UNIVERSITY of GUELPH

CHANGING LIVES
IMPROVING LIFE

2014 Graduate Student Research Symposium and Schofield Lecture

Ontario Veterinary College, November 2014

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Preface

It is our great pleasure to welcome you to the 2014 Graduate Student research symposium (GSRS). This volume contains the papers presented at 2014 GSRS held on November 12, 2014 in Guelph. There were 65 submissions. The program includes 24 talks and 41 poster presentations.

We would like to thank graduate students and faculty members for their participation and to the many sponsors of our research here at the Ontario Veterinary College. The Organizing committee hopes that you will find this symposium compelling and thought-provoking and that the symposium will provide you with a valuable opportunity to share ideas with other students and researchers.

Shayan Sharif, Associate Dean, Research and Innovation (Acting)
Elizabeth Lowenger, Office of the Dean
Barb Gaudette, Office of the Dean
Courtney Schott, Pathobiology
Neda Barjestah, Pathobiology
Kayla Price, Pathobiology
Nicole Weidner, Clinical Studies
Lauren Wallar, Population Medicine
Jennifer Giffin, Biomedical Sciences
Shannon Westgarth, Clinical Studies
Stephanie Hughes, Population Medicine
Faisal Alibhai, Biomedical Sciences

OVC
Ontario Veterinary College

OVC Research and Innovation

2014 Graduate Student Research Symposium
&
2014 Schofield Memorial Lecture

Wednesday, November 12
OVC Lifetime Learning Centre

Featuring:
Dr. Corrie Brown DVM, PhD, DACVP

Corrie Brown received her B.Sc. in Animal Behavior from McGill University and her DVM from Ontario Veterinary College at the University of Guelph. She completed a combined residency/ PhD in Comparative Pathology at the University of California at Davis. She was an assistant professor of pathology at Louisiana State University briefly before joining the U.S. Department of Agriculture at Plum Island, where, as Head of the Pathology Section, she specialized in the diagnosis and pathogenesis of transboundary animal diseases. In 1996, she joined the University of Georgia College of Veterinary Medicine as Professor of Veterinary Pathology. In 2003, she was honored with the university's highest teaching award, being named a Josiah Meigs Distinguished Teaching Professor. Dr. Brown has worked internationally in building animal health infrastructure and diagnostics for more than 25 years. She has conducted workshops on basic field necropsy and diagnostic techniques in 30 countries. Dr. Brown has served on many national and international expert panels about animal health and has received numerous awards for her efforts. She is happiest when working on animal health issues with veterinarians in a developing country setting.

Topic:
Lessons and Lesions from Around the World

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Schedule of Activities:

10:00 - 11:30 AM	**Poster Presentations**	**1707 B/C**
12:30 - 3:30 PM	**Oral Presentations**	**1713/1715**
3:30 - 4:30 PM	**Schofield Memorial Lecture**	**1714**
4:30 PM	**Awards Ceremony & Reception**	**1707**

GRADUATE STUDENT RESEARCH SYMPOSIUM – NOVEMBER 12, 2014

POSTER SESSION WITH GRADUATE STUDENTS, ROOM 1707 - 10:00 – 11:30

1	Jennifer Kylie	DVSc	The rabbit fecal microbiome: Antimicrobial use and the presence of antimicrobial resistance
2	Shannon Westgarth	DVSc	The Individual and Combined Effects of Long-chain n-3 Polyunsaturated Fatty Acids and Low-dose Aspirin on Platelet Function in Healthy Dogs
3	Miranda Abrahams	DVSc	Clostridium difficile prevalence in foals on ontario breeding farms: a longitudinal study
4	Miranda Abrahams	DVSc	Infectious agents in diarrheic foals on ontario breeding farms: a case control study
5	Lynn Williams	DVSc	Equine allogeneic umbilical cord blood mesenchymal stromal cells reduce acute synovial fluid nucleated cell count and induce low-grade sustained inflammation when evaluated in a LPS induced synovitis model
6	Fernanda Mantovani	DVSc	In vitro effects of epidermal growth factor receptor kinase inhibition on radiation response in canine osteosarcoma cell lines
7	Raishard Haynes	MBS	Establishing the Link Between Par6 and PI3K/Akt Signaling and Its Potential Role in TGFβ-induced Apoptosis
8	Stacey Butler	MSc	Properties of colorectal cancer stem cells and their role in chemoresistance
9	Ritesh Briah	MSc	Isoform specific Akt inhibition and lung cancer tumourigenesis In-Vitro
10	Sashen Rajagopaul	MSc	Pet Owner Attitudes and Practices Regarding Canine Cancer and Nutrition
11	Bhairavi Sivaramalingam	MPH	A scoping review of research on the effectiveness of food safety education interventions for consumers
12	Lauren Ramsay	MSc	Sodium intake in urban, peri-urban and rural Cambodia determined by 24-hour urine samples
13	Kelsey Spence	MSc	Social network analysis of a single equestrian show to determine the potential for infectious disease transmission in horse populations
14	Anson Wu	MSc	Comparative Analysis of the Genomes of the Fish Pathogen Flavobacterium psychrophilum
15	Allison M. Barre	MSc	Characterization of the Relationship between Two Bacterial Pathogens of the Swine Soft Palate Tonsil
16	Maryse Darch	MSc	Cytoprotective role of Cytochrome P450 2A5 in bilirubin metabolism during liver injury
17	Hawmid Azizi	MSc	Effect of docosahexaenoic acid (DHA) and epigallocatechin gallate (EGCG) on amyloid-precursor protein (APP) processing
18	Cristine Reitz	MSc	The Circadian Clock Regulates T Cells and Benefits Healing after a Heart Attack
19	Simone ten Kortenaar	MSc	Efficacy of 3TSR Fusion Proteins on Epithelial Ovarian Cancer
20	Casandra Merrill	MSc	Mitochondrial dynamics in slow and fast growing preimplantation bovine embryos
21	Zhi Hui Sim	MSc	Cyclooxygenase-2 in feline eyes with and without uveitis
22	Beryl Chung	MSc	Developmental regulation of nicotinic receptor signaling in mouse hippocampal CA1 pyramidal neurons
23	Joshua Antunes	MSc	The role of microRNAs in bovine ovarian development and angiogenesis
24	Jessica Walsh	MSc	Ear notching of mouse pups does not require analgesia as assessed by the Mouse Grimace Scale and behavioral scoring
25	Wesley Rose	MSc	A scoping review of the evidence for efficacy of acupuncture in companion animals
26	Abbie Viscardi	MSc	Development of a pig grimace scale for evaluation of pain and analgesia efficacy in preweaned pigs
27	Georgia Kritikos	MSc	Analysis of vitamin D3 concentration in commercial dog foods
28	Lauren Wallar	PhD	Determining community well-being priorities in Guelph, Ontario
29	Lauren Dawson	PhD	Recognition and management of patient fear in the veterinary clinic setting
30	Megan Strachan-Whaley	PhD	Combining Histone Deacetylase Inhibitors with Oncoviral Therapy as a Novel Treatment for Leukemia
31	Christopher Pinelli	PhD	Macrophages secrete factors that alter prostate cancer cell proliferation and growth behaviour in vitro
32	Laurence Tessier	PhD	RNA-seq profiling of the bronchiolar epithelium: What does the transcriptional landscape reveal about gene regulation in recurrent airway obstruction in horses?
33	Faisal Alibhai	PhD	Circadian Clock Disruption Worsens Cardiac Remodeling with Age
34	Graham Gilchrist	PhD	miRNA Expression Patterns in Bovine Oocytes and Zygotes
35	Anja Stojsin	PhD	Plasma anti-Mullearian hormone predicts ovarian diameter and antral follicles count based on the cattle breed
36	Andrea Thomas-Bachli	PhD	Relative performance of dead corvids and mosquito pools in surveillance for West Nile virus in southern Ontario, 2002 - 2008
37	Stewart Russell	PhD	The PIWI Pathway in the Early Embryo and Potential Isoforms of PIWIL1
38	Katie Clow	PhD	Understanding the current distribution of the blacklegged tick and the risk of Lyme disease in Ontario
39	Shirene Singh	PhD	Systemic Immune Responses to an Inactivated H9N2 Virus Vaccine with a Toll-like Receptor 21 Adjuvant
40	Shaimaa Abdelmegid	PhD	Proteomic Analysis of Bovine Milk from Cows infected with S aureus subclinical Mastitis
41	Katherine E. Bishop	PhD	Seasonal Changes in Prevalence of Acute Gastrointestinal Illness in Rigolet, Nunatsiavut, Canada

GRADUATE STUDENT RESEARCH SYMPOSIUM – NOVEMBER 12, 2014

12:30-13:22	**ORAL PRESENTATIONS I**	
	Room 1713	**Room 1715**
12:30-12:43 1	**Mythri Viswanathan (MSc)** Molecular and statistical analysis of Campylobacter and antimicrobial resistant Campylobacter carriage in mammalian wildlife and livestock species from Ontario farms (2010)	**Liz-Valery Guieu (DVSc)** Daily post-operative evaluation of peripheral blood and abdominal drain fluid from dogs with closed suction abdominal drains to identify septic peritonitis following gastrointestinal surgery in vitro
12:43-12:56 2	**Kevin J. Stinson (MSc)** Early mucosal antibody responses in a subclinical Johne's disease calf model	**Alexandra Mitcham (MSc)** Comparison of two methods for the assessment of impulsivity in companion dogs
12:56-13:09 3	**Alexandra Rasiuk (MSc)** Role of Type I Interferon Signaling in Regulating Cytokine Expression in Dendritic Cells and Macrophages	**Nicole Weidner (MSc)** Dietary vitamin D intake and vitamin D status in canine cancer patients
13:09-13:22 4	**Diane Gibbard (MSc)** Assessment of capsule-endoscopy technology as a diagnostic tool for imaging the small intestine of the horse	**Amanda Kubik (MSc)** The effects of iron deficiency and anemia on nursery pig performance
13:22-13:32	**BREAK**	
13:32-14:24	**ORAL PRESENTATIONS II**	
	Room 1713	**Room 1715**
13:32-13:45 1	**Jose Denis-Robichaud (PhD)** Survey of Management of Reproduction on Canadian Dairy Farms	**Mackenzie Slifierz (PhD)** The early-life microbiota of the domestic pig
13:45-13:58 2	**Monica Baquero (PhD)** Early effects of bovine γδ T lymphocyte subsets on macrophages infected with Mycobacterium avium subsp. paratuberculosis	**Neda Barjesteh (PhD)** Reduction of avian influenza virus shedding by administration of Toll-like receptor ligands in chickens
13:58-14:11 3	**Adina Bujold (PhD)** The expression of Actinobacillus suis adhesin genes in growth conditions that mimic the host environment	**Kristin Bondo (PhD)** Prevalence and characteristics of Salmonella found on the paws and in the feces of free-ranging raccoons (Procyon lotor) in southern Ontario, Canada
14:11-14:24 4	**Nelson Ho (PhD)** Effects of 3-bromopyruvate on human colorectal cancer cells	**Evan Crawford (DVSc)** Biofilm gene expression in Staphylococcus pseudintermedius
14:24-14:34	**BREAK**	
14:34-15:26	**ORAL PRESENTATIONS III**	
	Room 1713	**Room 1715**
14:34-14:47 1	**Elena Tsimakouridze (PhD)** Angiotensin converting enzyme chronotherapy benefits cardiac structure and function in a murine heart attack model	**Jacquelyn Jacobs (PhD)** Identifying behaviours associated with canine resource guarding
14:47-15:00 2	**Sarah Lepage (PhD)** Generation, characterization and multilineage potency of mesenchymal-like progenitors derived from equine ips cells	**Kayla Price (PhD)** Live Eimeria vaccination success in the face of artificial non-uniform vaccine administration in conventionally reared pullets
15:00-15:13 3	**Keith Russell (PhD)** Platelet lysate as alternative to fetal bovine serum in equine and canine mesenchymal stromal cell culture	**Shawn MacKenzie (DVSc)** Accuracy and safety of image guided percutaneous injection of gelified ethanol in the lumbo sacral intervertebral disc in dogs
15:13-15:26 4	**Allison Tscherner (PhD)** Regulatory mechanisms controlling microRNA availability during bovine in vitro oocyte maturation	**Tanya M. Rossi (PhD)** Cardiac Troponin Assays in Equine Medicine: Analytic and Clinical Validation
15:30-16:30 **LLC 1714**	**SCHOFIELD LECTURE:** **"Lessons and Lesions from Around the World"** **Dr. Carrie Brown** DVM, PhD, DACVP Professor of Veterinary Pathology University of Athens, Georgia	
16:30-17:00	**RECEPTION & PRESENTATION OF AWARDS**	

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Proteomic Analysis of Bovine Milk from cows infected with *S aureus* Subclinical Mastitis.

