its potential effect on lipid metabolism.

Keywords: Opioids, OPCs, myelin

28. Comparison of Antibody Presence Between Preconditioned and Traditionally Raised Cattle During the First 40 Days on a Feedlot

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Bovine Respiratory Disease (BRD) is a multifactorial disease and the leading cause of morbidity and mortality on feedlots. In many instances, metaphylactic administration of antimicrobials is used to both treat and prevent BRD. However, due to increasing antimicrobial resistance, this practice is becoming less sustainable. Therefore, methods such as preconditioning are being explored to reduce BRD incidence and the associated antimicrobial use. Preconditioning includes aspects such as two-stage weaning and a strategic vaccination schedule to prepare cattle for life on the feedlot. This study aimed to demonstrate a higher proportion of preconditioned animals would arrive on the feedlot with antibodies present than their traditionally raised counterparts. The vaccination protocol was evaluated by collecting serum samples from preconditioned calves at spring processing, weaning, arrival on the feedlot, 20 days after arrival, and 40 days after arrival. While traditionally raised cattle had serum collected at the last three time points. Enzyme-linked immunosorbent assays were used to detect antibodies against five viral BRD associated pathogens: Bovine Viral Diarrhoea Virus, Infectious Bovine Rhinotracheitis, Bovine Parainfluenza Virus Type 3, Bovine Respiratory Syncytial Virus, and Bovine Coronavirus (BoCoV). All viruses had antibodies present upon arrival at the feedlot. Except for BoCoV (stayed consistently high), all virus antibody titres increased during the remaining 40 days. The proportion of preconditioned calves with antibodies was higher than traditionally raised calves at arrival. This data shows that using this preconditioning protocol, preconditioned calves have, on average, a higher level of antibodies at the moment they are moved to the feedlot.

Keywords: Bovine Respiratory Disease, Cattle, Preconditioning, Vaccination, and ELISA

29. Human skin and nerve derived Schwann cells exhibit subtle transcriptomic and functional differences

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Schwann cells (SCs) support peripheral nerve regeneration, but the efficacy of this regeneration is limited. Introducing exogenous SCs to the site of peripheral nerve injury is an ongoing avenue of investigation as a cellular therapy to enhance endogenous repair. Approaches that utilize an accessible source of a patient's own cells would greatly facilitate clinical translation. Previous work demonstrates that skin-derived SCs are able to promote axonal growth and remyelination in murine models of nerve injury, however, it is not clear whether skin-derived and nerve-derived SC are functionally equivalent. To this end, we isolated SCs from small skin samples and then subjected them to high resolution single-cell mRNA sequencing (scRNA-seq) followed by battery of in vitro and in vivo assays. Transcriptomic analyses revealed ~95% similarity in gene expression between skin and nerve SCs, and gene regulatory network analysis showed similar overlap with the exception of immune regulatory family (eg. IRF) which was enriched in skin SCs. Analysis of secreted cytokines showed significantly higher expression of VEGF and collagen content in skin SCs; and TGF-alpha in nerve SC. *In vitro* assays revealed similarity in proliferation, migration and association with axons. Overall, our results showed that skin and nerve Schwann cells share mostly identical properties with subtle differences, but also highlight some inherent differences driven by tissue-specific functional demands. Our data further support skin as a viable source of Schwann cells to improve nervous system repair.

Keywords: Schwann cells, Nerve regeneration, Single-cell RNA sequencing

30. Efficacy of bacteriophage mediated colibacillosis control in laying hens

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, a poultry disease which results in significant losses to the Canadian egg-industry. Laying hen colibacillosis in Canada cannot be treated with antibiotics due to Canadian Food Inspection Agency regulations, necessitating novel APEC control strategies. Phage biocontrol has been successful in reducing APEC infection in broiler chickens, and we hypothesized that suitable phages would be effective in treating colibacillosis in a laying hen model. The study objectives were to isolate and characterize anti-APEC phages for use in a laying hen model. Hen fecal and human sewage water samples were processed to isolate phages targeting clinical APEC strains, and the host range and lytic activity of seven isolated phages were determined using microplate phage virulence assays. Phages were subjected to transmission electron microscopy to determine their morphology and pH tolerance assays were conducted to determine pH stability. Phage genomes were sequenced using the Illumina MiSeq platform, and annotated to predict protein function. Four of seven phages possessed strong activity against multiple APEC strains and phages were stable between pH = 3.5 and 9.0, with reduced survivability at pH = 2.5. Whole-genome sequencing data was generated for all seven phages indicating that there are four phages of the *Myoviridae* (*Felixounavirus*: 2; *Phapecoctavirus*:1; *Tequatrovirus*: 1), one of the *Autographiviridae* (*Teseptimavirus*), and two of the *Siphoviridae* families (*Sashavirus* and unclassified). These phages showed promise for controlling *in vivo* APEC infection, possessing high stability, strong lytic activity, and no genes encoding virulence traits or antimicrobial resistance.

Keywords: Bacteriophage, colibacillosis, biocontrol, genomics

31. Factors influencing the prevalence of Antimicrobial Resistant *Enterococcus* spp. in the Canadian Beef Cattle System: A Scoping Review to inform an Integrated Assessment Model

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Background. Antimicrobial resistance (AMR) occurs when bacteria change in a way that antimicrobials are no longer effective against it. The epidemiology of AMR and its transmission is complex, and involves antimicrobial use in human, animal, and environmental health. This research intends to examine the specific pathways between beef cattle and humans in Canada, and explore factors associated with resistance within beef production, using En*terococcus* spp. as an indicator.

Aim: The authors will identify measurable factors associated with an increase or decrease of AMR enterococci in the Canadian Beef Industry and reflect them in an integrated assessment model (IAM).

Method: PRISMA guidelines were followed in conducting a double-blinded scoping review. Five databases were searched for articles meeting seven inclusion/exclusion criteria, including reporting AMR enterococci odds ratio or prevalence following an intervention (N = 19). Authors extracted results and study design details in a standardized iAM.AMR database.

Results: Preliminary results found studies focused on macrolide use (n = 7), antibiotic free labelling (n = 5), copper supplementation (n = 3), third generation cephalosporins use (n = 1), tetracycline use (n = 1), grass fed diets (n = 1) and streptogramin use (n = 1).

Anticipated Conclusions: Interventions from the scoping review will populate the IAM. This project is a component of the national iAM.AMR initiative, which uses IAMs to examine AMR risks to humans and animals in the Canadian agri-food system. The model intends to provide a transdisciplinary platform in considering AMR and its intersection between human and animal health.

Keywords: Antimicrobial Resistance, One Health, Beef Cattle, Modelling, Scoping Review

32. Knowledge gaps in the understanding of antimicrobial resistance in Canada

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Current limitations in the understanding and control of antimicrobial resistance (AMR) in Canada are described in a comprehensive review. Three important areas were identified for review, including: (1) treatment optimization, (2) surveillance of antimicrobial use (AMU) and AMR, and (3) prevention of transmission of AMR. Without highlighting current gaps that exist in identified areas, sustained progress in AMR mitigation efforts cannot be made. Current AMR surveillance efforts in Canada are summarized and the need for increasing environmental consideration becomes clear. Using Canada as an example, this review emphasizes the importance and necessity of a One Health approach for AMR research and policy. Specifically, AMU in the human, animals, and environmental sectors cannot be thought of as separate actions, and a One Health approach in AMR understanding and current surveillance efforts is required. Judicious AMU is required across all sectors; however, this must be supported by research into best AMU reduction practices, understanding and supporting behavioural changes, economic considerations,

and increasing public knowledge. Overall, the presented evidence and knowledge gaps underscore the necessity for AMR policy decisions to be considered in a One Health framework, while also calling attention to the current issues that need to be addressed for realistic and meaningful progress to be made.

