CANADIAN EMERGING VETERINARY SCHOLARS SUMMIT

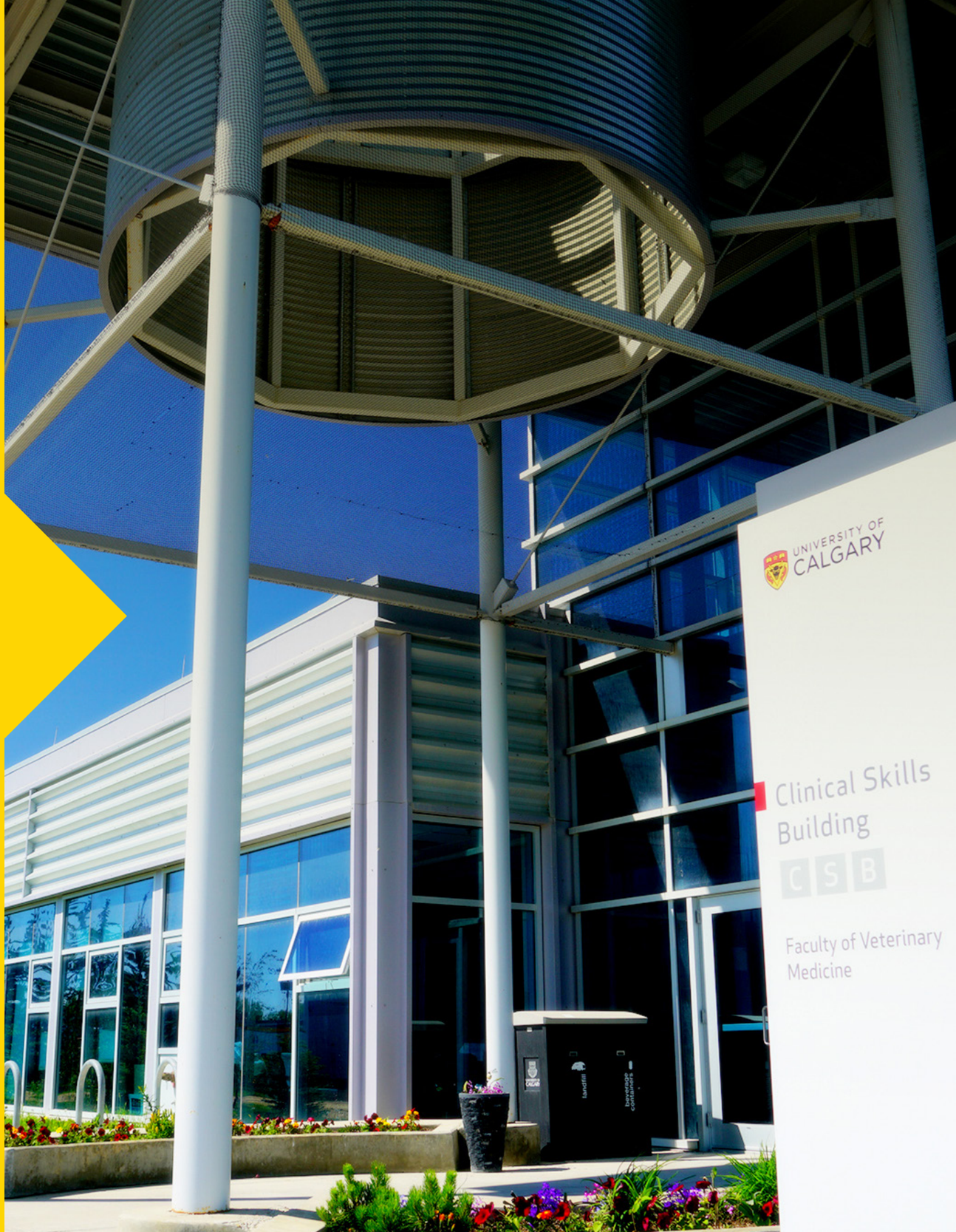
2019

UNIVERSITY OF CALGARY

FACULTY OF VETERINARY MEDICINE

WELCOME TO

CANADIAN EMERGING VETERINARY SCHOLARS SUMMIT



The Program

Friday, November 1, 2019

7:00 AM	Bus pick up at Hotel (travel to CSB)
7:30 AM	Registration
8:15 AM	Opening Remarks: Dr. Robin Yates
8:30 AM	Keynote Address: Dr. Jess McArt, ***From ski trails to cow tails: how to thrive as a chronic learner*** (chair Dr. Tuan Trang)

Session 1 — Chair: Dr. Angelica Galezowski

9:30 AM	Mariia Tokareva (Grad, WCVM) - Exploring the motivation of stall-housed sows and gilts to exit their stall
9:45 AM	Anam Hamza (Grad/DVM, AVC) - Recognition of Stress Levels in Hosptialized Equine Patients During Various Veteirnary Procedures: Adaption of a stabled horse stress scale to a cohort of Hospitalized Horses
10:00 AM	Marina Evangelista (Grad, UM) - Pain assessment in cats: The Feline Grimace Scale
10:15 AM	Marina Kashevska-Gozdek (DVM, OVC) - Optimizing serum concentration for primary canine cancer cell culture
10:30 AM	Velina Milkova (DVM, WCVM) - Expression analysis of EphA receptor tyrosine kinases in canine lymphoma and osteosarcoma
10:45 AM	BREAK

Session 2 — Chair: Dr. Tuan Trang

11:15 AM	Thomas Parmentier (Grad, OVC) - Effects of RNA modifications on neuronal gene expression in canine epilepsy
11:30 AM	Sarthak Sinha (MD/PhD, UCVM) - Molecular Dissection of Skin Regeneration and Scarring using single-cell Seqeuncing: Implications for Human and veterinary Medicine
11:45 AM	Jack Jarvis (Grad, AVC) - Auditory and Olfactory communicative deficits in the early life seizure model of autism
12:00 PM	Diana Powell (DVM, UCVM) - The Metabolic Phenotype of Prepubertal Porcine Spermatogonia
12:15 PM	LUNCH

Session 3 — Chair: Dr. Claire Windeyer

1:45 PM	Melissa MacKinnon (Grad, OVC) - An Evaluation of Incidence rates and antimicrobial resistance in human *E. coli* bloodstreem infections
2:00 PM	Kaleigh Eichel (DVM, UCVM) - An initial description of antimicrobial resistance in the environment on a cow-calf operation in Alberta
2:15 PM	Hélène Lardé (Grad, UM) - Evaluation of Antimicrobial Usage by Veterinary Practitioners and Dairy Farmers in Quebec Dairy Farms
2:30 PM	Camilla Queiroz (Grad, UCVM) - The application of “Nemabiome” deep amplicon sequencing to investiage gastrointestinal nematode species abundance and anthelmintic resistance in sheep flocks
2:45 PM	Jocelyne Chalifour (DVM, WCVM) - Do Pesticides Make Honey Bee Larvae More Suscpetible to European Foulbrood?
3:00 PM	BREAK

Session 4 — Chair: Dr. Dongyan Niu

3:30 PM	João Romero (Grad, AVC) - Simluation modelling of infectious salmon anemia spread in farmed salmon population in BC
3:45 PM	Mengying Liu (Grad, WCVM) - Development of a variant infectious bursal disease (varIBDV) immune complex vaccine to immunize broiler breeder to protect broiler progeny against var IBDV infection
4:00 PM	Roseann Kehoe (DVM, OVC) - Linking Morphotype and Genotype for *Eimeria leuckarti* (Equine parasite)
4:15 PM	Flavie Payette (DVM, UM) - Detection of Nicoletella semolina by qPCR in the airways of horses
4:30 PM	CLOSING REMARKS