Abdelmegid S.[1], Atalla H.[1,2], Mackay A.[1], Caswell J.[2], Kelton D.[3], Corredig M.[4] and Kirby G. [1]

[1,] Department of biomedical Sciences, Ontario Veterinary College, University of Guelph

[2,] Department of Pathobiology, Ontario Veterinary College, University of Guelph

[3,] Department of Population Medicine, Ontario Veterinary College, University of Guelph

[4,] Department of Food Science, Ontario Agriculture College, University of Guelph

S. aureus is the most common contagious pathogen associated with bovine subclinical mastitis. Diagnosis of *S. aureus* bovine mastitis relies mainly on bacteriological culture of milk samples along with non-pathogen specific screening test i.e. somatic cell count. The objective of the current study was to identify protein biomarkers of *S aureus* subclinical mastitis in dairy cows using a proteomic approach. Proteomic analysis was performed on milk proteins from healthy cows and cows suffering from *S aureus* subclinical mastitis. The analysis was performed using different techniques that were either; a) gel-based techniques i.e. (2D-GE) and (2D-DIGE) and b) gel-free i.e. LC-MS/MS. Composite and quarter milk samples were collected from 14 healthy (n=7) which tested negative to *S aureus* bacterial culture and infected cows with *S aureus* subclinical mastitis (n=7) which tested positive to *S aureus*. Whey fractions were prepared with differential centrifugation and then subjected to 2D-DIGE and LC-MS/MS and label free quantification to profile protein expression between the two groups. Preliminary results identified varying levels of expression of low abundant proteins i.e. Haptaglobin and Cathelicidin in mastitic milk fractions when compared to those fractions of the healthy milk. Results also indicated that the label free quantification provides a robust tool to screen the modulation of milk proteins profile mastitis and to identify diagnostic biomarkers of this disease. The previous proteomic studies used different processing and clean-up methods, while the methods reported in this study allowed the identification of minor milk proteins with minimal sample preparation.

Funding source: OVC grants

Infectious agents in diarrheic foals on Ontario breeding farms: a case control study

M Abrahams[1], H Staempfli[1], C Leutenegger[2], JS Weese[2].

[1] Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2] Molecular Diagnostics, IDEXX Laboratories, Inc., California, USA-----

Diarrhea is a common problem in foals, and while there are various possible causes, a large percentage of cases remain undiagnosed. The objectives of this study were to evaluate shedding of putative pathogens in diarrheic and healthy foals.

Fecal samples were collected from diarrheic foals (n=25) and compared to samples from the same foal collected within 2 weeks prior to and/or following onset of diarrhea (n=27), as well as healthy age matched foals from the same farm (n=10). Samples were tested by real-time PCR for equine rotavirus, equine coronavirus, *Clostridium difficile* (*cdtA* and *cdtB)*, *Neorickettsia risticii, Clostridium perfringens* alpha toxin, *Lawsonia intracellularis, Rhodococcus equi, Cryptosporidium* spp., and *Salmonella* spp.

One or more positive results were obtained for 14 (56%) diarrheic foals, 16 (59%) foals prior to or following the onset of diarrhea and 6 (60%) healthy controls. There was not a significant difference in the number of positive results between groups for each pathogen (all Wilcoxon P>0.35). Co-infections were identified 9/25 (36%) diarrheic foals and 9/27 (33%) foals prior to or following onset of diarrhea, but no healthy controls (P=0.02).

Enteropathogens were commonly detected in diarrheic and non-diarrheic foals, with co-infections differing between groups. These results highlight the difficult nature of diagnosis of neonatal diarrhea and the need for further study of co-infections.

Funding source: Equine Guelph

Clostridium difficile prevalence in foals on Ontario breeding farms: a longitudinal study

M. Abrahams, H. Staempfli, M. Walker, JS. Weese.
Ontario Veterinary College, Guelph, ON.

The objectives of this study were to longitudinally evaluate the prevalence of *C. difficile* shedding by foals, characterize *C. difficile* isolates and evaluate the impact of *C. difficile* shedding by mares on colonization of their foals.

Fecal samples (n=1830) were collected from 162 Thoroughbred and Standardbred foals and their dams at 2 week intervals from birth until approximately 4 months of age on 8 breeding farms in southern Ontario. Foals that developed diarrhea had additional fecal samples (n=50) collected at the time of disease occurrence. Fecal samples were cultured for *C. difficile* using standard culture techniques and characterized by PCR ribotyping and detection of toxin genes.

Of the samples processed to date, 40 of 604 (7%) samples were positive for *C. difficile*, 30/350 (8.5%) from foals and 10/254 (4%) from mares. Both diarrheic (4/30) and clinically healthy foals (26/30) shed *C. difficile.* 70% of foals shedding *C. difficile* were neonates (less than one month of age), and neonates were significantly more likely to shed *C. difficile* than older foals (p=0.036). Shedding was not significantly correlated with diarrhea in foals. All isolates were toxigenic and 21 different ribotypes were identified, including ribotypes which have been reported in human infections.

These results demonstrate that younger foals are significantly more likely to shed *C. difficile* and that shedding does not have an association with diarrheal disease, likely due to the large number of other potential pathogens causing foal diarrhea. The presence of ribotypes previously identified as human pathogens has further implications for biosecurity practices and public health.

Funding source: Equine Guelph

Circadian Clock Disruption Worsens Age Dependent Cardiac Remodeling Cardiac Function

Faisal J. Alibhai, Elena V. Tsimakouridze and Tami A. Martino

Cardiovascular Research Group, Department of Biomedical Sciences, University of Guelph, Ontario, Canada

Background: The circadian clock regulates cellular processes throughout the body over 24hrs to ensure normal heart function over the day/night cycle. Aging is associated with structural and function changes in the heart which increases the risk of cardiovascular disease. However, how the circadian clock regulates cardiovascular aging is not known.

Hypothesis: Maintenance of circadian clock function is crucial for healthy cardiovascular aging; disruption of the circadian clock promotes cardiac dysfunction with age.

Methods and Results: Cardiac structure and function were followed using echocardiography in transgenic circadian clock mutant and wild type mice 4-21 months of age. *Clock* mutant vs. wild type mice had significantly worse left ventricular (LV) diastolic (4.89±0.06mm vs. 4.35±0.03mm) and systolic dimensions (3.49±0.06mm vs. 2.90±0.04mm), and worse LV % fractional shortening (28.60±0.89% vs. 34.00±0.61%) and % ejection fraction (61.15±1.49% vs. 69.00±0.71%) by 21 months. Moreover, *clock* mutant had greater cardiac hypertrophy indicated significantly increased heart weight (HW) (208.38±7.48mg vs. 175.00±4.02mg) and HW/BW (5.90±0.39mg/g vs 4.70±0.39mg/g) at 21 months compared to wild type mice. Next, using *in vivo* hemodynamics we assessed LV contractile performance and blood pressure at 4, 8, 18 and 21 months and found *clock* mutant mice vs. wild type mice had significantly decreased LV developed pressure (81.17±4.44mmHg vs. 98.27±0.58mmHg) and mean arterial pressure (70.32±0.78mmHg vs. 76.75±0.99mmHg) at 21 months.

Conclusion: These data show that the clock is important for cardiovascular aging and disruption of the clock mechanism promotes cardiovascular dysfunction.

The role of microRNAs in bovine ovarian development and angiogenesis

Antunes, J. and Petrik, J.

Department of Biomedical Sciences

Background: Ovarian follicle development is a tightly regulated, multi-stage process. MicroRNAs have recently emerged as potentially being central players in regulating the ovarian cycle. Previous studies have identified numerous microRNAs associated with both ovarian follicular and luteal development as well as ovarian angiogenesis. However, their exact role in regulation still remains unclear.
Hypothesis: MicroRNAs are integral to the regulation of ovarian function and coordinate ovarian angiogenesis and follicular development, and altered miRNA expression contributes to the onset and progression of ovarian dysfunction and reproductive disorders in the bovine species.
Objectives: 1) Identify the expression of microRNAs in bovine ovarian structures. **2)** Evaluate the role of microRNAs in regulating ovarian angiogenesis and follicular and luteal development. **3)** Determine the contribution of microRNAs to dysregulated ovarian function associated with reproductive disorders.
Impact: The results from these studies will provide new data about the biological mechanisms that regulate ovarian angiogenesis and follicular and luteal development. Although changes in expression of pro- and anti-angiogenic factors have been described, there currently is a significant lack of understanding of how these factors are regulated, particularly in how they reciprocally inhibit each other's expression. The data from these studies will illuminate the roles of miRNAs in regulation ovarian angiogenesis and how they contribute to normal ovarian function and ovarian pathologies such as COD in cattle.

Funding Source: Ontario Veterinary College and CIHR

Effect of docosahexaenoic acid (DHA) and epigallocatechin gallate (EGCG) on amyloid-precursor protein (APP) processing

Hawmid Azizi and Bettina Kalisch

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario

Alzheimer's disease is the leading neurodegenerative disorder in the elderly population. Disproportionate processing of amyloid precursor protein (APP) in the brain is currently considered a major hallmark of Alzheimer ’s disease, resulting in neuronal death and impaired synaptic transmission. Through manipulation of the APP processing enzymes, alpha-secretase (ADAM9, -10, -17) and beta-secretase (BACE), the progression of amyloid-beta (Aβ) plaque formation may be reduced. DHA (the most abundant omega-3 fatty acid found in the brain), EGCG (a potent antioxidant), and catechin have recently been under investigation as promising therapies for the prevention and progression and Alzheimer’s disease. To determine the effect of DHA and EGCG in APP processing, human neuroblastoma were treated with various doses of DHA or EGCG and analyzed for ADAM9, -10 and -17 mRNA and protein expression. Treatment with DHA resulted in an increase in ADAM10 mRNA and protein expression. Treatment with EGCG also resulted in an increase in the expression all ADAM proteases. Combined treatments of EGCG with DHA resulted in a significant increase in ADAM10 mRNA and protein expression. The significant increase at the protein (mature ADAM10) level is suggestive that combined effects are synergistic relative to individual treatments with DHA and EGCG . The synergistic effects that are not seen at the mRNA level is indicative that DHA and EGCG are acting differently and that perhaps these treatments are affecting protein stability or are increasing the processing of ADAM10 to its mature form. Current work is also looking at how treatments are effecting soluble-APPα, the end-product of ADAM10.

Early effects of bovine γδ T lymphocyte subsets on macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis*

Baquero, M and Plattner, B

Department of Pathobiology

Mycobacterium avium subspecies *paratuberculosis* (*Map*) is the etiologic agent of bovine paratuberculosis also known as Johne's disease. This disease is characterized by a long subclinical phase (two or more years) followed by progressive granulomatous enteritis, failure of adequate nutrient absorption, diarrhea, weight loss and eventually death. The pathogenesis and immune responses of early *Map* infection remain incompletely understood; however it is believed that following initial *Map* infection by the fecal-oral route, some calves apparently successfully clear the pathogen while others become persistently infected. The importance of γδ T lymphocytes during early anti-mycobacterial immunity is recognized; however in bovine paratuberculosis the mechanisms by which distinct subsets ($WC1^+$ and $WC1^-$) of γδ T lymphocytes are involved in this process remain unclear. Our hypothesis is that bovine γδ T lymphocyte subsets differentially regulate host immunity during early *Map* infection and that this influences the outcome of the early infection. The first phase of the research seeks to understand how early γδ T lymphocyte subsets responses mediate macrophage function during early *Map* infection. To achieve this objective, monocyte-derived macrophages co-cultured in direct contact or in a transwell system with autologous $WC1^+$ or $WC1^-$ γδ T lymphocyte subsets were infected with *Map* (strain gc86) at a MOI of 10:1. After 72 hours of co-culture, *Map* viability was analyzed with flow cytometry. IL-17, IL-10, IL-4 and IFN-γ were quantified using ELISA and nitrites were measured using a commercial high-sensitivity assay. Our data shows that cytokine profile secretion by bovine $WC1^+$ and $WC1^-$ γδ T lymphocytes is distinct, suggesting that they may differentially modulate macrophage function (*Map* killing) during the first three days after infection with live *Map.* These data suggest that the function of bovine γδ T lymphocytes is different when co-cultured in contact or not with infected monocyte-derived macrophages.

Funding Source: OMAFRA, NSERC, Vanier CGS, Colciencias

Reduction of avian influenza virus shedding by administration of Toll-like receptor ligands in chickens

Neda Barjesteh, Bahram Shojadoost, Jennifer T Brisbin, Éva Nagy, Shayan Sharif

Department of Pathobiology, OVC

Avian influenza viruses (AIV) are of concern in the poultry industry. Outbreaks of AIV underline the urgent need for effective control means. Disadvantages of available vaccines against AIV have been reported. Therefore, other prophylactic strategies should be explored that rapidly elicit immunity against the virus. Toll-like receptors (TLRs) are innate immune molecules that could induce anti-viral responses. The application of TLR ligands as prophylactic agents in chickens is gaining more attention. We hypothesized that treatment of chickens with TLR ligands reduces the shedding of AIV from infected birds. In addition, the effect of TLR ligand dose and route of administration on the efficiency of TLR ligands to reduce AIV shedding were examined. Chickens were treated with TLR2, 4, 7 and 21 ligands using different doses and routes of administration, 18 hours before AIV infection. Moreover, the expression of some candidate genes was quantified at 3, 8 and 18 hours post-treatment with TLR ligands. The results revealed that route of administration and dosage affect the efficacy of TLR ligands to reduce virus shedding. Furthermore, varying effects were observed when different ligands were applied. Our results demonstrated that all TLR ligand treatments reduced AIV shedding, with the CpG-ODN being the most efficacious followed by Pam3CSK4 and LPS from *Escherichia coli* 026:B6. Moreover, TLR ligands induced the expression of genes involved in antiviral responses such as type I interferons and interferon-stimulated genes in chicken trachea and cecal tonsils. These results raise the possibility of treatment of chickens with TLR ligands as antiviral agents.