Keywords: Antimicrobial resistance, One Health, Stewardship, Surveillance

33. Sensing of intracellular Hcp Levels controls T6SS expression in *Vibrio cholerae*

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The type 6 secretion system (T6SS) is a bacterial weapon broadly distributed in Gram-negative bacteria and used to kill competitors and predators. Featuring a long and double-tubular structure, this molecular machine is energetically costly to produce and thus is likely subject to diverse regulation strategies that are largely ill defined. In this study, we report a novel quantity-sensing control of the T6SS that down-regulates the expression of secreted components when they accumulate in the cytosol due to T6SS inactivation. Using *Vibrio cholerae* strains that constitutively express an active T6SS, we demonstrate that mRNA levels of secreted components, including the inner-tube protein component Hcp, were down-regulated in T6SS structural gene mutants while expression of the main structural genes remained unchanged. Deletion of both *hcp* gene copies restored expression from their promoters while Hcp overexpression negatively impacted expression. We show that Hcp directly interacts with the RpoN-dependent T6SS regulator VasH and deleting the N-terminal regulator domain of VasH abolishes this interaction as well as the expression difference of *hcp* operons between T6SS-active and inactive strains. We find that negative regulation of *hcp* also occurs in other *V. cholerae* strains and the pathogens *Aeromonas dhakensis* and *Pseudomonas aeruginosa*. This Hcp-dependent sensing control is likely an important energy-conserving mechanism that enables T6SS encoding organisms to quickly adjust T6SS expression and prevent wasteful build-up of its major secreted components in the absence of their efficient export out of the bacterial cell.

Keywords: *Vibrio cholerae*, type 6 secretion system, regulation

34. Valuing wood bison, their health, and their landscape: Integrating values and valuation

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A large portion of the wood bison (*Bison bison athabascae*) population resides in and around Wood Buffalo National Park (WBNP) and has been infected with bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*) for nearly a century. Disease management efforts have focused on bison removal and resulted in public backlash. Alternative bison health management strategies, such as a vaccination program, have not been well evaluated. Our research addresses people's willingness to pay (WTP; contingent valuation) for various bison health management strategies. Other WTP research has related management preferences to an individual's background or demographics. Our novel work expands this relationship by integrating how someone thinks about bison and the landscape (i.e., underlying values, valued attributes) into WTP. We expect a person's background to inform their nature-related underlying values; for instance, a cattle producer's underlying values may be dominated by the use of natural areas whereas an environmentalist's underlying values may be dominated by nature having intrinsic or non-use value. An individual's underlying values then influence which features of the landscape are important and valued (e.g., large bison populations, ecological relationships, disease-free status). Alternative bison health management strategies will affect these valued features differently. A person's beliefs about these effects are expected to inform their management preferences, including their WTP. Understanding the relationship between a person's underlying values, valued features, and WTP allows managers to tailor bison health messaging and to predict which health management strategies will have stakeholder support and involvement.

Keywords: bison, wildlife, disease reservoir, valuation, management

35. Effective Johne's disease control using phage technology

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Johne's disease (JD) is an infectious chronic enteritis characterized by diarrhea, loss of body condition, and lower productivity in ruminants. Studies reported shedding of calves and transmission of MAP between MAP-positive and negative calves. However, calves are not included in JD control programs due to the low sensitivity of diagnostic tests in this group of animals. Currently, MAP culture and qPCR are used, each with their own challenges to be implemented in control strategies. We explored phage technology to be implemented in JD research to detect MAP-infected animals in early stage of infection. Laboratory validation of phage technology with blood samples spiked with MAP culture in different concentrations achieved a limit of detection of 10 MAP cells/ml. We now aim to evaluate the sensitivity and specificity of the phage technology on samples from naturally MAP-infected calves to improve results of disease control programs. The study will be implemented on 10 Alberta dairy farms with JD history. Blood samples for the phage assay and fecal samples for qPCR analysis will be collected every two mo from all female young stock (≤12 mo) to determine the accuracy of the new test to detect MAP-positive calves. Results from this study has the potential to impact the current JD control programs, as the significant accuracy of the phage technology will enable producers to detect MAP-positive animals, separate and remove them early in the course of the disease. Consequently, this will prevent production losses associated with MAP-positive animals and support reduction of within-herd MAP prevalence.

Keywords: Johne's disease, paratuberculosis, early detection, phage technology

36. Herd health and production management visits on Canadian dairy cattle farms: structure, goals and topics discussed

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Regular veterinary visits to improve herd health and production management (HH&PM) provide opportunities for constructive veterinarian-farmer conversations and to direct management to proactively optimize animal health and welfare. However, little is known about the structure of HH&PM visits. In this study, our aims were to: describe HH&PM farm visit structure; determine discussed dairy-specific topics; and assess whether the focus of visits aligned with farmers' priorities. Audio-video recordings of 70 HH&PM farm visits by 14 Canadian veterinary practitioners were analyzed.

Consistent with farmers' priorities, the focus of visits was cow fertility; however, dairy-specific discussions were generally relatively infrequent, with only 17% of the HH&PM visit duration spent discussing dairy-specific topics, and short, lasting an average of 2 minutes. Veterinarians raised topics related to the whole herd more often than farmers. Most frequent topics included cow fertility, udder health, calf health/management, and transition diseases. However, answers to an open-ended question revealed that additional aims of many farmers were to receive information, have questions answered, and identify and discuss problems. A farmer's belief that HH&PM farm visits were 'absolutely' tailored toward their goals was positively associated with number of discussions per visit and their conviction that they 'always' voiced their wishes and needs.

In conclusion, opportunities to broaden the focus of HH&PM farm visits and improve veterinarian-farmer communication should be identified and veterinarians trained accordingly. As a result of specific training, veterinarians might become better equipped to add further value in HH&PM farm visits.

Keywords: dairy cattle, herd health management, veterinary communication, farm advisory

37. A single amino acid polymorphism within the cervid prion protein alters chronic wasting disease pathogenesis

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Chronic wasting disease (CWD) in cervids is caused by prions, the infectious agents of transmissible spongiform encephalopathies. Prions consist of misfolded isoforms of the endogenous prion protein (PrP^C) abundantly present in the brain. PrP^C molecules are converted into prions upon contact, which then aggregates, leading to neurodegeneration and ultimately death. Many factors contribute to successful prion propagation, including single amino acid (aa) polymorphisms within the prion protein (PrP) sequence. A polymorphism from wild-type serine to asparagine at aa 138 is present in Canadian caribou populations. Studies show limited prion accumulation in the brains of 138SN and 138NN reindeer (i.e., caribou) orally infected with CWD prions, and fallow deer (wild-type 138NN) are fully resistant to natural prion infection. Thus, I hypothesized that this polymorphism alters CWD pathogenesis. First, I demonstrated that 138NN PrP^C is poorly converted into prions *in vitro*, a mechanism that possibly explains the potential resistance of fallow deer to CWD. Next, I infected gene-targeted mice expressing 138SS and 138NN cervid PrP^C with CWD-positive tissue homogenates. Mice expressing 138NN cervid PrP^C did not develop clinical signs nor harbored detectable proteinase-K resistant prions in the brain, as revealed by western blotting, while their 138SS counterparts successfully propagated prions and succumbed to the disease. Interestingly, extraneural prions were detected in both mouse lines, suggesting asymptomatic 138NN animals may still shed prions. Although CWD have not been reported in caribou so far, determining the susceptibility of these endangered and culturally important species to CWD is important for prevention and future control measures.