TRAVEL TO MOOSE MCGUIRE’S

5:00 PM	Bus pick up CSB
5:45 PM	Moose McGuire’s (Social Night)
8:30 PM	Bus from Moose to Hotel

Program continues the next day

The Program

Saturday, November 2, 2019

8:15 AM	Bus pick at Hotel (travel to CSB)
9:00 AM	Interactive Game (Dr. Angelica Galezowski)
10:00 AM	Career Panel (Moderator: Dr. Claire Windeyer; Panelists: Drs. Janice Berg, Jess McArt, Daniel Pang, Jen Davies)
11:00 AM	Science & Policy (Jennifer Dotchin)
12:30 PM	LUNCH
1:30 PM	Tour CSB
2:45 PM	Bus to hotel
5:00 PM	Bus to Zoo
5:30 PM	Reception at Calgary Zoo
5:30 PM	Cocktails
6:00 PM	Dinner
6:00 PM	Welcome Remarks (Dr. Baljit Singh)
6:10 PM	Buffet
7:00 PM	Doug Whiteside (panda presentation)
7:30 PM	Merck Awards presentation (Dr. Janice Berg)
8:15 PM	Concluding remarks (Dr. Baljit Singh)

Sunday, November 3, 2019 - *Free day*

Presentation Abstracts

Exploring the motivation of stall-housed sows and gilts to exit their stall

Mariia Tokareva, Jennifer Brown, Edmond Pajor, Yolande Seddon

Introduction: It was proposed within the 2014 Canadian Code of Practice for the Care and Handling of Pigs that existing stall barns could continue functioning after July 2024, if mated female pigs are periodically given a greater freedom of movement. Understanding the motivation of sows for a greater freedom of movement, and the benefits to the sow and her productivity from providing periodic exercise, is important for decision making around the implementation of this requirement.

Project description/methods: The objectives were to determine the motivation of stall-housed female pigs for access to three minutes of free time within an alleyway (M: movement), compared to that of a high value reward: feed (F: 30% of daily ration), and to understand whether prior stall experience influences the motivation for each reward (study 1). A second study explored how the motivation of sows to exercise is influenced by satiety (study 2). For study 1, stall-housed bred sows (n=12) and gilts (n=12) were trained to use a panel containing two buttons: i) active button (AB - push counts result in a reward of movement or feed), ii) dummy button (push counts not rewarded). The required number of AB presses during a 30-minute session increased by 50% each consecutive test day. Upon reaching their highest price paid (HPP - maximal AB push counts) for one reward, animals were retested for the alternate reward. This process was repeated for study 2, with 42 new sows tested for their motivation for exercise, assigned to one of three treatments: control (C), fed a standard gestation ration; moderately satiated (0.5 HF), receiving 50% of their ad-libitum high fibre feed (HF) intake additional to their ration; and fully satiated (ad-lib HF), given unlimited access to HF additional to their ration. Further research is being conducted to explore the effects of periodic exercise on sow productivity through comparing animals that receive different amount of exercise during gestation.

Key findings/significance: Sows showed a greater HPP for feed than movement. Sows also showed a greater HPP to access feed than gilts, but for movement it did not differ (sows F: 369.25±56.47 vs M: 68.5±13.61; gilts F: 211.67±47.73 vs M: 77.75±19.47, $P < 0.05$, mean±SEM). In study 2, control sows showed a greater HPP to exit the stall, than ad-lib HF sows. The HPP for 0.5 HF sows did not differ from C and ad-lib HF sows (C: 94.43±18.21, 0.5 HF: 66.43±15.88, ad-lib HF: 59.14±23.65, $P<0.05$, mean±SEM). Results suggest that stall-housed sows and gilts show a level of motivation for greater freedom of movement, however, this motivation is influenced by sow level of satiety. This implies that provision of HF feed, additional to the standard ration may be an option to increase the welfare of stall-housed sows, if periodical providing a greater freedom of movement is not viable. These results can be considered by the working committee of the Code of Practice when making a decision regarding the extra provisions for bred female pigs housed in stalls post 2024.

Source of funding: Agriculture Development Fund Saskatchewan.

Recognition of Stress Levels in Hospitalized Equine Patients During Various Veterinary Procedures: Adaptation of a Stabled Horse Stress Scale to a Cohort of Hospitalized Horses

Anam A. Hamza, Michael S. Cockram, William J. Montelpare, Karen L. Overall, Laurie A. McDuffee

In hospital, equine patients are exposed to unfamiliar environments and handlers. They undergo various treatment procedures, including routine actions that are not always recognized as stressful. Patient stress levels vary, and patients may display behaviour considered problematic for staff. In-hospital procedures are essential: stress is induced, and problematic behavior persists or worsens. Stress behaviour impedes the efficiency and ease of the procedure, and benign treatments can become dangerous for horse and handler. Immune function can be decreased by the physiological stress response. Handlers may use force for treatment, instead of employing pacific techniques that would calm the patient. The primary objective of this research is to determine a visual assessment method of identifying and measuring stress levels in hospitalized equine patients during various, minimally invasive procedures. If stress behaviour·was easily recognizable and quantifiable, techniques could be implemented to mitigate stress levels.

Using a cohort of 50 horses, of varying demographics, admitted to the Atlantic Veterinary College (A VC) Large Animal Hospital, we aim to create an assessment tool to recognize and quantify stress in hospitalized horses. Horses were chosen based on specific selection criteria: horses were non-painful, and were admitted for arthroscopy, castration, or breeding, were easily handled, and were admitted under in-patient status. Video footage, heart rate variability and salivary cortisol measures are collected for each subject before and after standardized physical examination, weighing, and blood collection. An ethogram and corresponding behaviour scale will be formulated from videoed behavioural and physiological data. Validity and practical usage will be subsequently assessed over a two-year period by imposing the scale upon all equine patients at the A VC Large Animal Clinic.

Preliminary findings include pilot data collected from seven horses, as well as behavioural and physiological data collected from 18 horses. Though no significant results have emerged from the current data set, some patterns have been noted. When comparing behavioural data, heart rate variability measurements, and salivary cortisol for each treatment, it is evident that each indicator of stress is lower during physical examination and blood collection, and higher during baseline and scale weighing. During both physical examination and blood collection the horses are in close contact with their handlers, whereas during baseline measurements, the horses are left alone in their stalls and receive no contact from handlers. The majority of equine patients enrolled in this study are sport or pleasure horses that experience high levels of human interaction. Therefore, can be inferred that handling by humans in a calm and appropriate manner during hospital procedures may help to reduce stress levels in hospitalized equine patients. These findings would be verified through increasing the sample size of equine patients.

Through the dissemination of findings to veterinary professionals and owners, we will provide research-based evidence to improve the welfare of horses at the AVC Veterinary Teaching Hospital and the equine community. This research should improve the quality of healthcare that horsemen and veterinary professionals provide to equine patients.

This research was made possible through funding from the Sir James Dun Animal Welfare n Centre at the Atlantic Veterinary College.