Funding Source: OMAFRA

Characterization of the Relationship between Two Bacterial Pathogens of the Swine Soft Palate Tonsil

Barre, A.M. and MacInnes, J.I.

Department of Pathobiology

The tonsils of the soft palate of swine play a major role in immune surveillance and paradoxically, are simultaneously a major site of colonization by many bacteria. *Haemophilus parasuis* and *Streptococcus suis* are common residents of tonsils in healthy pigs but can also cause severe disease. There is growing evidence that cohabiting organisms are able to form synergistic and/or antagonistic relationships through metabolic interactions, chemical signals, physical associations, and immunomodulation. Understanding the interplay between *S. suis* and *H. parasuis* will be an important first step in revealing some of the complex polymicrobial interactions in swine tonsils and could lead to the development of new approaches to reduce swine respiratory disease. Initial screening using a variety of virulent and avirulent strains of *S. suis and H. parasuis* co-cultured on agar plates has been used to identify interacting strains. Selected strains are being studied in both broth and biofilms to determine whether the presence of one species affects the growth or phenotype of the other. To further characterize these interactions, direct association assays using transwell culture plates will be used to determine if observed effects are proximity-dependent, while analyzing the culture supernatant will reveal the contribution of diffusible molecules. qRT-PCR experiments are also underway to evaluate the expression of selected virulence genes to determine if there are differences in co-culture vs. mono-culture. Preliminary results suggest that *S. suis* serotype 2 strains may benefit from the presence of *H. parasuis* serotype 3 strains, while *H. parasuis*-3 is negatively impacted by *S. suis*-2.

Funding Source: NSERC

Seasonal Changes in Prevalence of Acute Gastrointestinal Illness in Rigolet, Nunatsiavut, Canada

Bishop-Williams KE[1*], Berrang-Ford L[2], Edge VL[1], Ford J[2], Thomas MK[1], Shiwak I[3], RICG[3], IHACC Research Group[4], Harper SL[1]

[1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada
[2]Department of Geography, McGill University, Montreal, QC, Canada
[3]Rigolet Inuit Community Government, Rigolet, Nunatsiavut, Labrador, A0P 1P0, Canada
[4]Indigenous Health Adaptation to Climate Change Research Group
*Presenting and Corresponding Author

BACKGROUND: Acute gastrointestinal illness (AGI), including vomiting and diarrhea, can be caused by parasitic, viral, or bacterial pathogens whose transmission can include food, water and contact with various environments. Thus, the prevalence of AGI is often variable by season and affected by changes in weather patterns. As such, climate-related variability of AGI poses particular relevance for many Canadian Inuit because a close relationship with the land is central to well-being. Thus, variability in the climate and changes in season are particularly relevant to exposure and transmission of AGI.

METHODS: Utilizing data from six previously conducted census surveys in Rigolet, Nunatsiavut from fall 2011 to spring 2013, incidence and prevalence of self-reported AGI was estimated. A multi-level mixed effects logistic regression model was used to examine the association between seasonality and AGI when accounting for repeated measures.

RESULTS: During the 2-week recall periods, incidence rates ranged from 2.94 episodes/person-year in spring to 6.96 in winter. Prevalence estimates based on 4-week recall ranged from 3.24-4.19 episodes/person-year. Winter was the only season with significantly different rates of AGI than other periods of the year ($p<0.001$). Respiratory symptoms, season, and gender variables were significantly associated with the incidence of AGI in Rigolet. An adjusted annualized incidence rate of AGI was estimated at 2.71 episodes/person-year.

CONCLUSIONS: Prevalence estimates vary widely by season and are particularly high in winter. Winters are long and sometimes harsh in the Canadian Arctic, and may be impacting the significant relationship with other seasons. Respiratory conditions, such as influenza, potentially account for the seasonal increase of AGI in Rigolet. Additional work is needed to identify factors driving AGI in other seasons.

Funding Sources: Indigenous Health Adaptation to Climate Change (CIHR, NSERC, SSHRC, IDRC, Health Canada)Graduate Research Assistant Tuition Supplement (OVC)

Prevalence and characteristics of *Salmonella* found on the paws and in the feces of free-ranging raccoons (*Procyon lotor*) in southern Ontario, Canada.

Bondo KJ [1], Pearl DL [2,3], Janecko N [3,4], Boerlin P [1], Reid-Smith RJ [1,3,4], Parmley J [1,2,4], Jardine CM [1,2]

[1] Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[2] Canadian Wildlife Health Cooperative, Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[3] Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[4]Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada, N1G 3W4

Raccoons are common in urban and rural environments and can carry a wide range of bacterial agents, including *Salmonella*, that can affect human and livestock health. Although previous studies have reported that raccoons shed a variety of *Salmonella* serovars in their feces, it is unknown whether *Salmonella* can be carried and transmitted on the paws of raccoons. Our objective was to compare the prevalence of *Salmonella* on the paws and in the feces of raccoons in southwestern Ontario. Raccoons were sampled in a cross-sectional study from May to October 2012 where individuals were sampled repeatedly; 416 paired fecal and paw samples were collected from a total of 285 individuals. We detected *Salmonella* in 18% (75/416; 95% CI, 14–22%) and 27% (111/416) (95% CI; 22–31%) of paw and fecal samples, respectively. *Salmonella* was found both on the paws and in the feces in 10% (40/416) of raccoon captures, while 8% (35/416) had *Salmonella* on the paws but not in the feces, and 17% (71/416) had *Salmonella* in the feces but not on the paws. The final multi-level multivariable logistic regression model included these explanatory variables: sex, sample type, season, sex-season and sex-sample type interaction terms. Random intercepts were included to account for clustering by animal and location identification. We noted significant differences in the prevalence of *Salmonella* carriage between sexes that varied with sample-type and season. Because raccoons can carry *Salmonella* serovars known to infect humans and livestock on their paws and/or in their feces, they have the potential to mechanically and biologically disseminate *Salmonella* among livestock facilities and human recreational areas.

Funding Source: USDA National Institute of Food and Agricultural Grant; NSERC; OGS Scholarship (awarded to KB)

Isoform specific Akt inhibition and lung cancer tumourigenesis In-Vitro

Briah R., Franks S.E., Moorehead R.A.

Department of Biomedical Science, University of Guelph

Lung cancer has the highest cancer related mortality rates and NSCLC constitutes about 85% of cases. Akt is a signaling kinase involved in cell survival, proliferation, metabolism and migration. It has been shown to be hyper-activated in NSCLC cases and is associated with poor prognosis. Previously it was believed that all three isoforms of Akt played similar roles in cellular processes, however recent research is beginning to show differing roles. Our lab was previously able to demonstrate that Akt 1 knockout mice formed fewer tumours than Akt2 knockout mice and appeared to be less invasive as well. These results indicate that Akt1 targeted therapy may be more effective in lung tumour suppression than the pan-Akt inhibitors currently undergoing clinical trials. To investigate this theory we decided to conduct functional experiments on lung epithelial cell lines (A549, NCI-H358, LA4 and NBE-135) to assess tumourigenic properties. Through the metabolic WST-1 assay we were able to generate cell survival curves and determine EC50 dosages of Akt1, Akt2, and pan-Akt inhibitors to their respective cell lines. Results showed that Akt 1 inhibition was more potent than the other two inhibitors. Currently changes in cell cycle phase in treated cells are being investigated through flow cytometry. Apoptosis changes are being assessed through an Annexin V kit. The next steps include isomer specific protein analysis through western-blotting coupled with densitometry. If significant differences are found between treatments then changes in expression of downstream products of Akt such as GSK3β and NFκB will be investigated.

Funding source: CCSRI, CIHR, OGS

The expression of *Actinobacillus suis* adhesin genes in growth conditions that mimic the host environment

Bujold A and MacInnes J
Department of Pathobiology

Actinobacillus suis is a common commensal of swine tonsils, but unknown stimuli can lead to systemic disease. Its pathogenesis, including host colonization, is poorly understood. The objective was to measure expression of genes involved in attachment, a key factor in health and disease.

In healthy animals, *A. suis* is thought to exist in tonsils in biofilm and planktonic forms. Cells in biofilm likely persist in a lower oxygen/nutrient environment in stationary phase. Cells shed from biofilm assume planktonic form, with higher nutrient/oxygen availability. We hypothesize that *A. suis* will differentially express adhesins in these conditions, and that certain signals will lead to an invasive phenotype with a different complement of adhesins.

From 47 adhesin genes identified by bioinformatics of a virulent *A. suis* isolate, 9 were chosen for qPCR. Samples were taken from aerobic cultures at 60 and 180 min post-inoc (mpi) for exponential and stationary phase, respectively, and from anoxic static cultures at 60 and 210 mpi.

All genes were upregulated in one growth phase: type IV pilin *ppdD*, outer membrane proteins *ompA2* and *ompP2*, and fibronectin-binding *ybaV* in exponential, while biofilm-associated *flp*, fine-tangled pili *ftpA*, filamentous hemagglutinin *fhaB*, *ompA1*, and autotransporter *tibA* were upregulated in stationary. Most genes were upregulated in one growth condition: *ftpA*, *ompA1*, and *tibA* in aerobic, and *flp*, *ppdD*, and *ompA2* in anoxic growth. Time by treatment interactions were also observed for several genes.

Knockout mutants are being generated to determine roles in attachment and biofilm. Studies to elucidate cell types/receptors in tonsils have also begun.

Funding sources: NSERC, OVC Scholarship, OGS

Properties of colorectal cancer stem cells and their role in chemoresistance

Butler, S.J., Farias, N., *Coomber, B.L.

Department of Biomedical Sciences

Treatment of advanced colorectal cancer is impacted by drug resistance and tumour heterogeneity. Cancer stem cells (CSCs) are a subpopulation of tumour cells likely responsible for tumour recurrence due to their ability to self-renew, differentiate and evade current treatments. This project explores the responses of CSCs to chemotherapy and identifies therapeutic targets of the CSC population. We used serial culture to enrich for CSCs from two human colorectal cancer cell lines, HCT116 and SW480, based on their ability to form colonospheres in serum-free 3D culture. Limiting dilution analysis showed that CSC cell lines have significantly higher colonosphere formation ability compared to their respective parental cells. CSC cell lines were further characterized via qPCR for their expression of colorectal CSC markers. A significant upregulation in CD166 and EpCAM gene expression was seen in HCT-CSC and SW-CSCs compared to parental cell lines. Cell viability was analyzed via PrestoBlue assays in both monolayer and 3D culture in response to 5-fluorouracil, cisplatin and epirubicin. CSC and parent cell lines responded similarly to treatment in monolayer but CSC colonospheres were slightly more resistant to high doses of chemotherapy than their respective parental cells. Since ABC transporters often confer drug resistance to cancer cells, the expression of *ABCB1*, *ABCC1* and *ABCG2* was examined via qPCR. There was a significant upregulation in *ABCB1* and *ABCG2* in HCT-CSCs compared to HCT-P cells. SW-CSCs also had significant upregulation in *ABCB1* compared to SW-P cells. In the future we will explore ABC transporter and CSC markers expression in response to chemotherapy.

Funding Sources: NSERC, OVC Dean's Scholarship, OGS

Developmental regulation of nicotinic receptor signaling in mouse hippocampal CA1 pyramidal neurons

Chung, B.[1], Jacklin, D.[2], Bignell, W.[1], Winters, B.[2], Bailey, C. [1]

[1]Deptartment of Biomedical Sciences, University of Guelph
[2]Department of Psychology, University of Guelph

The normal development and function of the hippocampus depends on the interaction between the neurotransmitter acetylcholine (ACh) and the nicotinic class of ACh receptors (nAChRs). Although the α4 β2* isoform constitutes a major class of nAChR in the hippocampus, the ability of these receptors to mediate nicotinic signaling specifically in glutamatergic CA1 pyramidal neurons is not well understood. Interestingly, expression of individual α4β2* nAChR subunits within the rodent CA1 pyramidal cell layer is developmentally regulated and greatest during early postnatal life, suggesting that receptor function is greatest in these neurons at this time. We sought to determine whether functional α4β2* nAChRs are present on CA1 pyramidal neurons during the postnatal development of male CD1 mice. Whole-cell electrophysiological responses to ACh were recorded for visually-identified CA1 pyramidal neurons following pharmacological blockade of α 7-isoform nAChRs and muscarinic ACh receptors. We found that ACh elicited postsynaptic inward currents and facilitated neuronal excitation in CA1 pyramidal neurons, and that the magnitude of these nicotinic responses was greatest during the first two weeks of postnatal life. Nicotinic responses were resistant to antagonists of synaptic transmission and were inhibited by the α4 β2* nAChR antagonist dihydro-β-ethroidine, suggesting that responses were mediated by α4β2* nAChRs on recorded CA1 pyramidal neurons. These findings demonstrate that α4β2* nAChRs mediate nicotinic signaling in CA1 pyramidal neurons during hippocampal development, and suggest a role for these receptors in the establishment of hippocampal learning and memory networks.