Keywords: prion protein, polymorphism, chronic wasting disease, pathogenesis, transgenic mice

38. Understanding farmer behavior and their decision-making process in the context of cattle diseases: A review of theories and approaches

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Understanding farmer behavior is essential to successfully implement behavior change interventions that improve the uptake of best practices. A literature review was conducted to identify the theoretical underpinnings and analytical methodologies that have been used, and the key behavioral determinants described to understand farmer behavior in disease control and prevention on cattle farms. Studies conducted in 27 countries worldwide were reported, resulting in 166 peer-reviewed manuscripts. An increasing trend in understanding farmer behavior has been identified; however, no similar trend was apparent in the application of appropriate social science methods. Generally, the reviewed studies lacked theoretical frameworks (58%), or the Theory of Planned Behavior was used (14%) despite criticisms and limited explanatory power. The complexity of farmer behavior was illustrated by mapping key constructs in behavior change frameworks. Mainly personal constructs and the farmer-veterinarian relationship were described, but it cannot be concluded that these factors are key. Presumably, constructs belonging to the interpersonal and contextual environment were not studied.

Explicit theory can help identify influences on behavior change, understand behavioral mechanisms and shape intervention strategies. The focus has mainly been on identifying influences on behavior, and not on behavioral change. That theoretical underpinning is often missing may indicate a lack of understanding on how to design behavioral studies, resulting in an overrepresentation of similar studies. For sustainable behavior change, studies should include wider constructs. Moreover, using coherent frameworks, researchers can link the constructs to interventions, and thereby take the first step towards theory-driven, evidence-based interventions to influence farmer behavior.

Keywords: Infectious disease, behavioral determinants, farmers, veterinarians, cattle.

39. Exploring PDGFRα Signaling in Sertoli Cell Primary Cilia and Seminiferous Tubule Morphogenesis

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Primary cilia, important to cell signaling, are present on testicular Sertoli cells and their numbers decrease as the testis matures, suggesting ciliary signaling is important in testes morphogenesis. PDGFRα signaling is regulated through the cilia and is important to migration and adhesion in some other cells. We hypothesize PDGFRα in Sertoli cells is necessary to seminiferous tubule formation by signaling the cells to migrate towards or adhere to one another. Using porcine testes as a model, we investigated Sertoli cells' ciliary response to extracellular signals. Isolated and cultured cells were exposed to 50mM LiCl for 24h. Cilia immunocytochemistry and measurements indicated exposure to LiCl increased their length (3.58±2.58μm in treated cells, 2.69 ±1.68μm in control cells, mean ±SD; n=3; $P<0.05$). We performed a scratch assay, which showed the Sertoli cells migrated 4μm/h towards the 700μm scratch created on a cell monolayer. These confirm porcine immature Sertoli cells, which are migratory in-vitro, have cilia functional and responsive to extracellular signals. Next steps will employ CRISPR/Cas9-mediated gene editing and western blots to study cilia ablation's effect on PDGFRα signaling. Migration assays and in-vitro testicular organoids of control and PDGFRα-inhibited cells will be used to study the PDGFRα's effect on cell migration, tubule formation, and extracellular matrix deposition. To broaden our knowledge on ciliary enriched signaling pathways, proteomics can be performed on cilia isolated through sheer force. We expect that results from these experiments will shed light on the signaling pathways mediating the role of primary cilia in testicular tubule morphogenesis.

Keywords: PDGFR alpha, Primary Cilia, Sertoli cells, Seminiferous tubules

40. Host age and environment describes variation in gastrointestinal parasite communities of Sable Island horses

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Mixed infection by multiple parasites species is common in natural and semi-natural (e.g., livestock) systems. However, there are critical knowledge gaps on the processes that drive variation in mixed infections due to a lack of tools able to characterise them. Using novel molecular techniques, we investigated mixed infections of gastrointestinal strongyle parasites in a feral horse population on Sable Island, Nova Scotia. First, we characterised mixed infections in Sable Island horses to identify general patterns of infection. Next, we tested the role of horse age, sex, median location, and band associations in describing the variation in mixed infections among Sable Island horses. Finally, we tested if parasite communities within individual horses vary in a season. Results suggest that Sable Island horses can be infected by up to 24 different strongyle species, with an average of 13 species infecting a single horse. There are clear age-structured patterns of mixed infections; while adults have infections dominated by large strongyles, foals and yearlings have infections dominated by small strongyles. Environmental factors such as horse median location and band associations also explain significant amounts of variation in mixed strongyle infections. We further confirm that mixed infections in individual horses appear to stay consistent within a season. Our findings indicate that variation in mixed infections are a consequence of multiple host and environmental processes, with an emphasis on age-structured patterns of parasite infection. These results highlight the need to consider host ecology when assessing parasite infection risk.

Keywords: Parasitology, Mixed Parasite Infections, Epidemiology, Nemabiome, Sable Island Horses

41. Pathogenicity of the Canadian Delmarva (DMV/1639) Infectious Bronchitis Virus (IBV) Infection in Chickens Leading to Reproductive Tract Abnormalities

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Infectious bronchitis virus (IBV) is a gammacoronavirus that causes economic losses in the poultry industry. IBV is a respiratory virus, but it spreads and results pathologies in the kidney, oviduct, and gastrointestinal tract. The severity of lesions and tissue tropisms are primarily determined by infecting IBV strains and the age of the chickens at infection. Recently, we had isolated and characterized an IBV DMV/1639 isolate that has been dominant in the poultry operations in Eastern Canada over few years. It is unknown if this IBV strain results reproductive tract abnormalities. We hypothesized that this IBV strain is pathogenic in young and adult chickens. The aim of this study was to investigate the pathogenicity of Canadian IBV DMV/1639 isolate to the reproductive tract of 1-day-old and 26-week-old chickens. Appropriate controls, which remained healthy throughout the study, were kept for each experiment. In 1-day-old inoculated chickens, signs of sneezing, snicking, and tracheal rales were observed. Virus shedding continued up to 21- and 105-days post-infection (dpi) via oropharyngeal and cloacal routes, respectively. At 16 weeks of age, during necropsy, cystic lesions with serous fluid accumulation were detected in the oviducts. In 26-week-old inoculated chickens, egg production dropped to 40% starting at 5 dpi. Gross lesions involving ovarian regression and decreased length of oviduct were detected. Microscopical examination showed sloughing of epithelium, mononuclear cell infiltrations, and degeneration of the glands in the oviducts. The study demonstrates that the Canadian DMV/1639 isolate can negatively impact the reproductive performance of chickens irrespective of age at exposure.

Keywords: Infectious bronchitis virus (IBV), cystic oviduct, egg production

42. Biofilm Formation of O157 And Non-O157 Shiga-Toxigenic *Escherichia coli* Isolates on Microplates and Stainless-steel Coupons

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Food-borne illnesses caused by Shiga-toxin producing *E. coli* (STEC) has been associated with severe clinical manifestations ranging from bloody diarrhoea to hemorrhagic colitis and hemolytic uremic syndrome. Formation of biofilms aids in virulence and environmental persistence , increasing public health concern. STEC biofilms can form on various surfaces in food processing environments, increasing the risk of food contamination, and likelihood of people acquiring STEC via the food chain. Biofilm recalcitrance to sanitization and biocides, increases the need for new and effective methods that will help eradicate them.. Bacteriophages (phages) are ubiquitous in nature, host- specific, and possess cell lytic abilities, endolysin and de-polymerase enzymes that works against biofilms, hence, making them a strong potential alternative to traditional antimicrobials. This study will investigate 20 STEC strains (6 -O157 and 9 Non-O157isolated from Canadian food sources between the years 2016-2018, for their biofilm forming potential, as well as their genetic relatedness by whole-genome sequencing. Microplate assay and coupons staining using crystal violet visualization, biofilm quantification and Scanning electron microscopy (SEM) will be employed to assess biofilm biomass after 24, 48 and 72 h at 22°C and for 168 h at 10°C. Subsequently, biocontrol will be utilized to investigate phage effect/s on biofilm formation at respective incubation times and temperatures. Preliminary data, shows increased biofilm formed over time on both surfaces for all isolates, of which 2 -O157 strains isolated from pasteurized dairy products and unprocessed raw meat shows strong biofilm formation; indicating great concerns due high virulence and pathogenicity.