Pain assessment in cats: the Feline Grimace Scale

Marina C. Evangelista, Paulo V. Steagall

INTRODUCTION - Pain management is commonly neglected in cats. Facial expressions of pain have been identified in numerous species including laboratory animals, horses, piglets, sheep and ferrets through the development of “grimace scales”. This study aimed to develop and validate the Feline Grimace Scale (FGS), a facial expression-based scoring system, to detect pain associated with naturally-occurring conditions and investigate whether the FGS could be successfully implemented in a clinical context.

METHODS / KEY FINDINGS - Twenty healthy cats (non-painful = controls) and thirty-five client-owned cats of all ages, breeds and gender, suffering from pain of varying origin and intensity were studied at their presentation to the hospital. The cats were video-recorded undisturbed in their cages using a wide-angle camera. Painful cats received analgesic treatment and videos were repeated one hour later. Screenshots were obtained from the videos when the cats’ faces were visible. Two individuals compared images of painful cats and controls to identify differences in facial features between these two groups and the FGS was created. Five action units (AU) were identified: ear position, orbital tightening, muzzle tension, whiskers change and head position. Each AU receives a score of 0 (AU is absent), 1 (moderately present) or 2 (obviously present). Afterwards, four observers independently scored 110 images of control and painful cats on two rounds, 30 days apart. The FGS scores were higher in painful than in control cats; a very strong correlation with another pain assessment instrument was observed as well as good overall inter-rater reliability and excellent intra-rater reliability. The FGS detected response to analgesic treatment (scores after analgesia were lower than before) and a cut-off score was determined.
Later, the clinical applicability of the FGS for real-time acute pain assessment was evaluated. Sixty-five female cats undergoing elective sterilization were included in the second phase. Pain was evaluated before and after surgery in real-time by direct observation of the cats’ faces and videos were recorded simultaneously. The agreement between real-time and image scores, assigned by the same observer six months later, was calculated. Minimal bias and narrow limits of agreement were observed between both scoring methods.

CONCLUSIONS - The FGS is a simple, valid and reliable tool for acute pain assessment in cats. Real-time assessment slightly overestimates image scoring, however with minimal clinical impact. Our research laboratory is actively exploring other applications and limitations of the FGS (i.e. different sources of pain, the influence of sedatives, surgery and the effect of the presence of the observer in front of the cage during pain assessments).

This study received an unrestricted grant by Zoetis and an internal grant from Fonds en santé des animaux de compagnie (FSAC), Faculté de médecine vétérinaire, Université de Montréal. Dr. Marina Cayetano Evangelista is a recipient of the International Veterinary Academy of Pain Management fellowship (2017) and a scholarship from the Merit Scholarship Program for Foreign Students of the Ministère de l’Éducation et de l’Enseignement Supérieur du Québec.

Optimizing serum concentration for primary canine cancer cell culture

Marina Kashevska-Gozdek, Kaya Skowronski, Paul Woods

Cultured primary cancer cells allow researchers to test different therapies *in vitro* which is a necessary precursor to *in vivo* trials. However, they are challenging to grow *in vitro* because of the artificial environment so cell cultures must be grown in specific media containing supplements that replicate *in vivo* physiological conditions as closely as possible. Media contains antibiotics, sodium pyruvate as an energy source, and fetal bovine serum (FBS). FBS itself contains important growth factors and inhibitors. The purpose of our research was to determine the optimal FBS concentration to support primary cancer cell culture growth and survival while maintaining physiologically relevant conditions. We expected that 10% FBS would contain the ideal balance between growth factors and inhibitors to allow for the greatest cell survival and proliferation. The method involved plating each excised tumour sample in three separate plates containing either 5, 10 and 20% FBS. Confluency was tracked weekly and compared between plates. We found that most plates contained heterogenous populations of cell types but the majority had mainly mesenchymal neoplastic morphologies which preferred 20% FBS, likely due to the higher concentration of cell attachment promoting factors and growth factors. In conclusion, our results suggested that our protocols should be altered to initially plate tumour samples in 20% FBS. When cells have attached and are proliferating, the plate should be switched to low serum media to reduce fibroblast contamination and prevent FBS from altering cell metabolism to maintain physiologically relevant cells. The significance of this altered protocol is that it will increase the number and variety of surviving cancer cell lines in the ICCI tumour bank to advance cancer research. This research was funded by the Andrea Leger Dunbar OVC Fund, Pet Trust and Smiling Blue Skies.

Expression analysis of EphA receptor tyrosine kinases in canine lymphoma and osteosarcoma

Velina Milkova, Dr. Behzad Toosi, Dr. Vikram Misra, Dr. Tim Strozen

Erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinases (RTKs) represent the largest family of RTKs with 14 receptors. They are divided into EphA and EphB receptors based on their ligand-binding preferences and are involved in both normal physiology and disease. In the search for new targets for cancer diagnostics and therapies, Eph receptors are emerging as promising regulators of tumor development, invasiveness and drug resistance; however, the expression and potential roles of these receptors in companion animal malignancies have not been studied. In this project, we investigated the expression of EphA receptors in two major canine malignancies: lymphoma and osteosarcoma.

Expression of all EphA receptors (A1-A8 and A10) was evaluated at the RNA level by reverse transcriptase quantitative PCR (rt-qPCR) in two canine lymphoma and four canine osteosarcoma cell lines and their expression panel relative to housekeeping genes was mapped. The expression of EphA receptors at the protein level was investigated by Western blotting. Since no validated canine-reactive EphA antibodies are commercially available, multiple antibodies were tested for cross reactivity with canine EphAs first. Our results indicated the abundant expression of EphA2 in canine osteosarcoma cell lines while its expression was low in canine lymphoma lines. We verified the presence of the EphA2 receptor at the cell membrane of two canine osteosarcoma cell lines using an immunofluorescent staining approach. To expand on the clinical relevance of these findings, we also showed that a synthetic anti-EphA2 antibody developed for diagnostic or therapeutic purposes in humans successfully binds to EphA2 receptor expressed on all four canine osteosarcoma cells when analyzed by flow cytometry.

Our preliminary data from this project indicates an abundant expression of multiple EphAs in canine osteosarcoma. This identifies EphAs as promising targets for the development of new cancer diagnosis and treatment approaches in companion animals, similar to ongoing investigations in humans.

Funding: Allard Research Chair start-up research fund, Interprovincial Summer Student Research Awards

Effects of RNA modifications on neuronal gene expression in canine epilepsy

Thomas Parmentier, Fiona James, Craig Bailey, Dean Betts, Luis Gaitero, Jonathan LaMarre

Canine epilepsy is the most common brain disease seen in dogs. While current treatments aim to decrease the frequency of recurrent seizures, they do not alter the behavioral abnormalities exhibited by these dogs in between seizures. These behavioral problems exacerbate the seizures themselves and contribute to the decrease in quality of life of epileptic patients. How the brain of epileptic patients is remodeled during epilepsy has been a subject of intense research in human medicine, but no study has been conducted specifically in epileptic dogs due to the lack of *in vitro* models of canine epilepsy. Abnormal neuronal connections and migration in response to seizures are among the underlying causes of increased seizure frequency and behavioral abnormalities that can occur in epileptic humans and in laboratory models of epilepsy. Neurons acquire these abnormalities after seizures disturb the regulation of certain key genes. One key regulation mechanism, called m6A RNA methylation, is critical in normal brain development and neuron connection formation. To model epileptic-like seizures *in vitro*, recent research has led to the development of brain organoid cultures derived from pluripotent stem cells. Organoids recapitulate brain organization and electrical activity making it a promising model to study epilepsy. While brain organoids have been used to understand the mechanisms of several neurological diseases in humans, they have never been generated from dog stem cells and their ability to recapitulate canine epilepsy is unknown. In this study, we are developing canine brain organoids by modifying existing protocols developed in other species and induce seizure-like activity by different pharmacological manipulations. The epileptic phenotypes of the organoids is evaluated at the single cell level with whole-cell patch clamp as well as at the whole organoid level with live calcium imaging. Changes in neuronal proliferation and survival are evaluated through immunohistochemistry for specific markers. The RNA of the organoids subject to seizure-like activity is then extracted and the global level of m6A methylation quantified using a specific antibody. Using immunoprecipitation we will then analyze the methylation level of specific transcripts. By inhibiting methylases/demethylases enzymes and interact with the methylation of transcripts we will analyze the effect of RNA methylation on neuronal proliferation, survival and firing properties. Preliminary results indicate that cerebral organoids can be generated from canine embryonic stem cells and acquire spontaneous electrical activity after 50 days *in vitro*. This activity is also enhanced by the addition of glutamate in the medium.