Funding Source: NSERC Discovery Grant (CDB)

Understanding the current distribution of the blacklegged tick and the risk of Lyme disease in Ontario

Katie Clow, Nicholas Ogden, Robbin Lindsay, Pascal Michel, David Pearl & Claire Jardine

Lyme disease is the most common vector-borne disease in North America and is an emerging disease in Canada. This disease, which is caused by the bacterium *Borrelia burgdorferi,* is transmitted by the blacklegged tick (BLT) (*Ixodes scapularis*). In Ontario, there are eight recognized sites that have established populations of BLTs and endemic cycles of *Borrelia burgdorferi,* but BLTs have been found in a growing number of other areas in Ontario and the incidence of human Lyme disease is increasing. Our research aims to determine the current distribution of BLTs in Ontario and measure the biotic and abiotic factors associated with their occurrence in new areas of the province. We conducted active surveillance at 108 sites across 3 ecoregions in Ontario in the summer and fall of 2014. Each site was surveyed via drag sampling for three person-hours. Of the 105 sites sampled to date, BLTs were present at 27 sites. Ongoing work will determine if these represent newly established populations of ticks that harbour *Borrelia burgdorferi*. This research will contribute to our understanding of the risk of Lyme disease across the province. Enhanced risk maps will be developed to assist with targeted public health preventative measures. Abiotic and biotic data collected at each site will allow us to build statistical models to further understand and predict the spread of the BLT and pathogens associated with this tick species.

Biofilm gene expression in Staphylococcus pseudintermedius

Crawford E., Singh A.

Department of Clinical Studies

Reasons for the rapid emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) as a leading cause of surgical site infections are currently unknown. Biofilm formation may be an important factor; however, detailed examination of the relationships between, and understanding of the regulation of biofilm expression and virulence is lacking. We hypothesize that expression of biofilm and other virulence-associated genes will vary between surfaces and strains of MRSP, and will correlate to biofilm competence and *in vivo* virulence. This study initially confirmed the constitutive expression of several reference genes for use in a quantitative real time PCR assay, which was subsequently validated. This assay was then used to examine the expression of multiple genes including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), biofilm-associated genes, and a quorum sensing system. Expression was subsequently measured in logarithmic and stationary phases, as well as in biofilm from two strains on eight clinically relevant surfaces and in biofilms on stainless steel and titanium for 10 additional strains. Genes responsible for biofilm formation were upregulated in biofilm phases ($p<0.01$). Most MSCRAMMs studied were also upregulated in biofilm phases, with notable strain variations. There were some variations identified in expression of quorum sensing genes, but no clear trends. Upregulation of biofilm and MCRAMM genes in biofilms is expected. The other genes studied may have more complex determinants of expression, and further investigation of the in vivo relevance of these variations is warranted.

Funding Source: OVC Pet Trust, AKC Canine Health Research Fund

Cytoprotective role of Cytochrome P450 2A5 in bilirubin metabolism during liver injury

Darch, M.[1], Lou, Y.[2], and Kirby, G.[1]

[1] Biomedical Science, University of Guelph, Guelph, ON;

[2] Animal & Poultry Science, University of Guelph, Guelph, ON;

The Cytochromes P450 (CYPs) are a superfamily of enzymes involved in Phase I drug metabolism. In mice, CYP2A5 is the only enzyme from this superfamily induced during liver injury. Recently, CYP2A5 was shown to oxidize bilirubin (BR) to biliverdin (BV) in recombinant yeast microsomes expressing CYP2A5. BR is an antioxidant at physiological levels (<10μM), and is usually eliminated via glucuronidation by UDP-glucuronosyltransferase (UGT1A1) as BR accumulation is toxic. We hypothesized that the unique induction of CYP2A5 during liver injury protects against BR-mediated cytotoxicity by increasing BR metabolism. The effect of *Cyp2a5* gene knockdown on BR cytotoxicity was assessed in mouse hepatocytes in primary culture. Hepatocytes were transfected with *Cyp2a5* siRNA and treated with BR at increasing concentrations to 50μM for 12 hours. The expression of *Grp78*, *Xbp-1s* and *Chop* was assessed by qRT-PCR as cytotoxicity endpoints that reflected ER stress. Although BR did increase expression of the ER stress markers, *Cyp2a5* knockdown did not significantly alter their expression. Additionally, to determine the importance of CYP2A5 in BR metabolism relative to UGT1A1, an assay based on reversed-phase HPLC was developed. BV and BR isomers were separated on a C18 column and peaks were detected by a UV-visible detector set at 375nm and 450nm. Retention times for BV and BRIXα were 12.4 and 19.7 minutes respectively. In summary, *Cyp2a5* knockdown did not alter the expression of ER stress markers following BR treatment, and future studies will instead assess apoptosis as a cytotoxicity endpoint. The HPLC assay will be used to determine CYP2A5's efficiency as a BR oxidase in mammalian cells.

Funding Source: NSERC

Recognition and management of patient fear in the veterinary clinic setting

Lauren Dawson*[1], Cate Dewey[1], Elizabeth Stone[2], Michele Guerin[1], Lee Niel[1]

[1] Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada
[2] Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada

In order to receive veterinary care, cats and dogs are commonly brought into a novel environment, where they interact with unfamiliar animals and people, often in unusual ways. As such, most cats and dogs exhibit behavioural signs of fear in the clinic setting. To explore welfare in veterinary clinics, all companion and mixed animal veterinary clinics within a 100km radius of Guelph were invited to participate in a larger study. As part of this study, in person interviews were conducted with 22 veterinarians, each employed at a different clinic, during which participants were asked to describe how they recognize fear in their canine and feline patients. All questions were open-ended and content analysis was performed on all responses. Aggression, body position, and ear position were the three most commonly cited signs of fear (68%, 59%, and 32% for cats; 45%, 36%, and 32% for dogs, respectively). Most veterinarians (95%) listed other fear-related behaviours (e.g. hiding, freezing, avoiding interaction) independent of or in addition to aggression; however, few veterinarians identified more subtle signs of fear, such as yawning (9%) and lip licking (14%). Those surveyed also suggested the approaches and strategies they use to minimize and manage fear in their patients, such as offering treats, administering pheromones, and using towels. Overall, results suggest that veterinarians typically recognize overt indicators of fear; however, they might not observe or correctly interpret more subtle signals. The ability to recognize and manage fear has implications for ensuring that veterinary visits are a positive experience for pets, owners, and veterinary staff members alike.

Funding Source: Pet Trust Fund

Survey of Management of Reproduction on Canadian Dairy Farms

Denis-Robichaud, J., Cerri, R.L.A., Jones-Bitton, A., and LeBlanc, S.J.

Department of Population Medicine, University of Guelph, Guelph, Canada
Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada

Many dairy herds do not achieve their targets for reproductive performance. Despite many advances in technologies and management tools, producer attitudes towards, and frequency of implementation of these tools are not well documented. The objective of the present study was to survey current reproduction management practices in a representative sample of Canadian dairy farms. A survey was developed to assess general and reproduction management in dairy herds. March to May 2014, a bilingual survey was administered to Canadian dairy farmers using internet and mail. Mean, median, and interquartile range were calculated for continuous and ordinal variables, and frequencies were calculated for binary and categorical variables. A total of 833 surveys were completed. Heat detection by observation was used as part of the management strategy to inseminate cows in the vast majority of herds. Cows were observed for heat 3.3 times per day for 36 minutes, on average. Only a third of the respondents reported that no other task was accomplished during heat detection. More than two third of the respondents used prostaglandins or synchronization protocols as part of their reproduction management. Most respondents agreed that reproductive hormones were safe for consumers of dairy products, but less agreed that routine use of synchronization programs was acceptable to them, or to the consumers. Automated activity monitoring systems were used in a third of the herds. Most of the respondents used the system to flag cows in heat, but also rely on heat signs to decide to inseminate. Results from this survey highlighted the variability in reproduction management among Canadian dairy herds.

Funding Source: Dairy Research Cluster 2 (Dairy Farmers of Canada)

Assessment of capsule-endoscopy technology as a diagnostic tool for imaging the small intestine of the horse

Gibbard, Diane; Thomason, Jeff; Armstrong, Warren; Frost, Laura; Greco-Otto, Persephone, and Wilson, Angela.

Department: Biomedical Sciences

Common gastrointestinal tract (GIT) diseases in horses, such as ulcers and colic, often go undiagnosed because less than 10% of the alimentary tract can be examined using gastroscopy or *per rectum*. The use of unattached camera 'pills'—capsule endoscopy (CE) — has been successful in human medicine to diagnose a variety of GIT diseases and has the potential to view the inside of more than half of the horse's GIT. Previous equine studies have had limited success when deploying other types of wireless capsules. The goal of this study was to assess the value of using the human capsule, MiroCam®, in horses.

Image quality, duration of recording, clinical relevance and feasibility to track capsule location were assessed in two normal Standardbred horses that each underwent four CE trials, three using the standard capsule (SC) and one using a modified capsule (MC) with double the signal strength.

We developed a placement map for the EMG-style external electrodes, a feeding/fasting protocol for clearing the intestines while maintaining water intake, and a method for introducing a capsule. These procedures were varied slightly between trials in an attempt to optimize image acquisition and quality.

In the three SC trials, high quality video segments were obtained, with useful clinical information on visible lesions and parasites. But the segments lacked positional information and were of short duration (10s to 13 min), totaling 0.28% to 5.83% of the 12-hour recording sessions. From the SC data, the camera is not yet ready for use in horses. Images from MC trials, which may change this conclusion, are not available for this abstract but will be presented at the symposium.

Funding Source: Equine Guelph, OVC Dean's Fellowship Scholarship

Partners: Halton Equine Veterinary Services, Intromedic Inc.

miRNA Expression Patterns in Bovine Oocytes and Zygotes

Gilchrist G.C., King W.A., and LaMarre J.

Department of Biomedical Sciences

Mammalian oocyte maturation in preparation for fertilization involves many complex molecular processes. An immature oocyte is arrested in prophase I and must undergo germinal vesicle breakdown prior to progression of the cell-cycle through to metaphase II of meiosis after which it can be fertilized. During this process, transcriptional activity in the oocyte decreases. Many facets of these processes remain unknown. MicroRNAs (miRNA), a class of small non-coding RNA, could be important in regulating oocyte and embryo development. MicroRNAs have regulatory effects in cells by altering translational activities. Since miRNA populations are known to be dynamic in different cell types, we hypothesized that there would be subpopulations of miRNAs at different stages of oocyte maturation and after fertilization that regulate processes in a stage-specific manner. Bovine oocytes were collected, matured, and/or fertilized *in vitro* for analysis. The miRNA profiles of immature (GV) oocytes, mature (MII) oocytes, and presumptive zygotes (PZ) were determined using next-generation sequencing (NGS). Bioinformatics analysis revealed a population of miRNA that are differentially expressed across these three stages. One miRNA of interest is bta-miR-155, which is qPCR-validated, that was up-regulated during the oocyte maturation process and has predicted mRNA targets that are functionally involved in the regulation of the cell-cycle. The increase of this miRNA during oocyte maturation could alter the translation of their targets, which in the case of miR-155 may be an important regulator of the processes driving maturation and subsequent divisions beyond fertilization.

Funding Source: OMAFRA, NSERC and the OVC Scholarship Program

Daily post-operative evaluation of peripheral blood and abdominal drain fluid from dogs with closed suction abdominal drains to identify septic peritonitis following gastrointestinal surgery

Guieu L.V.S., Bersenas A.M.E., Brisson B., Holowaychuk M.K., Weese J.S., Ammersbach M., Beaufrere H., and Fujita F.

Septic peritonitis (SP) occurs in 6-35% of dogs following gastrointestinal (GI) surgery. Post-operative management with closed-suction abdominal drains (CSAD) may alter abdominal fluid characteristics. The aim of this study was to identify, in dogs with CSAD, objective laboratory markers to predict SP requiring relaparotomy after GI surgery. Client-owned dogs undergoing GI surgery (caudal to the stomach) with placement of a CSAD were prospectively enrolled (January 2012 - March 2014). Abdominal fluid and peripheral blood were collected pre-operatively and then daily until drain removal. Analysis of all samples included inflammatory cell count, PCV/TS, glucose (G), lactate (L), electrolytes and gases. Daily abdominal fluid analysis also included cytology, bacterial culture, and fluid volume. Outcome was SP requiring relaparotomy. The association between SP requiring relaparotomy and each laboratory variables including blood-to-fluid differences and ratios for selected variables (G, L, WBC and neutrophil count) was assessed using univariate mixed logistic regression models. A heterogeneous population of 31 dogs was included (*n*=16 prior celiotomy, *n*=24 pre-operative SP, *n*= 7 routine GI biopsy). Of these dogs 27 had an uneventful recovery while 4 dogs required relaparotomy. None of the blood and fluid laboratory variables had a significant effect on the odds of needing relaparotomy ($p>0.9$) including BFG, BFL differences and WBC and neutrophils count ratios. No objective laboratory markers were identified to predict the need for relaparotomy. BFG and BFL differences should not be used as predictors of SP in dogs with CSAD that undergo GI surgery.