43. Impact of ependymal cell metabolic perturbation on brain function

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Presenting Author: Nilesh Sharma (PhD Student)

Metabolic regulation is thought to be an important feature within stem cell niches. Recent work suggests that ependymal cells (ECs), that line the ventricular system of the brain, could be critical players in regulating the ventricular-subventricular zone (V-SVZ). ECs are multi-ciliated glial cells that are responsible for regulating the neural stem cell niche and propelling the cerebrospinal fluid. Transcriptional profiling of the adult SVZ niche showed that ECs are highly enriched in glucose transporter 1 (GLUT1) and leading us to hypothesize that ECs may regulate NSC behavior by modulating metabolism within the V-SVZ. To test this, I performed a conditional deletion of GLUT1 in adult ECs *in vivo* using aSMACreER^{T2}:ROSA^{TdTomato}:GLUT1$^{flox/flox}$ mice to delete GLUT1 in aSMA^{+} ECs. At 1-month post-GLUT1 deletion, an increase in overall proliferation (marked with Ki67) was observed within the V-SVZ niche. A sex dimorphic effect was observed on neurogenesis; with females displaying a reduction in the number of DCX^{+} neuroblasts, while males exhibited no change. Interestingly, this reduction was more pronounced in the anterior V-SVZ compared to posterior V-SVZ, suggesting sensitivity to GLUT1KO might be spatially dependent. There was also a marked increase in GFAP staining and an accumulation of lipid droplets within the V-SVZ post-GLUT1 deletion suggesting that disruption in glucose metabolism may perturb local lipid metabolism. Altogether, these results indicate that EC metabolism regulates the NSC niche and may play a broader role in maintaining brain homeostasis.

Keywords: Ependymal cells, Single Cell RNA Sequencing, Glut1, Metabolism, Neural stem cell niche

44. Identifying the neurodevelopmental differences of opioid withdrawal

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Stopping opioid medications can result in a debilitating withdrawal syndrome in chronic users. Opioid withdrawal can occur at all ages, but mechanistic understanding of this condition is predominantly derived from adult studies. We examined whether there are age-dependent differences in the behavioural phenotype and cellular indices of opioid withdrawal. We tested this by assessing the behavioural and cFos response (a surrogate marker for neuronal activation) to morphine withdrawal in C57BL/6J mice across key developmental stages-neonatal, adolescent, and adulthood. Mice in all age groups received escalating doses of morphine (10-50 mg/kg) over 5 days and withdrawal was precipitated by a single injection of the opioid receptor antagonist naloxone (2 mg/kg) two hours after the last morphine dose. In adult and adolescent mice, withdrawal behaviours were robust, with age-related differences in autonomic and somatic signs. In both groups, cFos expression was increased in spinally projecting neurons within the Periaqueductal Grey (PAG), Rostro-ventromedial Medulla (RVM), and Locus Coeruleus. Neonatal animals displayed both a distinct behavioural withdrawal and cFos expression profile. Notably, in young animals cFos expression was increased within the PAG and LC, but decreased in the RVM. In summary, naloxone challenge precipitated robust opioid withdrawal behaviours across all developmental stages with neonatal animals displaying differences in withdrawal behaviours and unique neuronal activation patterns within key brainstem regions.

Keywords: Neonatal opioid withdrawal; Neonatal abstinence syndrome; Neurodevelopment; Morphine

45. Prion infection is associated with reduced Rab7 activation and impaired vesicular trafficking

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Prion diseases are fatal and transmissible neurodegenerative diseases in which the cellular form of the prion protein 'PrP^{C}' misfolds into an infectious and aggregation-prone isoform termed PrP^{Sc} (prions). Infections, mutations in the gene encoding PrP^{C} as well as spontaneous misfolding can trigger the conversion of PrP^{C} into PrP^{Sc}. This results in the accumulation of PrP^{Sc} aggregates mainly in the neurons eventually leading to neuronal death. Among the cellular impairments observed in response to prion infection are elevated cholesterol levels and impaired lysosomal maturation and consequently, reduced PrP^{Sc} degradation. Rab7 is a protein critical for lysosomal maturation and vesicle trafficking including the transport of low-density lipoprotein (LDL). A significant reduction in the PrP^{Sc} levels was attained by the over-expression of a constitutively active mutant of Rab7 in persistently prion (22L)-infected neuronal cells. We also show that the amount of GTP-bound Rab7 is reduced in 22L-infected cerebellar granular cultures, indicating a defect in Rab7 activation. By deploying pulse-chase experiments with confocal microscopy, we observed impaired trafficking of LDL from early endosomes to the lysosomes which might lead to the defective feedback mechanism in the *denovo* cholesterogenic gene expression, that we observed in 22L prion infected neuronal cell lines. In summary, we conclude that a defect in Rab7 activation in prion-infected cells is causally linked to the impaired LDL transport and, as a consequence, elevated cholesterol levels. Hence, restoring the normal neuronal physiology by the over-expression of active Rab7 could be one promising remedy in prion diseases, for which currently no cure exists.

Keywords: Prion, Rab7, cholesterol

46. Economic impact of infectious bronchitis virus on Canadian poultry industry

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Introduction: Infectious bronchitis is a common, highly contagious, acute, and economically important viral disease of chickens caused by infectious bronchitis virus (IBV), a gammacoronavirus. It affects the respiratory system, reproductive organs, and kidney. Morbidity is 100% and mortality can go well up to 30% while egg production drops by up to 50% or more depending upon secondary infection by bacterial pathogens. Emerging IBV variants have led to outbreaks in vaccinated flocks due to lack of cross-protective immunity, which represents a concern for producers. We could not find a study of economic impact of IBV on the Canadian poultry industry.

Hypothesis/Objective: Vaccination to prevent IB in poultry layers has net positive economic benefits for Canadian poultry producers.

Methodology: A classical benefit-cost analysis approach was applied. We examined economic impact of IBV using three production categories under a range of IBV infection scenarios while considering possible control and response options. Initial analysis used secondary data (Agriculture and Agri-Food Canada); model relevance will be improved with primary data from Canadian poultry producers currently under collection.

Results: Preliminary results indicate high benefit-cost ratios (1.5-5.0) from adopting IBV vaccines, suggesting use of vaccines as a preventive strategy would be highly cost effective.

Conclusion: The study has clarified the value of vaccinating as a preventive or response strategy as well as part of a long-term biosecurity programme reducing potential losses from IBV. Understanding better the risk of economic losses will inform the development of timely and cost-effective disease control and preventive measures. The results can be used in the formulation of mitigation strategies with a view of minimizing the impact of IBV on egg and chicken production and fertility.