With these findings we hope to better understand of how seizures remodel the canine brain through changes in RNA methylation and pave the way for a non-invasive, patient-specific model of canine epilepsy to use for potential drug-testing.

Molecular Dissection of Skin Regeneration and Scarring using Single-cell Sequencing: Implications for Human and Veterinary Medicine

Sarthak Sinha, Elodie Labit, Sepideh Abbasi, Nilesh Sharma, Arzina Jaffer, Prajay Shah, Jo Stratton, Jeff Biernaskie

Introduction: Fibroblasts are the principal mesenchymal cells. Even within a tissue, fibroblasts exhibit considerable heterogeneity during homeostasis and in response to injury/disease. Mechanisms enacting fibroblast states are not known but will be essential for therapeutics that mitigate scarring and promote regeneration. Our previous fate mapping of fibroblast progenitors in the skin revealed that following a severe injury, their progeny exhibited striking functional divergence within the wound, resulting in regeneration of new hair follicles (HF) at center and scarring at periphery. We surmised this regenerative response is enabled through interactions between fibroblasts' epigenome, regulatory networks, and signals within each wound microenvironment.

Methods: Single-cell mRNA-Sequencing was performed at four wound stages to reconstruct spatiotemporal dynamics of fibroblasts and their interactions with immune cells. Single-cell Assay for Transposase-Accessible Chromatin (scATAC-Sequencing) was performed to dissect epigenetic mechanisms driving regenerative competence.

Results: Distinct cell compositions and secreted factors were enriched within each wound microenvironment. For example, resurgence of Ngp/Lcn2+ neutrophils was exclusive to the regenerative zone. As a result, fibroblasts activated embryonic gene programs (indicated by Crabp1, Fabp5, Prss35) driven by novel transcription factors (Retinoic Acid Receptors (RARs), Runx1, Hox proteins) to enable regeneration. Fibroblasts' regenerative propensity was pharmacologically modulable by topical application of drugs targeting RARs and Runx1. Integration of single-cell epigenomics with transcriptomics revealed (poorly defined) cis-regulatory and non-coding regions of the genome driving divergent mesenchymal fates.

Conclusion and Implications: Signals within wound microenvironments activate distinct transcriptional and epigenetic landscapes in fibroblasts to drive skin regeneration or scarring. Dissection of molecular mechanisms enacting distinct fibroblast states will lay the foundation for developing therapeutics that mitigate fibrosis and promote regeneration in veterinary and human medicine. As well, sequencing workflows developed can interrogate disease genomics on timescales compatible with clinical decision-making.

S.S. is supported by Vanier and Killam Studentships. Research described was supported by Canadian Institute of Health Research and Calgary Firefighters Burn Treatment Society.

Auditory and olfactory communicative deficits in the early life seizure model of autism

Jack Jarvis, Catherine Fiset, Logan Bigelow, Paul Bernard

Early life seizures (ELS) are associated with various detrimental neurological outcomes, including autism spectrum disorder (ASD) and intellectual disability. Following ELS rodents display chronic social deficits. We hypothesized that these social abnormalities may be the result of deficits in auditory communication (expressive and receptive). To determine if abnormal auditory function contributes to the social deficits observed in ASD associated with ELS, we developed a behavioural test paradigm to assess auditory communication in a rat model and contrasted it with olfactory communication performance. Two modalities of communication were chosen to help elucidate whether social abnormalities are a result of deficits in a specific mode of communication or a result of reduced social motivation.

Compared to mice rats exhibits more complex auditory communication, thus were preferable for the proposed research. Thirty-six rats were injected with kainic acid on post-natal day 7 (PNd7) to induce ELS, and 32 were injected with saline to serve as controls. Following PNd32, rats were habituated to the testing environment via extensive handling by experimenters and being allowed to explore the 8-arm radial-arm maze in which testing would occur. On PNd55 and PNd56, the movements of the rats in the radial arm maze were recorded in response to audio playbacks of aversive 22kHz and attractive 50kHz calls. On PNd 61, 62, 63 and 64 the movements of the rats in the radial arm maze were recorded when exposed to plain bedding, unfamiliar female scent, unfamiliar male scent and 10% phenethylamine, respectively.

All rats were attracted to 50 kHz calls, supporting previous studies, and ELS rats were significantly more attracted to the 50kHz calls than the Control rats. This effect was only apparent in males, which correlates with a higher incidence of ASD in males. Whether 22 kHz calls induce avoidance behavior in rats has been widely debated in the literature. Our results contradict current dogma and indicate that 22 kHz calls do not provoke avoidance. This suggests that rats are socially inquisitive animals and are generally attracted to calls from other rats, regardless of frequency ranges.

The results of the olfactory tests indicated a significant preference for the female scent among ELS rats. This indicates that deficit in communication extends to multiple modalities, suggesting a change in social motivation, not just a sensory deficit. The response to phenethylamine, a compound reported to be the element in predator urine that induces avoidance, contradicted the literature, with Control rats exhibiting increased approach behaviour compared to ELS rats.

Our results strongly support the hypotheses that ELS are not benign and contribute to long-term social and communicative abnormalities, similar to what is reported with the association between ELS and intellectual disability. Our findings also provide further support for the aggressive treatment of ELS, an issue which continues to be intensely debated in the field of pediatric neurology.

Source of funding: NIH Grant #5R21NS104604-2, UPEI Veterinary Summer Research Award.

Comparison of MALDI-TOF mass spectrometry identification scores using different bacterial growth media