Funding Source: Pet Trust

Establishing the Link between Par6 and PI3K/Akt Signaling and Its Potential Role in TGFβ-induced Apoptosis

Haynes, R. and Viloria-Petit, A.

Department of Biomedical Sciences

TGFβ signaling elicits stage-dependent effects on cell cycle progression, cell death and survival, and cellular attachment in breast epithelial cells. TGFβ signals via canonical (Smad) and non-Smad pathways, such as the PI3K cell signaling pathway and the Par6 polarity pathway. We previously showed that overactive Par6 signaling increases TGFβ-induced cell death, though the mechanism is unknown. We hypothesize that the Par6 pathway negatively modulates PI3K pro-survival signaling, promoting apoptosis. It may be possible to rescue the loss of pro-apoptotic and anti-proliferative TGFβ signaling in advanced-stage breast cancer by modulating such associated cellular pathways. Normal (Parental) Namru Murine Mammary Gland (NMuMG) cells, NMuMG cells overexpressing wild-type Par6 (WT) and NMuMG cells expressing a dominant-negative mutant of Par6 (S345A) were used to model normal, overactive and inactive Par6 pathways, respectively. Cells were grown in the absence (control) or presence of 5ng/mL TGFβ with or without 10μM of an inhibitor of Smad signaling for 48 or 144 hours to model the effects of TGFβ signaling at various stages. Protein lysates were analyzed by western blotting for the phosphorylated (p-) and native forms of Smad2, the PI3K signaling kinase Akt, and the anti-proliferative and pro-apoptotic Akt targets FoxO1 and FoxO3a. Akt and FoxO3a expression was constitutively lower in S345A cells. Inactive p-FoxO1 was constitutively lower in WT cells. Levels of active p-Akt were increased with TGFβ treatment in Parental cells. The results suggest that the TGFβ-Par6 signaling axis modulates PI3K/Akt signaling activity and the phosphorylation of FoxO transcription factors.

Funding Source: NSERC

Effects of 3-bromopyruvate on human colorectal cancer cells

Ho, N., Silva, A. and Coomber, B.L.

Department of Biomedical Sciences

The Warburg Effect describes the metabolic phenotype of cancer cells with heavy reliance on the glycolytic pathway for energy production regardless of oxygen tension. 3-bromopyruvate (3BP) is a promising anti-cancer compound capable of influencing the Warburg Effect in cancer cells. One of its targets, hexokinase II (HKII), is a glycolytic enzyme often overexpressed in tumors. Although 3BP has been shown to effectively kill cancer cells, the underlying mechanisms and its efficacy in human colorectal cancer (CRC) cells has not been thoroughly investigated.

We sought to determine whether targeting colorectal cancer metabolism could be exploited as a therapeutic strategy. A panel of human CRC cell lines was screened for the expression of different HK isoforms and the impact of 3BP on cell survival and signaling was examined. Through flow cytometry and western blot analysis, we found evidence of 3BP-induced cell death via both apoptotic and necrotic mechanisms. Changes in survival signaling pathways following 3BP treatment occurred rapidly in a dose-dependent manner and was site-specific. HKII dissociation from the mitochondria following 3BP treatment was visualized with immunofluorescence. Due to 3BP's ability to target HKII, we hypothesized that sensitivity to this compound could be affected by glucose availability. CRC cells grown in different glucose concentrations showed variable HKII expression and response to 3BP treatment.

Together, this study highlights the importance of HKII in the Warburg Effect and 3BP as a potential anti-cancer agent against human colorectal cancer cells that targets their unique metabolic signature.

Funding Source: NSERC, OGSs

Identifying behaviours associated with canine resource guarding

Jacquelyn A. Jacobs1*, Jason Coe2, Lee Niel3

Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Threats or aggressive behaviour in relation to valued food or objects, often termed resource guarding (RG), is a common form of canine aggression. Anecdotally there are behavioural indicators of RG that precede aggressive behaviour but these have yet to be examined in the scientific literature. This information could help pet owners recognize RG early and apply interventions to prevent escalation. Companion animal behaviour experts (n=85) were invited to participate in a Delphi survey to identify behaviours associated with canine RG. The Delphi survey method involves repeated stages of consultation with participants, wherein each round of questions is based on previous responses. A 42% and 80% response was achieved in the initial and second stage, respectively. Thirty-seven unique behaviours were initially reported and returned to participants in stage 2 for comment on expected observed frequency. Agreement was defined as ≥ 70% consensus. Body tension (81%), freezing (75%), and facial tension (70%) were suggested to always or often occur during RG. To confirm the behaviour frequencies reported, videos of dogs assessed for RG behaviour were collected (n=70) with 27 identified as displaying RG. Body tension was observed during assessments for 84.4±5.2% of RG dogs compared to 46.1±16.3% of non-RG dogs. Freezing instances were observed in 18% of RG dogs but never for non-RG dogs. Facial tension was difficult to observe from video recordings, and thus requires further investigation. The results suggest body tension and freezing are associated with RG as predicted by experts but high levels of variability between dogs may limit their usefulness for early identification of RG.

Analysis of vitamin D_3 concentration in commercial dog foods

Kritikos G[1], Weidner N[1], Atkinson JL[2], van Hoek I[3], Verbrugghe A[1]

[1]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, Canada [2]Department of Animal and Poultry Science, Ontario Agricultural College, University of Guelph, Ontario, Canada, [3]Royal Canin Research Center, Aimargues, France.

Background: Dogs must obtain vitamin D_3 through their diet, since they cannot synthesize it cutaneously. However, improper vitamin D_3 intake can lead to skeletal and extraskeletal health concerns. Thus, we examined vitamin D_3 concentrations in commercial dog foods to determine if they meet AAFCO recommendations.
Methods: Samples of 65 commercial dog foods were analyzed for vitamin D_3 via LCMS. Foods were compared based on moisture content, AAFCO nutritional adequacy, location sold, and on the size of their manufacturing company. Manufacturers' typical vitamin D_3 concentrations for each food were compared to analysed values.
Results: All foods met AAFCO minimum requirements, but 3 exceeded AAFCO maximum requirements. Wet foods contained significantly more vitamin D_3 than dry foods ($p=0.004$). Manufacturer size, selling location, and AAFCO nutritional adequacy did not have a significant effect on vitamin D_3 content ($p>0.05$). Manufacturers' vitamin D_3 concentrations were similar to those found by analysis ($p>0.05$), both for foods whose manufacturers measured final-product vitamin D_3 content and for foods whose manufacturers measured only the amount of supplement added before product completion.
Conclusions: In general, commercial diets seem to provide adequate amounts of dietary vitamin D_3. Manufacturer size, AAFCO adequacy, and selling location seem to have negligible effects on vitamin D_3 content, though wet and dry foods seem to contain differing amounts of vitamin D_3. Manufacturers' concentrations seem to be reliable estimates for vitamin D_3 content. Due to small sample sizes, further research is necessary to determine differences in vitamin D_3 content of commercial dog foods.

Funding Source: OVC Pet Trust, Royal Canin, and AAVN/Waltham Research Grant

The effects of iron deficiency and anemia on nursery pig performance

A Kubik[1], T O'Sullivan[1], J Harding[2], R Friendship[1]

[1]Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada,
[2]Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,

Piglets are born with limited iron stores and require iron supplementation within the first week of life. With updated management practices, piglets are now born into larger litters and grow at a faster rate compared to previous decades. It is therefore unknown whether the standard iron supplementation protocol is still sufficient in preventing iron deficiency and anemia. Iron deficiency and iron anemia may be defined as a blood haemoglobin concentration below 110g/L and 90 g/L respectively. The impact of iron deficiency and anemia results in slow growth and increased susceptibility to disease. The objectives of this project were to investigate if anaemia or iron deficiency is present in pigs at weaning and whether iron status affects post-weaning performance.

Twenty swine herds were conveniently sampled across Southern Ontario. Pigs (n=1095) were randomly selected 1-2 days prior to weaning. These pigs were tagged, weighed and serum and whole blood samples were taken. Three weeks later the same pigs were re-weighed and whole blood samples were taken to assess haemoglobin levels. The between herd prevalence of iron deficiency and anemia in pigs prior to weaning was 28% and 6% respectively. Three weeks after the initial visit, the between herd prevalence of iron deficiency and anemia increased to 43% and 18% respectively. Piglets injected with iron dextran had larger final weights compared to piglets injected with gleptoferron ($P<0.001$). Piglets that were anemic prior to weaning had smaller final weights compared to piglets with normal haemoglobin values ($p=0.002$). The efficacy of iron dextran and gleptoferron products for preventing iron deficiency is conflicting in the literature and further research would be an asset in understanding the benefits of each product. Understanding the consequences of iron deficiency and anemia is important for the swine industry and producers.

Funding Source: Ontario Pork

The rabbit fecal microbiome: Antimicrobial use and the presence of antimicrobial resistance

J. Kylie[1], R. Reid-Smith[2], S. McEwen[3], J.S. Weese[1], P. Boerlin[1], P.V. Turner[1]

Departments of Pathobiology[1] and Population Medicine[3], University of Guelph, Guelph, ON, Laboratory for Foodborne Zoonoses[2], Public Health Agency of Canada, Guelph, ON

There is increasing interest in defining the impact of antimicrobial use on gut flora, especially as it relates to the development of antimicrobial resistance (AMR) over time. AMR may impact both animal health and that of human handlers and caregivers. Rabbit populations are ideal for studying changes in the fecal microbiome as commercial meat rabbits are routinely fed antimicrobial supplements for enzootic diseases, while laboratory and pet rabbits are rarely treated. This study compared the composition of bacterial communities in hard feces (fecal microbiome) of laboratory, pet, shelter, and commercial meat rabbits, using high throughput sequencing of the V4 region of the 16S rRNA gene. Pooled fecal samples from groups of weanling and adult female rabbits were collected from 27 Ontario commercial meat rabbitries during both summer and winter months, as well as from three separate research laboratories, one animal shelter, and 54 healthy pet rabbits. Bacterial culture and AMR were also conducted to evaluate the presence of E. coli and Salmonella spp. isolates. Culture results demonstrated E. coli isolates in 92% of the samples collected, with no obvious differences in age or seasonality. Moderate AMR was present only in samples collected from commercial farms at a level of 19% and in 33% of shelter rabbit samples. Salmonella spp. isolates were identified exclusively in commercial meat rabbits. These results raise several questions regarding the role of asymptomatic rabbits as potential sources of enteric disease, how commercial farm practices contribute to development of AMR in rabbits, and the potential for cross-species and zoonotic transmission of agents to personnel working closely with rabbits.

Funding Source: OMAFRA

Generation, characterization and multilineage potency of mesenchymal-like progenitors derived from equine iPS cells

Lepage S, Nagy K, Sung HK, Kandel R, Nagy A, Koch TG

Department of Biomedical Sciences

In horses, mesenchymal stromal cells (MSCs) are used primarily in regenerative medicine studies to treat orthopedic injuries. However, these cells are limited in their expandability and differentiation capacity. Recently, the first equine induced pluripotent stem cell (eiPSC) lines were reported. Induction of iPSCs into MSC-like cells is an attractive option to using MSCs derived from other sources, as a much larger population of patient-specific cells could be generated to treat large orthopedic defects. However, the differentiation capacity of eiPSCs has yet to be explored. eiPSCs were induced to differentiate into MSC-like cells (termed eiPSC-MSCs) in MSC induction media. Upon induction, this cell population demonstrated a downregulation of genes associated with pluripotency and an upregulation of MSC-specific genes. As well, flow cytometry analysis revealed that eiPSC-MSCs uniformly expressed the same surface markers as equine MSCs derived from umbilical cord blood, further establishing eiPSC-MSCs as a MSC-like population. Furthermore, chemical induction of eiPSC-MSCs revealed mineralization and lipid droplet accumulation typical of early osteogenesis and adipogenesis, respectively. Chondrogenic induction of the eiPSC-MSCs remains a challenge.

We demonstrate that eiPSCs can be directly differentiated into an intermediate MSC-like population that is capable of undergoing adipogenesis and osteogenesis, which is characteristic of putative MSCs. Further studies are needed using an increased number of unrelated eiPSC lines to determine the generality of the findings, and whether chondrogenic potency is a clonal variability or a more general characteristic of these eiPSCs.

Funding Source: Equine Guelph and Partners

Accuracy and safety of image guided percutaneous injection of gelified ethanol in the lumbo sacral intervertebral disc in dogs.

Mackenzie, S., Brisson, B.A., Gaitero, L., Caswell, J.L., Chalmers, H.J.