Keywords: infectious bronchitis virus, economic impact, benefit-cost analysis

47. The pathophysiology of impaired skin wound healing in bovine digital dermatitis

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Active digital dermatitis (DD) is an ulcerative lesion on cattle feet that causes painful lameness, leading to animal welfare and economic concerns, with no effective treatment yet. These lesions, mostly colonized with *T. phagedenis*, begin as acute M2-stage and frequently progress to chronic M4-stage, that may eventually resolve or revert to active inflammation by mechanisms unknown. To understand why ulcerative inflammation persists in DD and impedes wound healing, populations of bovine macrophages were identified in the cattle foot skin by flow cytometry, targeting *classical* ($CD14^{++}CD16^{-}$) and *intermediate* ($CD14^{+}CD16^{+}$) and *non-classical* ($CD14^{-}CD16^{++}$) macrophages. We showed that classical (pro-inflammatory) macrophages were abundant in M2 DD lesions compared to healthy (M0) skin. We also detected higher matrix metalloproteinase (MMP) activity in bovine M2-DD lesions (220-510 pM/min/µg), compared to healthy foot skin (approximately 180 pM/min/µg). Next, to assess the recruitment of neutrophils and different macrophage phenotypes in DD in a more controlled condition, we subcutaneously injected *T. phagedenis*, isolated from DD, in the dorsal skin of mice. *T. phagedenis* induced-abscesses were infiltrated with neutrophils that persisted till day 14 post-infection. Pro-inflammatory ($Ly6C^{high}$ $CCR2^{+}$) and reparative ($Ly6C^{low}$ CX_3CR1^{+}) macrophages were recruited, that peaked on day 4 and were reduced at days 7 and 14 post-infection. We conclude that an aberrant infiltration of neutrophils and pro-inflammatory macrophages with dysregulated MMP activity dominate the non-healing ulcerative lesions in active DD. Since *T. phagedenis* is an opportunistic pathogen, effective pro-healing therapies targeting these infiltrating cells and MMPs can potentially offer antibiotic-free therapeutic alternatives against DD.

48. Monocytes regulate leptin-driven vascular dysfunction to promote healing during infection

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Monocytes are circulating immune cells that are recruited to sites of infection, and upon tissue entry monocytes convert into macrophages or dendritic cells that take on various functions depending on the local microenvironment. During infection, monocytes are thought to be key for bacterial eradication, but this is hard to reconcile with the fact that there are hundreds of neutrophils recruited for every one monocyte. Here, using a low dose bacterial skin infection model and intravital microscopy, we show that monocytes are recruited in significant numbers, surround the infection, and convert to monocyte-derived macrophage which persist in the infection for weeks after bacteria are cleared. The monocytes did not contribute towards bacterial clearance but were critical for tissue repair by restricting hypodermal adipocyte expansion and production of the adipokine hormone leptin. In CCR2-deficient mice which lack circulating monocytes, there was increased hypodermis thickness (fat cell number) and an elevated leptin level which drove significant overgrowth of dysfunctional blood vasculature in the infections. This led to delayed healing with a thickened scar. Treatment with a leptin antagonist or leptin's natural inhibitor, ghrelin, reduced vasculature overgrowth in CCR2-deficient mice and thus improved healing of infections. Our study links monocytes to the regulation of leptin which leads to appropriate revascularization during wound healing.

Keywords: monocyte, infection, wound healing, leptin, ghrelin

49. Modular molecular epidemiological toolkit for studying anthelmintic resistance using next-generation sequencing technology

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Presenting author: Rebecca Chen (student)

New DNA sequencing technologies are revolutionizing our ability to undertake surveillance and study how drug resistance emerges and spreads, but the application of these approaches to anthelmintic drug resistance in nematodes is still in its infancy. This is partly due to a lack of easily usable tools to analyze these large DNA sequencing datasets for molecular epidemiological studies.

We have therefore developed a modular and flexible bioinformatic toolkit written in R programming language to study the molecular epidemiology of anthelmintic resistance. This toolkit takes raw short-read amplicon sequencing data and applies several different modules to process the data. The results are then visualized via haplotype networks and geospatial maps. This toolkit has been designed with flexibility in mind to allow different molecular markers to be easily incorporated as our molecular understanding of anthelmintic resistance grows.

We have used benzimidazole in small ruminant gastrointestinal nematode species to develop the toolkit as several important resistance mutations in the isotype-1 β-tubulin drug target are well characterized. We illustrate its use on two different large-scale isotype-1 β-tubulin amplicon sequencing datasets from sheep nematode populations to compare early-stage (*Nematodirus battus* from 150 UK sheep farms) and late-stage (*Haemonchus contortus* from 130 North American sheep farms) benzimidazole resistance. The haplotype networks and geospatial maps illustrate the localized emergence of resistance in its early stages and the more complex patterns in late stage resistance suggestive of derivation from multiple origin regions in the late-stage population. This toolkit should provide a valuable resource for molecular epidemiology analysis of anthelmintic resistance.

Keywords: molecular epidemiology, drug resistance, next-generation sequencing, small ruminant, gastrointestinal nematode

50. Engineered endolysins against multidrug resistant bacteria causing bovine respiratory disease

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Bovine respiratory disease is a leading cause of economic loss in beef and dairy industries despite the extensive use of vaccines and antimicrobials. The primary bacterial pathogens associated with bovine respiratory disease are *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* [1]. Bacterial pathogens associated with bovine respiratory disease (BRD) are becoming increasingly multi-drug resistant (MDR). Thus, alternative strategies against MDR bacteria are needed. Bacteriophage derived lysins are an alternative strategy against MDR in treating BRD. Endolysins are produced at the end of the lytic replication cycle when bacteriophage hydrolyze the host cell wall to release their progeny. This is the first time to our knowledge, that endolysins derived from *M. haemolytica* phages are being engineered and assessed for antimicrobial activity against BRD pathogens. The primary research goal was to investigate the effects of endolysins derived from *M. haemolytica* phages in the control of bacteria associated with BRD. To assess the antimicrobial activity of engineered endolysins, enzymatic activity was calculated based on its ability to degrade peptidoglycan in a muralytic activity assay. Gram negative bacteria can limit the activity of endolysins as the outer membrane blocks access to the peptidoglycan layer. Thus, an outer membrane destabilizing agent such as EDTA was added to improve the access of endolysins to peptidoglycan. Growth inhibition and minimum inhibitory concentration assays were also conducted to assess efficacy of the engineered endolysin. It is expected that the endolysin will have broad spectrum activity against the bacterial pathogens involved in BRD.

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Keywords: bovine respiratory disease, endolysin, antimicrobial

51. Expansion of porcine germ cells in stirred suspension bioreactors is partially mediated by the Wnt/β-catenin pathway

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Male childhood cancer survivors often experience infertility due to gonadotoxicity from chemotherapies. For such individuals, expansion of germ cells (GCs) from testicular biopsies may be used to restore fertility. Thus, a reliable GC expansion system is needed. Here, we present a suspension culture system of porcine GCs using stirred suspension bioreactors (SSBs). GCs from 1wk old pigs were cultured at rotation speeds of 80, 100 or 120rpm and in static culture as control for 2wks. Proliferation rates (% $EdU^{+ve}UCHL1^{+ve}$(GC marker) cells) were respectively 10.9±1.22%, 12.4±1.68%, 17.8±2.9% and 11.5±2.1% (n=3,$p<0.05$). Rotational speed of 120rpm supported the highest proliferation and was used for subsequent experiments. Previous studies reported higher GC proliferation under low O_2 tension. However, GCs in SSBs at 10% O_2 proliferated less than at 21% O_2 (14.9±0.9% vs.17.8±2.99%, n=3,$p<0.05$). Based on expression of GFRα1 (undifferentiated), GCs cultured at 10% O_2 remained undifferentiated, while cells at 21% O_2 started differentiating. Previous studies reported that shear forces activate Wnt/β-catenin pathway. SSBs (21% O_2) had more GCs with nuclear β-catenin (Wnt/β-catenin activation) than static cultures (2.0±0.6% vs.1.27±0.25%, n=3,$p<0.05$). Expression of Wnt/β-catenin target Axin2 was 2-fold higher in cells cultured in SSBs compared to static cultures (n=3,$p<0.05$). Downregulation of Wnt/β-catenin in SSBs with XAV939 displayed a trend towards reduced proliferation. However, treatment of static cultures with CHIR99021 (activator) did not increase proliferation compared to controls (14.67±0.58% vs.12.96±0.29%, n=3,$p>0.05$) implying a role for additional mechanotransduction pathways affecting proliferation in SSBs. In summary, enhanced proliferation of GCs in SSBs is partially mediated by the Wnt/β-catenin pathway.