Carly Lilley, Matthew Saab, Cynthia Mitchell, Javier Sanchez, JT McClure

Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and inexpensive diagnostic tool which has been shown to be an ideal technology for bacterial species identification. MALDI-TOF MS measures the mass of proteins in a sample, producing a characteristic spectrum of peaks which is compared to a reference library of spectra. The relatedness of a sample spectra to the reference spectra is expressed as an identification score ranging from 0-3.000. Despite its expanding usage, several operating factors can influence the MALDI-TOF MS score. This study tested the effects different growth media have on the identification scores provided by the MALDI-TOF MS, as different growth conditions may influence the proteins expressed by bacteria. Isolates of *Escherichia coli* (n=21), *Salmonella* spp. (n=21) and *Campylobacter* spp. (n=26) previously identified by biochemical assays or PCR were cultured on different media (Tryptic soy agar with 5% sheep blood (BTSA), Luria Bertani (LB) and MacConkey agar (MAC) for£. coli and *Salmonella* spp.; *Campylobacter* blood free (CBF) and Mueller-Hinton agar with 5% sheep blood (BMHA) for *Campylobacter* spp.) and were prepared using the direct-smear method with HCCA matrix overlay. Analysis of the isolates was performed on a Bruker microflex LT MALDI-TOF mass spectrometer using MALDI BioTyper. Non-parametric Dunn tests were carried out to compare culture media within a bacterial species group.£. coli isolates had statistically significant lower (p<=0.05) scores on MAC (mean= 2.241) than LB (mean= 2.479) and BTSA (mean =2.399). *Salmonella* spp. isolates scored significantly higher (p<= 0.05) on LB (mean= 2.512) than MAC (mean = 2.446), while scores on BTSA (mean = 2.469) were not significantly different than either of the other two media. There was no significant difference between the *Campylobacter* spp. identification scores on CBF (mean= 2.274) and BMHA (mean= 2.212), although isolates did not grow as well on CBF media. Overall the majority of isolates on all media scored above the cut-off for probable species identification, with many scoring in the range deemed highly probable species identification. This suggests that while some media may provide higher identification scores than others for certain bacteria, all were capable of providing species identification with MADLI-TOF MS. Based on these identification scores we would recommend LB as the medium of choice for the identification of *E. coli* and *Salmonella* spp. by MALDI-TOF MS. Despite no statistically significant differences in the identification scores, BMHA would be recommended over CBF for the growth of *Campylobacter* spp. due to the improved growth on this media.

Funding sources: AVC Internal Research Funds, NSERC Undergraduate Summer Research.

The Metabolic Phenotype of Prepubertal Porcine Spermatogonia

D. Powell, A. Voigt, I. Dobrinski

Spermatogonial stem cells (SSCs) exist among undifferentiated spermatogonia in the testis and are the basis of male fertility. They can be isolated and transplanted to re-establish spermatogenesis and may be a powerful tool for infertility treatment. Prepubertal stem cell progenitors are highly sensitive to cancer treatment, and childhood cancer-survivors often suffer from infertility in adulthood. Spermatogonial stem cell research, especially prepubertal, is crucial to protect patient fertility. Downstream applications require high stem cell numbers and therefore a functional *in vitro* system for expansion, however, SSC culture in large mammals has been limited. Due to anatomical and physiological similarities between pigs and humans, we used the pig as a model. Our aim was to establish a metabolic phenotype of prepubertal spermatogonia to better understand their metabolic needs and stressors in culture. Germ cells have a tight metabolic interaction with Sertoli cells that perform glycolysis, and adult SSCs were reported to rely on anaerobic glycolysis as well. Preliminary results, however, indicated that prepubertal spermatogonia have highly active mitochondria which decreases with maturation. Therefore, we hypothesized that prepubertal spermatogonia mainly rely on oxidative phosphorylation (OXPHOS) and undergo metabolic maturation. Early prepubertal spermatogonia from1-week old pigs were compared to Sertoli cells to investigate metabolic interaction in a prepubertal model, and to late prepubertal spermatogonia from 8-week old pigs to investigate metabolic maturation with age. Extracellular and intracellular (C13 tracing) flux studies were performed with HPLC-MS and gene expression profiling with qRT-PCR. Compared to Sertoli cells (SC), 1-week old spermatogonia (GC) consumed little glucose (2.75% ± 2.836 vs SC 68.47% ± 7.42, n=4, p=0.0014), but significantly more pyruvate (71.08% ± 5.109 vs SC 19.39% ± 5.950, n=3, p=0.0030), and showed significantly less lactate production (GC 1122 ± 18.09, SC 3908 ± 281.6, n=3, p=0.0098). Intracellular flux tracing also indicated a higher incorporation into lactate (7.857 % ± 1.410) after 48 hrs in 1-week old compared to 8-week old spermatogonia. In addition, 1-week old spermatogonia also showed a lower expression of rate determining enzymes such as HK-1, GAPDH, and PYK compared to Sertoli cells. Compared to both groups, they additionally expressed higher levels of PGK-2 and LDH-C—two enzymes previously described only in primarily aerobic differentiated germ cells. Additionally, 1-week old spermatogonia expressed higher levels of TCA, OXPHOS, and anti-oxidative metabolism related enzymes than Sertoli cells and 8-week old spermatogonia. Consequently, we concluded that early prepubertal spermatogonia, at the age of 1-week, mainly rely on anaerobic metabolism, supplied by pyruvate in culture and potentially lactate *in vivo*, and that spermatogonia undergo metabolic maturation to a more quiescent and anaerobic metabolism during the establishment of the stem cell niche. Future studies include a more extensive metabolic flux analysis of early and late spermatogonia and an investigation of lactate consumption *in vitro*. This will help to design a culture system that supports the specific metabolic needs of prepubertal and may aid to distinguish SSCs from the population of undifferentiated spermatogonia based on metabolic phenotype.

Supported by NIH/ORIP (9R01OD016575-12) and Alberta Innovates Summer Research Studentship.

An evaluation of incidence rates and antimicrobial resistance in human *Escherichia coli* bloodstream infections

Melissa C MacKinnon, Scott A McEwen, David L Pearl, Elizabeth C Parfitt, Kelsey Pasquill, Lisa Steele, Kevin B Laupland

Introduction – In humans, the most common cause of bloodstream infections (BSI) is *E. coli*. In order to calculate the incidence rate of infections, a population-based approach must be used. Previous studies in Canberra (2000-04), Calgary (2000-06) and Auckland (2005-11) have demonstrated incidence rates of *E. coli* BSI of 28.0, 30.3 and 52.0 cases/100,000 people, respectively. Resistance to ciprofloxacin and resistance conferred by ESBL isolates is an important and evolving issue for *E. coli* BSI.

Objective – The study used a population-based approach in a geographically isolated Canadian region over a 7-year period to evaluate factors associated with changes in the incidence rate and variation in antimicrobial resistance of human *E. coli* BSI.

Methods – All incident *E. coli* BSI that occurred between April 2010 and March 2017 were obtained from a surveillance database in the western interior region of British Columbia. A multivariable Poisson regression model was used to assess the associations between study year, age category and sex (male/female), and *E. coli* BSI rate. Possible interaction between age category and sex was evaluated. Study year met the assumption of linearity and was modelled as a continuous variable. Age was modelled with three categories: children (≤19 years); adults (20-69 years); and elderly (≥70 years). Among cases of *E. coli* BSI, Fisher's exact tests were used to compare the proportions of ESBL isolates and ciprofloxacin-resistant isolates in study years 5-7 to those in years 1-4.

Key Findings – During the study, there were 668 incident *E. coli* BSI in 635 patients. The overall incidence rate was 53.2 *E. coli* BSI / 100,000 person-years. Based on the multivariable Poisson model, the rate significantly increased 1.40 times over a 7-year period (95% CI 1.07-1.83). There was a significant interaction effect between age category and sex ($p<0.0001$). In general, when the age categories compared were the same or males had a lower age category compared to females, then males had a lower incidence rate. When comparing different age categories separately within males and females, higher rates were seen with increasing age category. The odds of both ESBL and ciprofloxacin resistant *E. coli* BSI were significantly higher in years 5-7 compared to years 1-4 (OR 3.22, 95% CI 1.74-5.95; OR 1.53, 95% CI 1.06-2.21, respectively).

Conclusions – The incidence rate of *E. coli* BSI increased during the study, and age and sex interacted. There were a higher proportion of ESBL and ciprofloxacin-resistant *E. coli* in the final three study years compared to the first four years. The study provides important current Canadian information on the factors influencing the incidence rate of *E. coli* BSI and the temporal variation in types of antimicrobial resistance that are important to human health.