Department of Clinical Studies

Radiopaque gelified ethanol, marketed as Discogel®, has been described as a useful agent for the treatment of disc herniations in humans. The agent is injected into the disc via image guidance and is intended to cause the protruded disc material to recede. The aims of the current study are to evaluate the safety of the injected ethanol preparation in dogs and to evaluate the effect on intervertebral disc size. Injections of the lumbosacral discs of research dogs were performed using CT (*n*=9) or fluoroscopic guidance (*n*=1). Pre and post injection MRI and neurological examination was performed to assess for changes related to the injected preparation over time. The accuracy of gelified ethanol placement was documented. Follow-up MRI and CT was performed >1 year after injection in 5 dogs. Percutaneous injection of the lumbosacral intervertebral disc was successful in delivering radiopaque gelified ethanol to the nucleus pulposus in all dogs with no clinical adverse reactions. Leakage of the injected material into the epidural space was present in 4 dogs. There was no significant difference between those injections with and without contamination in regards to: time (p=0.738), needle repositioning (p=1.000), resistance to injection (p=1.000), satisfaction with injection (p=0.667), and Pfirrmann grade (p=0.667). At 1 year follow up 4 dogs had no change in disc size. One dog had worsening of intervertebral disc protrusion but this was not clinically significant. These findings indicate that injection of radiopaque gelified ethanol into the nucleus pulposus of the lumbosacral disc of dogs is well tolerated even in the presence of epidural contamination.

Funding Source: OVC Pet Trust and ACVR Resident Research Award.

In vitro effects of epidermal growth factor receptor kinase inhibition on radiation response in canine osteosarcoma cell lines

F Mantovani, J Morrison, AJ Mutsaers*

Department of Clinical Studies, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph

Introduction

External beam adiation therapy plays an important role in the palliative treatment of canine osteosarcoma, with transient improvement in analgesia observed in the majority of cases. The addition of radiation sensitizing agents may further improve outcomes for these patients. Epidermal growth factor receptor (EGFR) expression has been documented in canine osteosarcoma and correlated to prognosis. However, effects of EGFR inhibition in radiation responsiveness have not been characterized. This study examined the effects of the small molecule EGFR inhibitor erlotinib on canine osteosarcoma radiation responses, target and downstream protein expression in vitro.

Methods

Canine osteosarcoma cell lines D-17, Abrams, and Dharma were utilized. Radiation was delivered by a Varian trilogy linear accelerator. Doses of 0,2,4,6,8,10 Gy were utilized for clonogenic survival assays, and colonies were stained with crystal violet and enumerated after 10-14 days. Cell lysates were also obtained following 2 Gy radiation at time points from 0.25 to 72 hours for protein profiling. Cell viability, clonogenic survival and EGFR, HER2, Akt, phosphor-Akt, and VEGF production assays were conducted to assess single agent and combination treatment efficacy.

Results

Erlotinib in low micromolar doses inhibited clonogenic survival as a single agent treatment, and further inhibited clonogenic survival in cells following radiation. Radiation stimulated Akt phosphorylation within the first 2 hours post-radiation. VEGF in conditioned media was increased following both radiation and especially erlotinib treatment.

Conclusion

Preliminary results in canine osteosarcoma cell lines suggest there may be a role for EGFR inhibition to improve canine osteosarcoma treatment responses to radiation therapy.

Mitochondrial dynamics in slow and fast growing preimplantation bovine embryos

Merrill, C., Perkel, K and Madan, P

Biomedical Sciences, Ontario Veterinary College, University of Guelph, Ontario, Canada

Metabolomic assessment of bovine embryo viability is a potential tool to non-invasively predict early embryo mortality. The early embryo primarily depends on the mitochondria for energy production. Our lab has previously shown that metabolite levels differ in slow and fast growing embryos at various stages of growth. It is therefore our goal to observe mitochondrial function during the preimplantation stages of development to elucidate potential molecular mechanisms behind impaired embryonic development. Bovine oocytes underwent in vitro fertilization (IVF) and embryos were separated into slow and fast growing groups at the zygote, 2-cell, 4-cell, 8-cell, 16-cell, morula and blastocyst stages. Embryos were stained with live probes TMRM (mitochondrial membrane potential) and CM-H2DCFDA (reactive oxygen species; ROS) and time-lapse images were obtained using a confocal microscope. Embryos that arrested at the 2- and 4-cell stages had lower TMRM fluorescence than those that continued to develop. Impaired embryos also displayed higher ROS fluorescence. This indicates that early arresting embryos may be associated with inadequate mitochondrial activity. Mitochondria were distributed in uniform and peripheral patterns in fast and slow growing embryos respectively. A peripheral pattern is related to embryos with lower developmental potential. The data collected so far suggest that embryos with low mitochondrial activity develop slower or completely arrest. This can lead to a better understanding of impaired embryonic development and an improvement in the success of IVF. Further studies will delineate the mitochondrial genes involved in developmental delay and arrest of embryos.

Funding Sources: Ontario Veterinary College, NSERC, Dairy Farmers of Ontario, CFI

Comparison of two methods for the assessment of impulsivity in companion dogs

Mitcham, A., Niel, L.

Department of Population Medicine

Impulsivity is a multifaceted trait related to the level of control an individual has over their behaviour. High impulsivity is characterized by increased urgency and sensation seeking, and a lack of premeditation and perseverance. While impulsivity has been suggested to contribute to canine behaviour problems such as aggression, no studies to date have empirically assessed these relationships, partly due to the complexity of the associated behaviour tests. Our objective was to compare two different methods for assessing impulsivity in companion dogs: the conventional delay aversion task was compared to the recently developed cylinder detour task. Subjects were 18 healthy, companion dogs of various breeds that were boarding or attending daycare at a local kennel. For the delay aversion task dogs completed 6 sessions of 6 trials each in which they had to choose between a small, immediate reward and a large, but increasingly delayed reward. For the cylinder detour task, dogs completed 3 sessions of 10 trials in which they had to resist approaching food behind a transparent barrier, instead selecting a detour to successfully access the food. The average pass rate in sessions 1 and 2 of the detour task showed a moderate, positive correlation with performance on the delay aversion task when assessed using the area under the preference curve ($r=0.66$, $p<0.05$), but was not related to the calculated maximum delay increment the dog was willing to tolerate ($r=0.037$, $p>0.05$). This suggests that the tests are examining a similar facet of impulsivity in dogs, and that the detour task is a reasonable replacement for the complex and time-intensive delay aversion task.

Funding Source: NSERC

Macrophages secrete factors that alter prostate cancer cell proliferation and growth behaviour *in vitro*

Pinelli C, Petrik N, Wood G

Department of Pathobiology

Prostate cancer is the second most common malignancy in men, and is responsible for nearly 4000 deaths per year in Canada. Most mortality occurs when androgen-dependent disease progresses to androgen-independence, also known as castration-resistant prostate cancer (CRPC). CRPC is an aggressive form of prostate cancer whose development is associated with heightened prostate inflammation. Previous in vivo mouse studies in our lab demonstrated that inflamed prostate tumours have shorter tumour latency with increased invasion compared to non-inflamed tumours, with peritumoural macrophages potentially playing a central role. Current experiments involve in vitro investigation of this mechanism, using human cell lines. Androgen dependent (LNCaP) and independent (PC3) prostate cancer cell lines, as well as monocyte cell lines (THP1) differentiated into macrophages, were grown by themselves, and in co-culture together, to generate conditioned media to be harvested for subsequent experiments. Naive LNCaP and PC3 cells were exposed to a variety of conditioned media types, and demonstrated decreased cell proliferation rates when exposed to macrophage conditioned media compared to conditioned media generated by cancer cells alone. These naive cells also demonstrated altered cellular morphology and growth behaviour when exposed to macrophage conditioned media, were poorly adherent to the plate, and formed cellular aggregates. This phenomenon was present in both cell lines, in androgen replete and depleted culture conditions. Further experiments are necessary to elicit the cause of this change in behaviour, as well as to further characterize the altered phenotype of these cells.

Funding Source: Prostate Cancer Canada Movember Discovery Grant

Live *Eimeria* vaccination success in the face of artificial non-uniform vaccine administration in conventionally reared pullets

K. R. Price[1*], M. A. Hafeez[1], J. Bulfon[1], J. R. Barta[1]

[1]Dept. Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1

Live vaccines to protect against coccidiosis, an enteric disease caused by *Eimeria* spp. oocysts, are administered commonly via coarse spray of water/gel vaccine droplets. Variation in dose ingested by each chick is typical; some are missed completely. Fecal-oral transmission, "cycling", of oocysts in the environment has been shown to boost vaccine success and expose non-uniformly dosed chicks to vaccine oocysts. We hypothesized that non-uniformly vaccinated chicks could acquire immunological protection by exposure to oocysts cycling in the cage environment and that 40% cage floor coverage (CFC) with a durable material could improve this protection against challenge in vaccinated and non-uniformly vaccinated pullets by enhancing cycling. Day of age chicks (*n*=1,232) were randomized into 6 groups: vaccinated (vaccine oral gavage); contact-vaccinated (saline oral gavage, commingled with vaccinated chicks); sham-vaccinated (saline oral gavage, reared in separate groups) and reared on 0 or 40% CFC. Fecal samples were collected regularly (0-30 days) to assess oocyst shedding. Lesion scores, body weights and total oocyst output quantified protection against single or mixed *Eimeria* spp. challenge infections (30-42 days). Vaccinated and contact-vaccinated birds had enhanced cycling when reared with 40% CFC and were, in general, protected significantly better against challenge versus pullets on 0% CFC. Modifying the cage environment with 40% CFC to promote oocyst cycling enhanced protection against coccidial challenge following live *Eimeria* vaccination and non-uniform vaccination was redressed because protection against challenge was elicited in non-vaccinated, commingled pullets.

Funding Source: NSERC, OMAFRA

Pet Owner Attitudes Regarding Canine Cancer and Nutrition

Rajagopaul S[1], Parr J[1], Woods JP[1], Coe JB[2], Pearl DL[2], Verbrugghe A[1]

[1]Department of Department of Clinical Studies, [2]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

Background: Following cancer diagnosis, reduced appetite and metabolic changes can occur, leading to suboptimal nutrient profiles. Individualized nutrition support is recommended to remedy complications and maintain optimal nutrition. To facilitate veterinary-owner communication with respect to nutritional care, the present descriptive study examined owner attitudes related to diet and supplement use for dogs with cancer.

Methods: 75 owners of canine patients that presented at the OVC Animal Cancer Center in 2014 participated in in-person or phone interviews. Information was collected about the dog's diet, feeding behavior, nutritional supplement use, owner attitudes and information sources that were used.

Results: 69%, 8%, and 4% of owners fed primarily conventional, home-cooked or raw-meat diets, respectively. 13% fed a combination of conventional and home-cooked, while 6% fed a combination of conventional and raw. Half of the owners expressed distrust in conventional pet food. A diet change within 6-months of diagnosis was reported in 25% of dogs. Loss of appetite, which was seen in 36% of dogs, may be one reason for this diet change. 40% of dogs also received various nutritional supplements. Finally, 85% of owners listed the veterinarian as a valuable source of nutritional information.

Conclusion: Owners' attitudes influence dietary practices, which can affect cancer-associated complications and lead to nutrient imbalances. Thus, it is essential to educate veterinarians about owner attitudes and dietary practices to improve veterinary-owner relationships and increase owner compliance when making nutritional recommendations for dogs with cancer.

Sodium intake in urban, peri-urban and rural Cambodia

Lauren C. Ramsay[1], Christopher V. Charles[2], Cate E. Dewey[3], Alastair J.S. Summerlee[1]

[1]Dept. Biomedical Sciences, Ontario Veterinary College, University of Guelph
[2]Michael DeGroote School of Medicine, McMaster University
[3]Dept. Population Medicine, Ontario Veterinary College, University of Guelph

The cross-sectional study took place in four provinces in Cambodia: Kampot, Kandal, Kampong Chhnang and Phnom Penh. This study involved the collection of 24-hour urine samples, as well as a spot urine sample collected on the final morning of data collection to assess sodium and potassium excretion. Demographic, anthropometric, blood pressure, physical activity level and socio-economic data were collected in a face-to-face interview. A Student's *t*-test indicated that the mean urinary sodium excretion found in the 24-hour urinary sample of the entire sample population (M=5615.93mg, 95% CI [3377.13, 3854.72]) is nearly three times higher than the WHO recommended intake of 2000mg of sodium, $p<0.001$. The mean potassium excretion in 24-hours in our sample population was 88.61mmol/day (SD=71.78), which is equivalent to 3455.79mg/day. Sodium excretion has a positive linear relationship with potassium excretion: mg Sodium excretion = 3852.02 + 0.51(mg Potassium excretion). A one-way analysis of variance indicated that no significant differences in sodium intake was found between blood pressure groups, $p=0.879$. The major contributors to the high-sodium diet were added seasonings including: salt, fish sauce, MSG, and prahok (a locally made fish paste fermented with salt). There was no relationship between sodium excretion and blood pressure.

Funding Source: General Purpose Account AJS Summerlee

Role of Type I Interferon Signaling in Regulating Cytokine Expression in Dendritic Cells and Macrophages

Rasiuk, A., Walsh, S., and Bridle, B.