Keywords: bioreactor, germ cell, spermatogonia

52. The Epidemiology of Hoof-Related Lameness in Western Canadian Feedlot Cattle

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The epidemiology of hoof-related lameness (HRL) in western Canadian feedlots, with a focus on digital dermatitis (DD), was described and analyzed to help inform recommendations on HRL prevention and control. The data in this study were accessed from 28 western Canadian feedlots that placed cattle in 2014-2018, inclusive. The total population was 1,796,176 cattle, with an annual placement average of 12,830 cattle per feedlot. These data were accessed through iFHMS Consolidated Database, provided by Feedlot Health Management Services by TELUS Agriculture, and manipulated using Microsoft® Office Access 365 ProPlus and Microsoft® Office Excel 365 ProPlus. Epidemiological analysis determined that lameness accounts for 25.7% of all treatments, 71.7% of which is localized to the hoof, corresponding to 18.6% of all treatments in western Canadian feedlots. The most common HRL diseases are infectious bovine pododermatitis (foot rot (FR)); DD; and toe-tip necrosis syndrome (TTNS). These diseases account for 89.6%, 7.9% and 2.4% of HRL, respectively. Between 2014 and 2018, HRL prevalence ranged between 1.93% and 3.09% of the population, with FR consistently having the highest prevalence. HRL and DD were tested for their associations with several risk factors using © Ausvet 2021 Epitools software. Based on this univariate analysis, acquisition source has the largest influence on the odds of developing HRL and DD, followed by population size. Using SAS® (Version 9.4, SAS Institute Inc, Cary, North Carolina) statistical software, these preliminary findings will be subjected to a multivariate statistical model, to provide adjusted OR values and statistical significance for this study.

Keywords: Digital dermatitis, lameness, feedlot cattle health, epidemiology

53. Understanding the mechanisms of new inhibitors against *Vibrio cholerae*

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Antimicrobial resistance (AMR) has become a serious public and economic threat due to an increased rate of bacteria acquiring resistance against multiple antibiotics. In addition, the rate of bacteria acquiring AMR supersedes the rate of new antibiotics discovered resulting in more deadly AMR infections. The Pathogen Box is an open-source library of drug-like compounds that could be screened for potential new antibiotics. We have screened the Pathogen Box against *Vibrio cholerae*, the cholera-causing pathogen, and successfully identified two compounds (C1 and C2) that inhibit the growth. Although C1 has been reported to target a broad range of microbes including eukaryotes, its exact molecular mechanism is yet to be identified. We have used RNA-sequencing and whole genome sequencing of spontaneous resistance mutants to show broader impact of C1 on *V. cholerae* and to narrow down the possible targets in cellular processes. On the other hand, C2 has been shown to work as a trimethoprim analog against the dihydrofolate reductase (DHFR) of *Cryptosporidium spp.* and *Acinetobacter baumannii.* We have observed that C2 exhibits strong inhibitory activity even at a 11-fold lower concentration than trimethoprim. We have also confirmed that C2 targets the dihydrofolate reductase (DHFR) of *V. cholerae* through overexpression of trimethoprim-resistant DHFR. Our data towards understanding the underlying mechanisms of these compounds has the potential to unlock the development of antibiotics with better efficacy.

Keywords: Antibiotic resistance, Pathogen Box, Molecular mechanism

54. Distinct wound microenvironments remodel the regulatory landscape of dermal fibroblasts to unmask either a regenerative or fibrotic response

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Dermal fibroblasts exhibit considerable heterogeneity during homeostasis and in response to injury. Understanding the mechanisms controlling fibroblast plasticity will be essential for developing therapeutics that mitigate fibrosis and promote regeneration. Fate mapping of interfollicular fibroblast progenitors marked by quiescence-associated factor *Hic1 (Hypermethylated in cancer-1)* following skin wounding revealed that their progeny generated the bulk of reparative fibroblasts and exhibited functional divergence, mediating regeneration at the wound center and scar formation in the periphery. We surmised that divergent healing responses were enabled through acquisition of regionalized regulatory landscapes in *Hic1*-lineage fibroblasts. Single-cell RNA- and ATAC-Seq analysis revealed that central fibroblasts reactivated embryonic cellular retinoid-binding proteins (e.g. Crabp1, Fabp5), whereas peripheral fibroblasts differentially expressed (e.g. Dlk1, Sca1, Mest). Intriguingly, both central and peripheral fibroblasts expressed comparable mRNA levels of transcription factors (TF) *RARα* and *RUNX1*. However, SCENIC-inferred gene regulatory network activity suggested these TFs were exclusively active within central fibroblasts. Pairwise correspondences in sc-ATAC-Seq measurements revealed that regeneration-competent fibroblasts harboured an enhanced genome-wide motif accessibility for both *RARα* and *RUNX1,* suggesting the epigenome enabled (or occluded) activity of master regulators by modifying cognate motif accessibility. Consistent with their role as master regulators of regenerative competence, pharmacological inhibition of Retinoic Acid (RA) and RUNX1 ameliorated regeneration, whereas exogenous RA augmented regenerative capacity (>1.5-fold increase in hair regeneration post-wounding). Comprehensive regulatory landscapes controlling fibroblast fate/function can be explored intuitively on our publicly available Wound Atlas (*biernaskielab.ca/Wound_Atlas*). Together, our work reveals molecular targets to exploit latent, but modifiable, regenerative capacity of dermal fibroblasts to improve wound healing outcomes.

Keywords: Tissue regeneration; Fibrotic Scarring; Single-cell Sequencing; Lineage tracing

55. Molecular and computational tools for cost-effective, non-invasive genomic analyses in feral horses

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Though the cost of obtaining genotype data has steadily dropped, obtaining genomic data for entire populations has remained prohibitively expensive, especially for wildlife and conservation management programs. Furthermore, few methods offer a non-invasive approach to sample host genomic data. Targeted sequencing has emerged as a cost-effective alternative for obtaining host genotypes requiring little host DNA. Concurrently, advances in genome imputation have enabled the imputation of several thousand genotypes up to the density of a reference population containing several hundred thousand genotypes. Our goals for this experiment were twofold: we sought to test whether targeted sequencing can recover a pre-specified list of genotypes in terms of coverage and accuracy compared to commercially available genotyping arrays, and whether genome imputation can further increase genotype information at high accuracy. Using fecal swabs from a population of feral horses, we compared the agreement for 279 target genotypes between the NuGEN Allegro Targeted Genotyping kit and three standard equine genotyping arrays (Illumina GGP65, GGP65Plus and Affymetrix Axiom). For imputation, we masked genotypes to random subsets (1000 – 20000) and varied the number of individuals in the reference population. Targeted sequencing achieved accuracies of 90-95% for 272 out of the 279 targeted genotypes. With 10000 genotypes, imputation accuracy approached unity and only depended on the number reference individuals. These results demonstrate that cost-effective, non-invasive genotyping methods are within reach, enabling large scale genetic monitoring of wildlife populations in the near future.