Sources of Funding - Dr. MacKinnon's PhD program is supported through scholarships from the Canadian Institute of Health Research, the University of Guelph and the Ontario Veterinary College. The surveillance database was supported in part by the Strand Infectious Diseases Innovation Fund, Royal Inland Hospital Foundation.

An initial description of antimicrobial resistance in the environment on a cow-calf operation in Alberta

Kaleigh Eichel, Sylvia Checkley, Karen Liljebjelke

Introduction: Antimicrobial resistance (AMR) is a global health concern that requires a One Health approach to understand the interactions between the environment, animals, bacteria, and humans at their interfaces. Components of the beef production system, such as the feedlot, processing plant, and retail meat, have been examined by previous studies as potential sources of bacteria with AMR that cause food-borne illnesses in humans that are difficult to treat with antimicrobials. However, there has been little research exploring the complex interactions of fecal bacteria from cattle with the water and soil on cow-calf ranches. The concern is that bacteria can develop resistance through several mechanisms: in cattle treated with antimicrobials, or in the soil or water containing antimicrobial residues, or through co-selection with metal resistance, or through horizontal gene transfer of resistant genes. The resistant bacteria can then develop a resistome in the environment and/or the cattle on the ranch, allowing AMR to persist and potentially propagate on the ranch. AMR bacteria can then disseminate through the food system and become a food safety and public health concern.

Project Description/Methods: This project is an initial description of antimicrobial resistance in fecal bacteria, *Escherichia coli* and *Enterococcus*, in the water, soil, and cattle manure of a cow-calf operation in Alberta with typical low-antimicrobial use. The purpose of this project was to characterize AMR on the ranch and develop procedures to isolate *E. coli* and *Enterococcus* from environmental samples of water, soil, and manure. Samples of water, soil, and manure were filtered, hydrated, diluted, and spread onto selective media agar and selected colonies were biochemically tested and genetically analyzed to confirm species. Isolates were tested for antimicrobial susceptibility using the disk diffusion method to determine if isolates are resistant, susceptible, or intermediate to select antimicrobials. The antimicrobials were selected based on their importance in human health and use in cattle medicine. Data was analyzed qualitatively for epidemiological significance based on resistance profiles, sample type, and spatial location on the ranch.

Key findings/significance: Resistance to β-lactams, potentiated β-lactams, and tetracyclines was found in presumptive *E. coli* isolates from manure and water samples. Resistance was also found in presumptive *Enterococcus* isolates from water, soil, and manure samples. Similar resistance profiles have been found on similar cow-calf operations. This research is significant because these results provide the first year of data for long-term surveillance of antimicrobial resistance on this ranch. Laboratory methods for isolation of fecal bacteria from environmental samples were also developed.

Source of Funding: University of Calgary, Faculty of Veterinary Medicine

Evaluation of Antimicrobial Usage by Veterinary Practitioners and Dairy Farmers in Québec Dairy Farms

Marie Archambault, Simon Dufour, David Francoz, Hélène Lardé*, Jonathan Massé, Jean-Philippe Roy

Introduction: With increasing interest in evaluation of the impact of antimicrobial usage (AMU) on antimicrobial resistance, international health organizations have highlighted the importance to monitor AMU in human and veterinary medicine, as well as in agriculture. In order to implement adapted action plans in terms of reduction of AMU and promotion of a more judicious AMU, it is essential to know and follow the quantity, nature, and ways antimicrobials (AMs) are used.

Project description/methods: Main objectives were (1) to evaluate AMU practices of veterinarians and producers working with dairy cattle in Québec, (2) to compare different methods of quantification of AMU in dairy farms, and (3) to provide baseline data prior to implantation of new strategies on AMU management. Members of the Association of Veterinary Medicine Practitioners of Québec were surveyed on their practices through an online questionnaire during the fall 2018. In 2017, 101 dairy farms were recruited and followed for one year. A method called "garbage can audit" was set up in order to quantify AMU. This method was compared to other quantification methods which have shown potential as monitoring system: (1) veterinary invoices, (2) treatment records on farm, and (3) data obtained from the provincial program ASAQ. The 101 producers completed an exhaustive questionnaire on their AMU habits.

Key findings/significance: Canadian Defined Daily Doses (DDDbov) and Defined Course Doses (DCDbov) were established in order to measure AMU in cattle, and were used throughout the project. The main route of administration identified was the intramammary route. Category I AMs (very high importance in human medicine) were mainly used for the treatment of mastitis. With the exception of acute mastitis and neonatal diarrhea, veterinary practitioners rarely performed tests to identify a causative agent before treatment. Treatment protocols were not always present or applied, even for the most common infectious diseases. According to the garbage can audit method, a median of 208 DCDbov was used per standardized 100-cow farm per year. Veterinary invoices were an effective way to quantify reliably AMU. Benefits of this study are numerous: (1) This study provides an overview of current AMU in Québec dairy farms; (2) This study allowed us to propose a monitoring system based on the extraction of veterinary invoices (from the software VetExpert® in Québec); (3) The data pinpointed that future action plans should be focused on reduction of intramammary treatments, and promotion of ancillary tests and treatment protocols.

Source of funding: This work was carried out thanks to financial support from the Agri-Food Innov'Action Program, a program resulting from the "Growing Forward 2" agreement between the Québec Department of Agriculture, Fisheries and Food (MAPAQ) and Agriculture and Agri-Food Canada (AAFC). Hélène Lardé was supported by the Alexander Graham Bell Canada Graduate Scholarship (Doctoral Program) from the Natural Sciences and Engineering Research Council of Canada (NSERC), and by scholarships from the "CREATE in Milk Quality Program" of the NSERC and from the "Op+lait Regroupement pour un lait de qualité optimale" of the "Fonds de Recherche du Québec - Nature et Technologies" (FRQNT).

The application of "Nemabiome" deep amplicon sequencing to investigate gastrointestinal nematode species abundance and anthelmintic resistance in sheep flocks

Camila Queiroz, Russell Avramenko, Elisabeth Redman, Fabienne Uehlinger, Michel Levy, John Stuart Gilleard

Introduction: Anthelmintic resistance is a threat to sustainable parasite control worldwide in both animals and humans and is a one of the most important disease challenges faced by the sheep industry worldwide. The parasitic nematodes *Haemonchus contortus* is both a major economic and welfare problem in sheep and the leading parasitic nematode model in which to study anthelmintic resistance. In Canada, despite a sub-optimal climate, *H. contortus* and related nematode species are widespread and anthelmintic resistance has emerged as a major problem. Conventional methods of detecting resistance such as the Fecal Egg Count Reduction Test (FECRT) are still used, however, its results can be difficult to interpret. The Nemabiome approach is based on "microbiome-style" next-generation deep sequencing of ITS-2 rDNA and allows large-scale analysis to determine which nematode species are becoming resistant to anthelmintic drugs. We are also applying deep amplicon sequencing to screen for single nucleotide polymorphisms (SNPs) associated with benzimidazole drug resistance of several gastrointestinal nematode species. This approach allows large scale screening and can detect the emergence of resistance at its earliest stages.