Department of Pathobiology

As a first line of defense against viral infection, cells produce a set of cytokines known as type I interferons (IFN). These include IFNα and β, and are detected by cells expressing the IFNα/β receptor (IFNAR). Activation of the IFNAR signaling pathway may play a vital and unexpected role in shutting down production of a wide range of cytokines in cells. Previous data show that when mice lacked the IFNAR on their leukocytes, normally well-tolerated viral infections quickly became fatal. Results suggest that this was due to a massive overproduction of a vast array of cytokines (*i.e.* a cytokine storm). This suggests that the IFNAR signaling pathway may serve as a master switch for the negative regulation of cytokine production.

In this project, cytokine responses were studied using *in vitro* monoculture models. Dendritic cells (DCs) and macrophages (known to be mediators of anti-viral immunity) were treated with various attenuated viruses, or viral or bacterial mimics, with or without blocking the IFNAR pathway. Monotypic cultures did not recapitulate the phenomenon of cytokine dysregulation. However, dramatic increases in cytokine production occurred with the organotypic co-culture of DCs or macrophages with lymph node and aortic tissues. This novel model needs to be fully characterized. Then, the pathways downstream of IFNAR-mediated signaling will be investigated in hopes of identifying molecules responsible for the regulation of various cytokines. Discovery of new mechanisms controlling cytokine responses may facilitate the development of strategies to suppress excessive inflammation and cytokine storms, which represent significant medical complications.

Funding Sources: NSERC, OGS, OVC

The Circadian Clock Regulates T Cells and Benefits Healing after a Heart Attack

Reitz C, Tsimakouridze E, Alibhai F and Martino T

Department of Biomedical Science

Rationale: Cardiovascular disease (CVD) is a leading cause of death worldwide. Following myocardial infarction (MI; heart attack), an immune response is initiated to facilitate infarct healing. The role of the circadian mechanism in T cell-mediated wound healing and how this can benefit patients are unknown.

Objectives: We hypothesize that T cells are recruited to infarcted myocardium and the circadian clock is critical in coordinating this recruitment.

Methods and Results: We used an established murine model of MI. First, we showed that T cells were recruited to the heart at day 7 post-MI ($55.0\pm8.2/0.25mm^2$) as compared to SHAM ($1.5\pm0.1/0.25mm^2$) with immunohistochemistry. Second, we demonstrated that T cell recruitment was regulated by the diurnal environment, as T cell infiltration was reduced in diurnal disrupted mice ($26.0\pm1.8/0.25mm^2$ vs. $55.0\pm8.2/0.25mm^2$). Third, total immune cell ($2.8x10^5\pm4.2x10^4$ $CD45^+$) and T cell ($3.8x10^3\pm3.5x10^2$ $CD45^+CD3^+$) infiltration were higher in day 3 MI hearts vs. SHAM ($1.4x10^4$ $CD45^+$; 0 $CD45^+CD3^+$) shown by flow cytometry. Fourth, RT-PCR revealed increased mRNA expression of T cell cytokines *il-17a* (1.2±0.002 fold) and *il-10* (3.8±0.4 fold) in day 3 MI hearts over SHAM. Lastly, we identified T cell activation sites by tracking intramyocardially injected microspheres to mediastinal lymph nodes.

Conclusions: This is the first study to show that T cell recruitment to the heart post-MI is regulated by the diurnal environment and is altered with diurnal disruption. These findings have implications for patients with diurnal disturbances (e.g. shift workers). Our future studies will target T cell recruitment to open new avenues to benefit CVD patients.

Funding Source: Canadian Institutes of Health Research (CIHR)

A scoping review of the evidence for efficacy of acupuncture in companion animals

W.J. Rose[1], J.M. Sargeant[2,3], W.J.B. Hanna[1], D.F. Kelton[2,3];

[1]Department of Biomedical Sciences, [2]Department of Population Medicine, [3]Centre for Public Health and Zoonoses, University of Guelph, ON, CAN.

Acupuncture is a popular alternative or complementary therapy to conventional practises in veterinary medicine. Evidence related to its efficacy in animal patients, however, appears to be sparse. A previous systematic review was unable to draw conclusions on the effectiveness of acupuncture in animals due to the poor quality of some of the existing studies and the low number of trials published at that time[1]. For these reasons we are undertaking a formal scoping review of veterinary acupuncture used in companion animals. To this end, we are locating and categorizing all of the relevant data now available on acupuncture used as an intervention or preventive treatment in cats, dogs, and horses. Cell based or mechanistic studies are being excluded. We are using standardized search terms to survey the literature in a variety of databases (CAB, PubMed, AGRICOLA, Science.gov, Web of Science, CINAHL and TOXNET). Acupuncture has been endorsed as efficacious for a wide range of conditions and therefore all outcomes are being included. This review will provide information on what is available in the scientific and grey literature to inform knowledge synthesis efforts.

Cardiac Troponin Assays in Equine Medicine: Analytic and Clinical Validation

Rossi T.M, Pyle W.G, Pearl D.L, Maxie M.G, Kavsak P.A, Physick-Sheard P.W.

Department of Population Medicine

In 2000, troponin assays were adopted as the test of choice for detection of myocardial injury in humans. This decision was made after extensive testing, and followed a 60-year search for a biomarker of myocardial damage with sufficient analytic sensitivity and specificity. This led to a proliferation of assays for use in human medicine, each requiring extensive testing and validation before being made available for human diagnostic use. The search for ever more analytically sensitive assays continues.

Adoption of troponin testing in veterinary medicine followed shortly after its development, providing a much-needed means of detecting and following myocardial damage. However, the application of tests in equine veterinary medicine has exclusively involved the use of assays designed for, and clinically validated in, human patients. There is no mandated requirement for test validation in veterinary medicine and, while many of these assays have been shown to be capable of detecting equine troponin, the wide diversity of available tests, lack of validation, absence of protocols for their use, and lack of standardisation make their application problematic.

The objectives of my project are to: i) validate sensitive and specific cTnI assays for use in the horse, ii) using validated assays to plot the magnitude and time course of cTnI elevation and clearance after maximal effort, iii) to examine cTnI levels pre-and post-maximal effort in a population of racehorses competing in scheduled competition, and iv) to compare the magnitude and time course of cTnI elevation in horses experiencing arrhythmia with that of horses from the same maximal intensity event that did not exhibit arrhythmia.

Funding Sources: Equine Guelph, Siemens Diagnostics

Platelet lysate as alternative to fetal bovine serum in equine and canine mesenchymal stromal cell culture

Russell, K and Koch, T

Department of Biomedical Sciences

Mesenchymal stromal cells (MSC) must be expanded to sufficient numbers *in vitro* for emerging tissue regeneration therapies. Presently, expansion media (EM) for MSC is supplemented with fetal bovine serum (FBS). However, potential issues associated with FBS are high cost, high variability between batches, and risk of complications from bovine antigens. Platelet lysate (PL) has shown promise as an alternative supplement to FBS in human studies.

To determine how equine and canine MSC proliferate in EM enriched with PL compared to FBS, we performed dose-titration studies. Canine cultures were assessed for effects of long-term exposure to PL.

Pooled PL was produced through a double centrifugation method, followed by a freeze/thaw cycle. Pooled FBS was also prepared. Proliferation assays were performed with equine cord blood-, canine adipose-, and bone marrow-derived MSC in EM with 5% to 60% PL or FBS.

MSC proliferated with a dose-dependent response with no significant difference found between PL and FBS up to a 30% concentration in equine and 10% in canine. Beyond these thresholds, proliferation fell in the PL-cultured cells, while a continued dose-dependent proliferation response was noted in the FBS-cultured cells. In long-term PL cultures, spontaneous adipogenic differentiation was discovered as well as MSC intolerance to overcrowding.

We determined that PL can support the proliferation of MSC to some extent. However, when platelet-derived products and MSC are applied simultaneously as regenerative agents, their effect may not be additive as one might expect. As PL may cause spontaneous differentiation of MSC, PL cannot be recommended as a suitable supplemental alternative.

Funding Sources: Pet Trust Fund, OVC Office of the Dean, Morris Animal Foundation

The PIWI Pathway in the Early Embryo and Potential Isoforms of PIWIL1

S. Russell, G. Gilchrist, J. LaMarre

PIWI proteins comprise a subfamily of the Argonaute proteins that bind to specific 26-32 nucleotide RNAs (piRNAs). They are required for the suppression of deleterious genetic elements called retrotransposons in the germline. Recent studies have demonstrated the presence of these proteins and/or their associated piRNAs in early embryos. We postulate that the PIWI pathway is present in bovine early embryos and that it is involved in the reprogramming events of embryogenesis.

We identified *PIWIL1* and *PIWIL2* transcripts in the bovine gonads, but also in the oocyte and early embryo, a novel finding. The transcript levels across stages were compared, showing dynamic regulation of the PIWI proteins. Cloning and expression of *PIWIL1* in Hela cells demonstrates that we have an antibody able to detect bovine PIWIL1 protein. Interestingly, 3' RACE of PIWIL1 shows two potential isoforms present in the oocyte and embryo context.

In an attempt to identify novel small non-coding RNA sequences, deep sequencing was performed on large pools of oocytes and early embryos. Small RNA sequences were mapped back to the genome and classified using piRNA features. Our preliminary analysis returned putative piRNAs with variable expression between the oocytes and presumptive zygote and the potential to target retrotransposons.

These data demonstrate that a subset of the PIWI-like proteins along with potential associated piRNAs are present in early bovine embryos. Future studies will investigate piRNA expression and binding to PIWIL1 and PIWIL2, the expression profile of potential targets such as retrotransposons, and the roles these each play in early embryo development.

Funding Source: NSERC and OMAFRA

Cyclooxygenase-2 in feline eyes with and without uveitis

Sim, Z., Pinard, C., Plattner, B., and Bienzle, D.

Department of Clinical Studies

Uveitis, inflammation of the uvea, is one of the leading causes of blindness in cats. Regardless of the etiology, feline uveitis requires timely anti-inflammatory therapy to eliminate pain and preserve vision. Uveitis therapy often includes the use of non-steroidal anti-inflammatory drugs that inhibit prostaglandin release via the cyclooxygenase pathway. An important therapeutic target is cyclooxygenase-2 (COX-2), which is induced and upregulated in many tissues during inflammation. To date, no studies evaluating COX-2 expression in feline ocular tissues have been published. Therefore, the objective of this study is to localize and quantify COX-2 expression in feline eyes with and without uveitis. Specific aims are evaluation of ten normal and thirty uveitic cat eyes that will be categorized by type of inflammation into lymphocytic-plasmacytic uveitis, neutrophilic uveitis, and iris melanoma-associated uveitis. The severity of inflammation will be graded histologically. Location and intensity of COX-2 expression will be microscopically assessed using immunohistochemical staining of formalin-fixed paraffin-embedded eyes. In addition, enzyme immunoassay will be applied to detect COX-2 in aqueous humor collected via anterior chamber paracentesis from normal and uveitic eyes. The hypothesis of this study is that there is constitutive expression of COX-2 in feline eyes, which is upregulated with inflammation. Results of this study will help determine whether COX-2 is a suitable therapeutic target for feline uveitis.

Funding Source: OVC Pet Trust

Systemic Immune Responses to an Inactivated H9N2 Virus Vaccine with a Toll-like Receptor 21 Adjuvant

Shirene M. Singh*, Tamiru Alkie*, Douglas Hodgins*, Jennifer Brisbin*, Bahram Shojadoost* and Shayan Sharif*

*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Commercial vaccines against avian influenza viruses (AIV) in chickens consist mainly of inactivated AIV, requiring parenteral administration and the use of an adjuvant. Limited information is available on effective adjuvants for use in poultry. The Toll-like receptor (TLR) 21 ligand, CpG ODN, has been established to be immunostimulatory in chickens. Therefore, the objective of this study was to investigate the adjuvant potential of a high (20 µg) and low (2 µg) dose of both CpG ODN 2007 and 1826 when administered with a formalin-inactivated H_9N_2 AIV. Hemagglutination inhibition (HI) as well as IgY and IgM responses were evaluated in 90 SPF chickens after two intra-muscular administrations of the vaccine formulation at the age of 7 and 21 days, respectively. The results suggest that the vaccine formulation containing CpG ODN 2007 was more effective at generating both neutralizing and antigen-specific responses than the formulation with CpG ODN 1826. Moreover, a greater IgY and IgM response was elicited by the vaccine formulation containing the low dose of CpG ODN 2007 compared to the high and low dose of 1826. Hence, CpG ODN 2007 (2 µg) is currently being evaluated for delivery in a poly lactic co-glycolic acid (PLGA)-encapsulated microsphere compared to the un-encapsulated form in the same vaccine formulation.