Keywords: targeted sequencing, genome imputation, genotyping

56. Engineered type six secretion systems deliver active exogenous effectors and Cre recombinase

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Genetic editing has revolutionized biotechnology but delivery of endonuclease genes as DNA can lead to aberrant integration or overexpression, leading to off-target effects. Here we develop a mechanism to deliver Cre recombinase as a protein by engineering the bacterial type six secretion system (T6SS). Using multiple T6SS fusion proteins, *Aeromonas dhakensis* or attenuated *Vibrio cholerae* donor strains, and a gain-of-function cassette for detecting Cre recombination, we demonstrate successful delivery of active Cre directly into recipient cells. Most efficient transfer was achieved using a truncated version of PAAR2 from *V. cholerae*, resulting in a relatively small (118 amino acid) 'delivery tag'. We further demonstrate the versatility of this system by delivering an exogenous effector, TseC, enabling *V. cholerae* to kill *Pseudomonas aeruginosa*, including multidrug resistant clinical isolates. This implicates that *P. aeruginosa* is naturally resistant to all native effectors of *V. cholerae* and that the TseC chaperone protein is not required for its activity. Moreover, it demonstrates that the engineered system can improve T6SS efficacy against specific antibiotic resistant pathogens, proposing future application in microbiome manipulation or as a next-generation antimicrobial. Inexpensive and easy to produce, this protein delivery system has many potential applications ranging from studying T6SS effectors to genetic editing.

Keywords: Bacterial secretion systems, Interspecies interactions, Antibiotic resistance, Genetic editing, Protein delivery

57. Effect of selective removal of high proviral load cattle on the herd prevalence of bovine leukosis

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Bovine leukosis, caused by bovine leukemia virus (BLV), is a common viral disease of cows. Time between infection and disease is long, and leukosis cases are uncommon, but BLV infected animals have reduced immunity, milk production and longevity. It is difficult to detect infected cows without laboratory methods. Controlling BLV from dairy herds by test and cull strategy is impractical because of the high within-herd prevalence. BLV produces a copy of itself and build that in the genome of the cow, this is a provirus. The proviral load (PVL), measured as the amount of proviruses in the host's white blood cells, is associated with BLV transmission. The objective of this study therefore is evaluating the impact of removing just the high proviral load (HPL) cows from the herd on the BLV herd prevalence. Milk or blood samples were collected from the milking cows in eleven dairy herds across Alberta. The samples were tested for antibodies against BLV and the proviral load was determined in the BLV-positive cows. Of 2,238 dairy cows sampled, 770 tested positive for BLV antibodies, the within-herd prevalence ranged from 9.22% to 52.24% (median=34%). The proviral load distribution in the positive cows is reported in table 1. We will follow up with these farmers annually to evaluate the impact of this strategy for two more years.

Table 1: Proviral load distribution

	High proviral load	Moderate proviral load	Low proviral load	Proviruses not detected
Number of cows n (%)	88 (11.43)	227 (29.48)	332 (43.12)	123 (15.97)

Keywords: BLV, proviral load, control, prevalence

58. Zoonotic bacteria in free-ranging urban white-tailed jackrabbits (*Lepus townsendii*) and feral European rabbits (*Oryctolagus cuniculus*) in Calgary, Alberta

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Many emerging infectious diseases of human health significance originate in wildlife hosts and recent studies have identified urban-adapted wildlife species as carriers of zoonotic pathogens. Among these, *Clostridium difficile,* extended spectrum beta-lactamase (ESBL) producing bacteria, *Francisella tularensi*s, and Methicillin-resistant *Staphylococcus aureus* (MRSA)*,* are bacterial pathogens of clinical relevance to human health. In Canadian prairie cities, white-tailed jackrabbit (*Lepus townsendii*) and feral European rabbit (*Oryctolagus cuniculus*) populations are flourishing, however, little is known about their role in zoonotic pathogen transmission. The objective of this study is to determine the prevalence of *C. difficile,* ESBL-producing bacteria*, F. tularensis*, and MRSA in urban rabbits and hares in Calgary. We collected tissues, fecal samples, and nasopharyngeal swabs from 154 road-killed urban hares and rabbits in Calgary, Alberta. Samples were tested as follows: culture and typing of feces for *C. difficile* and ESBL, PCR of spleen for *F. tularensis*, and swab culture for MRSA. We identified six positive cases (3.9%) of *C. difficile*, six cases (3.9%) of ESBL-producing *Klebsiella oxytoca*, and one case (0.6%) of *F. tularensis* spp. *Holarctica*. None of the individuals tested were positive for MRSA, which indicates the prevalence in the population is less than 2% (95% confidence interval). From these results we can concluded that urban rabbits and hares in Calgary are competent carriers of some fecal and vector-transmitted zoonotic pathogens. Urban areas are likely to continue expanding, therefore, better defining the potential risk of zoonotic pathogen carriage posed by urban wildlife to human health is of particular importance.

Keywords: Urban, Wildlife, Zoonosis, Leporidae, Bacteria

59. Identifying the brain-specific cholesterol metabolizing enzyme (Cyp46A1) as a potential therapeutic target in prion diseases

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Prion diseases are a group of fatal, infectious, and incurable neurodegenerative disorders caused by misfolding of the cellular prion protein (PrP^{C}) into the infectious isoform (PrP^{Sc}). Prion diseases affect both humans and animals. Creutzfeldt-Jakob disease (CJD) is the most common human prion disease. Animal prion diseases include scrapie in sheep/goat, mad cow disease which has been transmitted to humans from cattle, and chronic wasting disease, a contagious prion disease in cervids. Currently, no treatment is available for prion diseases.

Cellular cholesterol is known to impact prion conversion, which in turn results in an accumulation of cholesterol in prion-infected neurons. The brain-specific enzyme, cholesterol 24-hydroxylase (CYP46A1) converts cholesterol into 24(S)-hydroxycholesterol that exits the brain. Herein, we aimed to determine the Cyp46A1 levels in *in vitro* and *in vivo* prion disease conditions.

We have demonstrated for the first time that Cyp46A1 levels are reduced in the brains of prion-infected mice at advanced disease stage, in prion-infected neuronal cells and in post-mortem brains of sporadic CJD patients. We have used the Cyp46A1 activator efavirenz (EFV) for treatment of prion-infected neuronal cells and mice. EFV is an FDA approved anti-HIV medication effectively crossing the blood brain barrier. EFV significantly mitigated the PrP^{Sc} in prion-infected cells. Notably, oral administration of EFV treatment after intracerebral prion inoculation of mice significantly prolonged the lifespan of animals.

Our results suggest that Cyp46A1 is a novel therapeutic target and that its activation through repurposing the anti-retroviral medication EFV might be a valuable treatment approach for prion diseases.

Keywords: Prion diseases; CYP46A1; Efavirenz; Infectious isoform (PrP^{Sc})

60. Dogs contribution to *Echinococcus multilocularis* lifecycle in the urban centers of Alberta

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As a result of human impact on natural landscapes, contact between wildlife, humans and domestic animals becomes increasingly common. This phenomenon has consequences for the ecology and transmission rate of parasites. Such is the case for *Echinococcus multilocularis* (*Em*), a multiple-host tapeworm whose life cycle involves rodents as intermediate hosts and canids, including foxes and coyotes as definitive hosts. Moreover, the shared habitat range between *Em* wild hosts and domestic dogs in urban landscapes has favoured this tapeworm to establish enzootic lifecycles globally. Previous studies in Alberta, have highlighted an unexpectedly high prevalence of intestinal echinococcosis in urban coyotes and foxes, whereas an unknown number of dogs contribute to the lifecycle of *Em* as dual participants. Dogs can act either as definitive hosts contracting intestinal *Em* infections and spreading the helminthic eggs through their faeces, but also as intermediate hosts, developing a multi-organ infection known as Alveolar Echinococcosis (AE). Studying Echinococcosis prevalence in dogs is important because of their potential contribution to *Em* spillover to humans and wildlife. Our project will estimate the intestinal and serological prevalence of *Em* in the canine populations of Calgary and Edmonton. We will assess the risk of exposure to *Em* for animal health practitioners and dog owners. Finally, we will identify the risk factors contributing to the occurrence of both forms of Echinococcosis in Albertan dogs. Surveillance of *Em* in canine populations is crucial given their ability to act as sentinels for humans and contribute to the environmental contamination by this parasite.