Methods: From 2014-2018 we have received fecal samples from producers throughout western Canada to examine parasite populations before and after anthelmintic drug treatments. We also visited sheep farms located in Alberta and Saskatchewan to perform FECRT, currently the standard method for anthelmintic resistance diagnosis. Fecal samples were collected from groups of 20 ewes before and after treatment with different anthelmintics, the number of nematode eggs per gram of feces quantified and populations of first stage larvae harvested. The phenotypic classification as "resistant" and "susceptible" was determined by the % reduction in eggs per gram after treatment. We then applied ITS-2 rDNA nemabiome sequencing to determine the drug resistance status of each parasite species and isotype-1 β-tubulin amplicon sequencing to screen for benzimidazole resistance mutations.

Results: We found *H. contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* to be the most abundant parasite species in western Canadian sheep flocks with the former species being the most commonly resistant to both benzimidazoles and ivermectin. The isotype-1 β-tubulin F200Y allele was present at high frequency in all *H. contortus* populations, and at lower frequencies in most *T. circumcincta* and *T. colubriformis* populations. Hence resistance is already widespread in *H. contortus* and emerging in the other two species. We also have also used the related phenotypic and genotypic data to validate the use of the F200Y allele as a benzimidazole resistance diagnostic marker as an alternative to the *in vivo* FECRT test. The next step is to perform haplotype network analysis of resistance and susceptible alleles to investigate the patterns of emergence and spread of benzimidazole resistance.

Funding: AITF (scholarship) and AAF (grant).

Do Pesticides Make Honey Bee Larvae More Susceptible to European Foulbrood?

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Introduction: European foulbrood (EFB) is a disease of larval honey bees (*Apis mellifera*), caused by the gram-positive bacteria *Melissococcus plutonius* (*M. plutonius*). This bacterium infects the digestive tract of developing bees, leading to starvation and death. Recently, EFB outbreaks have been associated with honey bees pollinating blueberries. Propiconazole is a triazole fungicide that is commonly used on blueberry fields. This pesticide is also applied to canola in Saskatchewan in combination with the neonicotinoid insecticide thiamethoxam. Foraging honey bees collect nectar and pollen contaminated with sublethal doses of these pesticides which may immunosuppress the larvae.

Project Description/Methods: There were two main objectives for this project:

- To establish a European foulbrood larval infection *in vitro*
- To determine if honey bee worker larvae are more susceptible to European foulbrood when exposed to propiconazole, thiamethoxam, or their combination

Newly hatched worker bee larvae were transferred into the laboratory and reared *in vitro* for one week. A pure culture of *Melissococcus plutonius* (0 CFU, 40 CFU or 400 CFU) was used to infect the larvae on the first day. The larvae were fed daily with control or pesticide-contaminated diet containing thiamethoxam (1 or 10 mg/L); propiconazole (14 mg/L); or the combination of thiamethoxam (1 mg/L) and propiconazole (14 mg/L). Larval survival was recorded daily. Results were analyzed using a one-way ANOVA.

Key Findings/Significance: Larval infection with European Foulbrood was successfully replicated *in vitro*. *Melissococcus plutonius* was identified and isolated from the infected larvae, demonstrating Koch's postulates of disease causation. There was no significant effect of thiamethoxam treatment on larval survival after infection with 40 CFU of *Melissococcus plutonius* ($F(2,12) = 1.17$, $P = 0.3348$) when compared to control larvae. Similarly, exposure to propiconazole or the combination of thiamethoxam with propiconazole did not significantly increase larval mortality after infection with 40 CFU of *Melissococcus plutonius* ($F(2,10) = 0.73$, $P = 0.5073$) relative to controls. Being able to reproduce an EFB infection *in vitro* is beneficial since researchers can manipulate environmental variables in a more controlled setting. Future studies can look at different strains of EFB found in Canada and their associated virulence. *In vitro* models of EFB can also be used to better understand this disease and determine the cause of its association with blueberry pollination. Considering that the concentrations of thiamethoxam tested in this study were 270-2700 times environmental concentrations, and the propiconazole dose represents worst-case field exposure, it is unlikely that honey bee larvae exposed to pesticides in these environments would be predisposed to European foulbrood.

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Simulation modelling of infectious salmon anemia spread in farmed salmon population in British Columbia

João Romero, Tariq Halasa, Derek Price, Krishna Thakur, Ian Gardner

Introduction: Infectious salmon anemia (ISA) outbreaks have caused significant economic losses in the salmon farming industry worldwide, given its complexity for disease management. The introduction of ISA in a na"i've population (e.g., farmed salmon on the British Columbia (BC) coast, Canada) can be devastating. However, it is hard to estimate their magnitude without prior knowledge of the disease dynamics in local populations. A useful approach to tackle this limitation is to resort to mathematical models that simulate disease transmission, based on parameters obtained from previous outbreaks in different locations. Stochastic models that account for the spatial transmission of disease are particularly helpful in these situations since these allow to assess the extent of disease spread under different conditions. These models provide critical information that assists regulatory agencies in establishing decision-making policies.

Objectives: The present study aimed to simulate ISA spread in a farmed salmon population on the BC coast of Canada, according to different number of initially infected farms (index farms) and varying location of these farm sites.

Methods: The most recent implementation of the public domain DTU-DADS simulation framework, the DTU-DADS-Aqua, was adapted to account for disease spread in marine environments. A SIR model was used to model the spread of ISA between salmon farms in BC. Since we do not have prior information on disease dynamics in BC, parameters were estimated based on the characteristics of 2003 ISA outbreak in New Brunswick. Connectivity between farms was incorporated in the model as a weighted seaway distance. Bay management area (BMA) affiliation of the farms was used to build different outbreak scenarios, in which the source of infection would originate from each BMA at a time and in varying number of farms.
Findings: Our results suggest that a higher number of index farms correspond to heavier infection pressure, and, therefore transmission of infection to a greater number of subsequent farms.

Source of funding: Ocean Frontier Institute.

Development of a variant infectious bursal disease (varIBDV) immune complex vaccine to immunize broiler breeder to protect broiler progeny against varIBDV infection

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Introduction: Infectious bursal disease (IBD) is an economically important disease of 3 to 6 week old broiler chickens. Infectious bursal disease virus (IBDV) is classified as apathogenic or pathogenic. The pathogenic serotypes of this virus are further classified as classical, very virulent or variant. Classical are vaccine strains with no mortality, very virulent (vv) lead to high mortality and variant (var) cause severe immunosuppression. vvIBDV have not been reported in Canada while varIBDV is widespread. Due to the severe immunosuppression caused by varIBDV, secondary infections, reduced weight gain and poor flock performance is reported. In an attempt to control varIBDV in Canada, broiler breeder parents are hyperimmunized to transfer maternal antibodies (MtAb) to broiler progeny. Unfortunately, circulating varIBDVs are ever changing and are able to escape the immunity derived from currently available vaccines. Therefore, the objective of our study was to develop a broiler breeder immune complex (Icx) vaccine to protect broiler progeny against varIBDV.

Materials and Methods: The virus used in this study (varIBDV-SK09) was propagated in specific pathogen free leghorns. Bursa of Fabricius was collected, pooled and homogenized to obtain a virus stock. The virus stock was then titrated by embryo infective dose 50 (EID50). The immune complex vaccine was prepared by mixing varIBDV-SK09 (1 x 103 EID50) and antisera (ELISA titer of 200). An inactivated varIBDV-SK09 vaccine was prepared by inactivating in 0.2% formalin solution followed by three dialysis for a final dose of 1 x 104 EID50 and a live vaccine was prepared by preparing 1 x 102 EID50 for each dose. Both the inactivated and live vaccines were used a positive controls.