Keywords: Prevalence, risk of exposure, emergent zoonosis, *Echinococcus multilocularis*,

61. Proliferation of porcine undifferentiated spermatogonia in co-culture conditions

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Spermatogonial stem cells (SSCs) must be expanded in vitro to obtain enough cells to develop treatments for male fertility preservation. Expanding porcine undifferentiated spermatogonia, containing SSCs, would translationally benefit human SSC expansion. Peritubular myoid cells (PMCs) and testicular endothelial cells (TECs) secrete glial- cell derived neurotrophic factor (GDNF), and other factors that promote SSC maintenance and self-renewal, but have not been investigated as feeders for porcine germ cell culture. We hypothesize that porcine germ cells co-cultured with PMCs and/ or TECs will be able to proliferate for as long or longer than with other reported feeders or feeder- free conditions. PMCs, TECs, and Sertoli cells isolated from prepubertal porcine testis tissue were passaged to 83-96% purity, and pig fetal fibroblasts (PFFs) were obtained from 40 day old porcine fetuses. Feeders were mitotically arrested and seeded at 65,000 cells/mL, and undifferentiated spermatogonia isolated from prepubertal porcine testis tissue were seeded on feeders at 500,000 cells/mL. Edu incorporation showed that after 1 week, proliferation of germ cells was 14.7± 1.25% on PMCs, 14.6± 2.71% on Sertoli cells, 10.6± 2.12% on TECs, 15.8± 1.8% on PFFs, and 7.5± 1.31% in feeder- free controls (n=3). After 2 weeks, proliferation was 13.7± 1.53% on PMCs, 14.0± 0.55% on Sertoli cells, 9.3± 0.93% on TECs, 14.3± 1.30% on PFFs, and 5.4%± 0.84% in feeder- free controls (n=3). Results for co-cultured cells after both 1 and 2 weeks support the hypothesis. Identification of the most supportive feeder will inform feeder- free culture development and will aid progress towards human SSC expansion.

Keywords: co-culture, spermatogonial stem cells, exosomes

62. Seasonal epidemiology of major gastrointestinal nematode species in the northern semi-arid climatic zone of western Canada using the ITS-2 nemabiome metabarcoding approach

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The prevalence of Gastrointestinal Nematode (GIN) parasites in northern semi-arid climatic zones is not as well understood as the other climatic regions. The population dynamics of GIN in these northern regions might be increased in the future for several reasons, including the emergence of anthelmintic resistance, global warming and changes in grazing management. This study investigated the species-specific seasonal prevalence of GIN free-living stages on pasture in northern semi-arid climatic regions. A field study was conducted on three organic farms in Alberta over the 2019 grazing season. Grass samples were collected every three weeks from June to October 2019 from around 72 fecal pats in each farm. Detailed meteorological data at farm level were recorded by solar powered weather stations. Finally, ITS-2 nemabiome metabarcoding was used to determine the GIN species composition such that species-specific time series data were obtained. We found on most pastures, L3 could not be recovered until six weeks after fecal deposition and L3 count peaked at the 9th week after fecal deposition. A large number of larvae remained in fecal pats at the end of grazing season suggesting this is an important refuge for larvae under these climatic conditions. ITS-2 nemabiome metabarcoding showed that *Cooperia oncophora* and *Ostertagia ostertagi* were the two predominant species, with *Nematodirus helvetianus* and *Trichostrongylus axei* also present. Epidemiological patterns were the same for all four species. Output of this study will now be utilized to validate a mathematical model (GLOWORM-FL) that predicts pasture larval contamination based on various climatic conditions.

Keywords: nematode, cattle, epidemiology, nemabiome metabarcoding,

63. The role of astrocytes in the propagation of prions

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Neurodegeneration induced by misfolded-proteins is a major cause of many neurodegenerative diseases. Establishing therapeutic strategies for such diseases is hampered by a heterogeneous pathophysiology, with added complexity by the presence of various cell types (neurons, astrocyte, and microglia) and their distinct roles in disease pathogenesis. Astrogliosis (reactive astrocytes) is present in most neurodegenerative disorders, including prion diseases, prototypical protein-misfolding diseases featured by neurodegeneration and astrogliosis. The role of astrocytes in prion propagation remains unclear, mainly due to lack of prion infection models for astrocytes.

We aimed to elucidate the role of astrocytes in the propagation of prions by i)- establishing an *in vitro* prion infection model of astrocytes, and ii)- analyzing the biochemical properties of astrocyte-propagated prions *in vitro* and in mouse bioassays.

We inoculated immortalized astrocytes (C8D1A) with three mouse prion strains (22L, RML and ME7), followed them for multiple passages, and intra-cerebrally infected mice with cell homogenates containing C8D1A-propagated prions. Prion propagation was examined by immunoblotting, RT-QuIC, and immunofluorescence and immunohistochemistry for mouse brains.

Interestingly, C8D1A cells propagated prion strains differentially, with 22L showing maximum, RML minimal and atypical, and ME7 no prion propagation. Single cell cloning was performed on 22L-infected C8D1A cells to select persistently infected clones. Surprisingly, inoculation of astrocyte-propagated 22L and RML prions into mice resulted in typical and very similar prion disease in both groups.

These results suggest the loss of the **"astrocyte-specific differential propagation of prions"** feature observed *in vitro* in the presence of other cell types in the central nervous system.

Keywords: neurodegeneration, protein misfolding, prion disease, astrocytes, C81A

64. Development of a Method for Detection of Bovine Mastitis Pathogens by Combining Isothermal Amplification and Split Trehalase Technologies

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Currently, options for on-farm detection of bovine mastitis pathogens are limited. Yet, a rapid, highly specific and sensitive diagnostic method for identifying causative agents of intramammary infections would be very useful.

SpoIIID, a *Bacillus subtilis* regulator, has affinity for a specific DNA consensus sequence. TreA, a glycolytic enzyme, can be split into two domains and conditionally reassembled, resulting in restored trehalase activity. This study aims to combine SpoIIID-DNA binding and split TreA complementation to differentiate mastitis caused by either gram-positive or gram-negative bacteria.

First, we investigated conditional complementation initiated by specific SpoIIID-DNA binding. Second, we optimized the reagent formulation of this reaction. Next, this method was applied to detect the outcome of PCR and loop-mediated isothermal amplification (LAMP) by incorporating the SpoIIID recognition site into primers.

Our results showed that fusion proteins HisTreAN-SpoIIID and HisTreAC-SpoIIID could be recombinantly produced. The SpoIIID parts specifically bound to oligonucleotides with tandem recognition sites with high specificity, resulting in detectable complementation of fused split TreA domains. Optimal salt and DNA concentration, direction of binding sites, and linker length between recognition sites were determined. Moreover, incorporation of DNA sequences in primers of PCR and LAMP assays lead to amplicons that activate the DNA-binding split TreA reagents. We are now planning to integrate this technology in a LAMP assay for detecting gram-positive and gram-negative bacteria.

This study will result in LAMP-based rapid diagnostic assays for bovine mastitis pathogens, which will be useful to identify mastitis-causing pathogens directly on farm and inform appropriate treatment options.

Keywords: Bacteria; Diagnosis; LAMP; Split enzyme; Bovine mastitis

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