Broiler breeder parents (n=20/group) were randomly divided into six groups [1 = saline, 2 = Icx (one dose), 3 = Icx (two doses), 4= Icx + inactivated, 5 = live and 6 = live + inactivated]. Broiler breeders were vaccinated at 13 weeks of age (live and first Icx dose) and 16 weeks of age (inactivated and second Icx dose).

The efficacy of the vaccines were studied by broiler progeny studies. Briefly, broiler progeny were obtained from each broiler breeder group (n=15/group), and compared with commercial broilers (n=15). All broilers were orally challenged with 1 x 102 EID50 of varIBDV (homologous and heterologous strains) at 6 days of age. Bursae were collected for viral load detection.

Key Findings: The results of this study demonstrate that broiler breeders vaccinated with an Icx varIBDV-SK09 vaccine are able to control varIBDV infection in broiler progeny until 3 weeks of age until MtAb decline. Additionally, the viral load in broiler progeny with MtAb was delayed. However, this vaccine may not provide complete protection after MtAb decline, therefore a broiler vaccine is necessary to develop in addition to a broiler breeder vaccine to control varIBDV in Canada.

Funding: Funding provided by Chicken Farmers of Saskatchewan (Saskatchewan Chicken Industry Development Fund), Saskatchewan Agriculture Development Fund, Natural Sciences and Engineering Research Council of Canada (NSERC), Alberta Livestock and Meat Agency (ALMA), Alberta Chicken Producers and personal funding from MITACS.

Linking Morphotype and Genotype for *Eimeria leuckarti*

Roseann Kehoe (OVC 2021)

Eimeria leuckarti is a protozoan parasite infecting equids worldwide. This protozoan parasite infects the enterocytes of the small intestine and can cause clinical signs of diarrhea and enteritis in foals as young as four weeks of age. *Eimeria leuckarti* oocysts are ovoid, thick-walled, dark brown, and unusually large (94µm X 79µm). Other *Eimeria* species infecting equids have been described possessing morphologically similar oocysts (*E. solipedum*, *E. uninugulata*). Their status as valid species is in question. Through this project, the morphotype and genotypic analysis of *Eimeria leuckarti* oocysts has demonstrated no species or strain differences. This project also generated the baseline mitochondrial DNA data from large, heavy walled oocysts.

Detection of Nicoletella semolina by qPCR in the airways of horses

Flavie Payette, Audrey Charlebois, Valerie Dubuc, Mathilde Leclere

Background: Nicoletella semolina was first identified in horses in 2004. Apparent low prevalence (≤ 6%) could be due to its fastidious growth and difficult identification using biochemical tests. Whether N. semolina is part of the normal microbiota or acts as a primary pathogen remains unclear.

Objectives: To develop a molecular technique to identify N. semolina and to determine its prevalence in the nose, mouth and bronchoalveolar lavage fluid (BALF) of 6 healthy and 6 severely asthmatic horses housed on pasture, and then indoors while fed hay.

Methods: qPCR primers were designed to target an amplicon in the sodA gene region to specifically amplify N. semolina. Results: When horses were on pasture, 3/12 oral and 5/12 nasal washes were positive for N. semolina, while 7/12 and 8/12 of the oral and nasal washes, respectively, were positive when horses were housed indoors (25 to 66 % positive samples). N. semolina was not detected in BALF. In addition, N. semolina was present in large amount in the tracheal wash of a foal with pneumonia (along with Rhodococcus equi). Him and his dam were still positive for N. semolina 3 months later.

Conclusion and relevance: The use of qPCR will simplify the identification of N. semolina in future epidemiological studies and could lead to higher prevalence than previously observed. Although no association with severe equine asthma was observed in this small number of horses, its association with airway inflammation and as a potential pathogen should be investigated.

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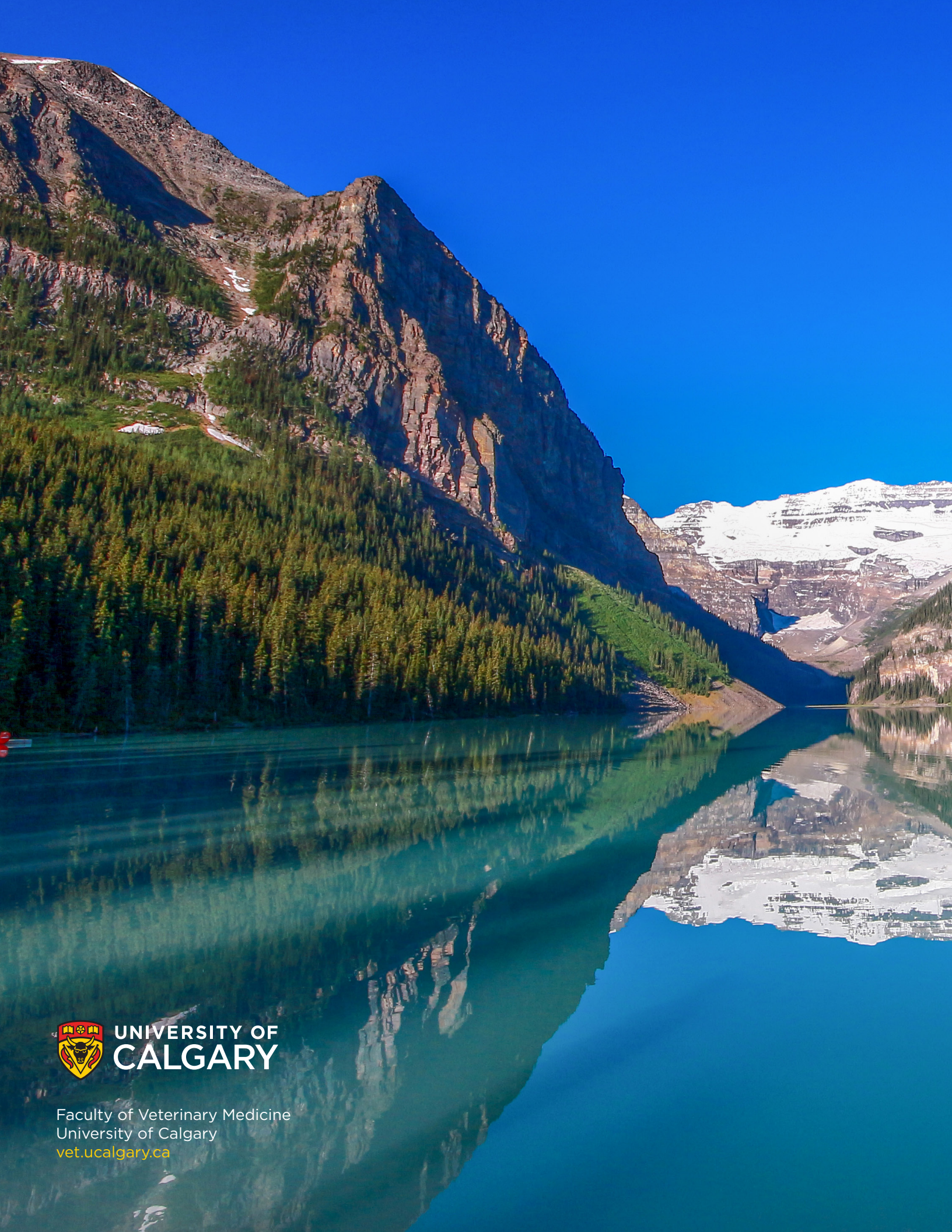

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