Funding Source: OMAFRA

A scoping review of research on the effectiveness of food safety education interventions for consumers

Bhairavi Sivaramalingam[1,2], Ian Young[1], Mai T. Pham[1,2], Lisa Waddell[1,2], Judy Greig[1], Mariola Mascarenhas[1], Andrew Papadopoulos[2]

[1] Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, 160 Research Lane, Suite 206, Guelph, Ontario, Canada
[2] Department of Population Medicine, University of Guelph, 50 Stone Road, Guelph, Ontario, Canada

Improper food handling by consumers at home is a major cause of foodborne illness worldwide. Therefore, effective education strategies are required to change consumers' food safety attitudes and behaviours. The purpose of this scoping review was to identify and characterize all primary literature examining the effectiveness of consumer food safety education interventions. To ensure that the results would be applicable to end users, stakeholders were engaged to provide input on the review scope, methods, and results. Using a comprehensive search strategy, 10 online databases were searched. Relevant articles were characterized by two reviewers. The characteristics of 246 relevant articles will be presented, of which 150 were quantitative, 66 qualitative, and 30 mixed-method research studies. Most studies (64.2%) were published in the United States, used an uncontrolled before-and-after study design (31.3%), and investigated community-based continuing education workshops and training sessions (52.0%). Research gaps were found in the number of randomized controlled studies conducted, academic- and school-based courses and curricula investigated, and in interventions targeting high-risk populations (e.g. pregnant women, immuno-compromised) and using new media channels (e.g. social media). Key opportunities to enhance the utility of future primary research investigating consumer food safety interventions include: basing studies on behaviour change theories and formative research; engaging the target population in the research; using validated instruments to measure outcomes; and reporting intervention characteristics and outcomes completely.

Funding source: Laboratory for Foodborne Zoonoses and Public Health Agency of Canada

The early-life microbiota of the domestic pig

Slifierz, M., Friendship, R., and Weese, S.

Department of Pathobiology

The microorganisms that populate the body are collectively known as the microbiota and it is well established that these microbial communities are important drivers of host health and metabolism. The present longitudinal study aims to characterize the gut and nasal microbiotas in young pigs using next-generation sequencing.

Ten pigs were followed from birth to 7-weeks of age, and weekly nasal and fecal samples were collected. DNA extracted from the samples was used to amplify the 16S (V4) rRNA gene and products were sequenced using the Illumina MiSeq platform.

The gut microbiota contained19 phyla and 489 genera of bacteria, with distinct age-related changes in the community structure and membership. The 3 dominant phyla (Firmicutes, Proteobacteria, Bacteroidetes) accounted for >90% of all bacteria. Richness and diversity were significantly lower during the first week of life but progressively increased with age and a relatively stable microbiota was evident at 2-weeks post-weaning. As expected, weaning was a significant driver of the transformation of the gut microbiota.

The nasal microbiota was equally rich and diverse, containing 22 phyla and 676 genera of bacteria, with some age-related shifts in community structure. The 3 dominant phyla (Proteobacteria, Firmicutes, and Actinobacteria) accounted for >97% of all bacteria. Interestingly, the nasal microbiota appeared to mature more rapidly and showed stability from the first day of life, although there were changes among the rare taxa.

Overall, our knowledge of the porcine microbiota is expanding and it can offer important insight into disease susceptibility and prevention.

Funding source: Ontario Pork

Social network analysis of a single equestrian show to determine the potential for infectious disease transmission in horse populations

Spence, K.L., Goh, B., O'Sullivan, T., and Greer, A.L.

Department of Population Medicine

The highly mobile nature of equine populations presents a challenge to the prevention and control of infectious diseases. Frequent participation in show and sporting events provides opportunities for horses to come into contact with new locations and horses, where the introduction and spread of diseases can occur. There is little information available regarding the connectivity of horses in Ontario that would allow for assessment of epidemic potential in the population. The objective of this pilot study was to visualize the network of potential contacts resulting from a single equestrian show in order to understand the influence of network structure on disease transmission. Horse show participants completed a survey asking about their horse's "home facility", travel patterns, and infection control procedures. The population of interest travelled frequently and came from home facilities of various sizes (between 2 and 85 horses). Most participants travelled less than 50 km to reach the show location, while fewer travelled longer distances (50 km – 370 km). Horses attending the show were highly vaccinated, with 92% of horses having received four or more vaccinations. Infection control was an important issue for participants and individuals reported high compliance with most infection control practices while away from home. These results will be used to develop infectious disease transmission models to simulate the impact of introducing equine infectious diseases into the described network. Understanding the interaction between network structure and disease dynamics is important for identifying strategies for effective equine infectious disease control.

Funding source: Equine Guelph

Early mucosal antibody responses in a subclinical Johne's disease calf model

Stinson, KJ and Plattner, BL

Department of Pathobiology

Johne's disease (JD) is a chronic progressive enteric infection of ruminants cause by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Cattle are infected at a young age by ingesting MAP shed in milk or feces of an infected animal, and undergo a lengthy subclinical period which can last years. During this time, MAP is shed asymptomatically in the feces of the animal. This represents a major source environmental MAP contamination and transmission within the herd. In Canada, JD, which is invariably fatal, affects an estimated 30-60% of herds, with an annual economic impact of $15M. The host immune response during early intestinal MAP infection is not well characterized, and MAP-specific antibody responses are typically not seen until onset of clinical disease. However, it is apparent that many animals exposed to MAP do not develop JD, suggesting an early host immune response capable of clearing the infection. Using a calf subclinical enteric MAP infection model, we tested the hypothesis that local intestinal MAP-specific antibodies could be detected prior to systemic serum antibodies. We measured MAP-specific antibodies in serum and intestinal mucosal scrapings using ELISA and found that infected animals showed significantly higher MAP-specific IgG and IgA antibody levels in intestinal mucosa compared to uninfected controls while no significant serum antibody responses were detected. To the best of our knowledge, this represents the first report of antibody production within the mucosa of MAP infected animals prior to the onset of clinical JD; though more research is required to understand the implications of these findings.

Funding Source: OMAFRA, ELANCO, OGS, OVC

Systemic and local Anti-Mullearian hormone reflect subspecies specific cattle fertility

Anja Stojsin[1]*, Kiana Mahboubi[1], Nathlia N. Costa[4], Tobias C. Sovernigo[2], Marcos A. Filho[2], Daniel J. Gillis[1], Michael S. Neal[3], Paulo R. Adona[2], Moyses S. Miranda[4], Otavio M. Ohashi[4], Laura A. Favetta[1], Pavneesh Madan[1], W. Allan King[1]

[1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [2]Central Avançada de Biotechnologia em Reproducion, Universidade Norte do Paraná - UNOPAR, Londrina, Paraná, Brazil, [3]ONE Fertility, Burlington, Ontario, Canada, [4]Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil

This study was conducted to evaluate the level of plasma anti-Mullerain hormone (Pl AMH), follicular fluid AMH (FF AMH) and granulosa cell AMH (GC AMH) as a predictor of the oocyte quantity and quality in two cattle subspecies, *Bos indicus* and *Bos taurus*. Parameters measured were antral follicle count (AFC), oocyte number, blastocyst rate and number of calvings per animal to date (NC) in a non-stimulated cycle. 2-D ultrasound examination and plasma collection was performed on *Bos indicus* (n=30), Bos Taurus (n=10), and crossbreed (n=10) cows of similar reproductive age in vivo. Subspecies specific Pl AMH mean (ng/ml) was: *Bos indicus*=0.77±0.09; *Bos taurus*=0.33±0.24; crossbreed=0.63±0.07, significantly higher in *Bos indicus* compared to *Bos taurus*, and correlated to subspecies specific AFC and NC. Slaughterhouse ovaries for *Bos indicus* (n=12) and *Bos taurus* (n=59) were collected and Pl and FF AMH concentrations and GC AMH RNA transcript levels were measured and found correlated with AFC and oocyte number. Subspecies specific FF AMH mean (ng/ml) was: *Bos indicus*=4934.3±568.5; *Bos taurus*=2977.9±214.1, significantly higher in *Bos indicus* compared to *Bos taurus*. In regards to blast rate we did not detect significance between Pl, FF, GC AMH. This study provides evidence of both Pl and FF AMH to be accurate and reliable measure of a bovine fertility in a non-stimulated cycle. *Bos indicus* cattle is found superior to *Bos taurus* cattle in both Pl and FF AMH levels therefore AMH parameter can be used as a subspecies specific fertility marker.

Funding Sources: Funding Sources: OVC Doctoral Scholarship, The Natural Sciences and Engineering Research Council of Canada (NSERC), Canada Research Chair (CRC) and the North/South Animal Biology and Reproductive Biotechnology Consortium DFAIT-CAPES, Canada/Brazil Doctoral Student Research Exchange

Combining Histone Deacetylase Inhibitors with Oncoviral Therapy as a Novel Treatment for Leukemia

Megan Strachan-Whaley, Dr. Bridle

Department of Pathology

Acute lymphoblastic leukemia (ALL) is uncontrolled proliferation of immature lymphoblasts, and is the most common cancer diagnosed in children. While current treatments for leukemia have increased the five year survival rate for children with B cell ALL, the prognosis is less positive for T cell leukemias and is worse for adults with the disease. Current therapies are expensive, long-term, and have a profoundly negative impact on quality of life. This study seeks alternative treatment to target malignant cells. Oncolytic viruses (OVs) can be effective in treating solid tumours, but OVs perform poorly in models of leukemia because the cells are widely dispersed among normal tissues with anti-viral defenses that can quench the infection. Histone deacetylase inhibitors (HDIs) can modulate the replication of OVs in solid tumours to enhance efficacy. We propose that only limited infection with an OV will induce a robust anti-viral response that can sensitize leukemic cells to the cytotoxic effects of class I HDIs. It is predicted that the virus will induce an immune response that renders leukemia cells susceptible to HDI-mediated cell death. Oncolytic maraba virus and viral mimics in combination with class I HDIs are being tested for the systemic treatment of leukemic cells in syngeneic murine models of B-cell and T cell ALL. Converting limited infections of leukemia cells with an OV into potent systemic therapies represents a paradigm-changing way to address safety and efficacy issues associated with conventional treatments for ALL.

Efficacy of 3TSR Fusion Proteins on Epithelial Ovarian Cancer

Simone ten Kortenaar[1], Jack Lawler[2], Jim Petrik[1]

[1]Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph Ontario Canada. [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston Massachusetts, USA.

Epithelial ovarian cancer is the most common and most deadly of the gynecological disorders, with late stage detection and inadequate treatment options contributing to its high mortality rate. Angiogenesis is a key mediator of cancer development, and as such represents an attractive therapeutic target for the inhibition of tumour growth. Thrombospondin-1 has been shown to have anti-angiogenic and apoptotic effects on vasculature and ovarian tumour cells. Current issues with thrombospondin-1 mimetic peptides include the short half-life of these compounds, which limits their efficacy. We therefore sought to evaluate the efficacy of a newly developed thrombospondin-1 mimetic fusion protein known as Fc-3TSR. Fc-3TSR is comprised of a linker protein that joins two peptides containing the 3 type I repeats (3TSR) of the TSP-1 gene. We believe Fc-3TSR will have increased anti-tumour and anti-angiogenic effects due to its increased molecular size. Initiating its anti-angiogenic and apoptotic effects through the CD36 receptor on both tumour and endothelial cells, the longer half-life of Fc-3TSR may significantly improve its anti-tumour properties. Results to date demonstrate an increased ability of Fc-3TSR to induce apoptosis and inhibit proliferation in ovarian tumour cells when compared to 3TSR. In addition, Fc-3TSR has been demonstrated to potently reduce the invasiveness of ovarian tumour cells and regulates factors important for angiogenesis and tumor cell survival. We have shown that 3TSR can potently induce regression of advanced stage ovarian cancer in a mouse model of disease and the increased half-life of Fc-3TSR may induce regression even more dramatically.

Funding Source: OGS

RNA-seq profiling of the bronchiolar epithelium: What does the transcriptional landscape reveal about gene regulation in recurrent airway obstruction in horses?

Laurence Tessier, Olivier Côté, Mary Ellen Clark, Dorothee Bienzle

Department of Pathobiology, University of Guelph, Guelph, Canada, 50 Stone Road, Guelph, Ontario, Canada N1G 2W1.

Recurrent airway obstruction (RAO) is a chronic inflammatory lung disease affecting mature horses similar to asthma of humans. The airway epithelium is the first tissue to respond to irritants and analysis of gene expression in the bronchiolar epithelium may provide crucial information regarding the pathogenesis of RAO.

We hypothesized that expression of genes and proteins involved in epithelial repair and inflammation differs in horses with and without RAO exposed to an asthmatic challenge. Furthermore, we also hypothesized that some of these differences originate from gene variants.

The response of the epithelium after exposure to an inhaled challenge was determined in horses with and without RAO by transcriptome sequencing (RNA-seq) to identify differentially expressed genes and exons, and potentially associated variants. Genome-based alignment, differential expression and variant analysis were performed with Tophat2, R and Genome Analysis Toolkit software, respectively. The significance level was set at a False Discovery Rate (FDR) of 0.05.

We found 101 differentially expressed genes involved in immune response, inflammation and cell cycle in horses with RAO compared to normal horses. Differentially expressed exons (20,816) and variants were also identified, but further analysis is required to determine their potential association with RAO. In depth investigation of these candidate genes and gene regulatory mechanisms will be performed. This work may identify key events of the pathogenesis.

Funding source: Equine Guelph, NSERC, CRC program, OVC scholarship

Corvid and mosquito pool surveillance data for the detection of West Nile virus in Ontario, 2002-2008: a comparison using survival analyses and spatial scan statistics.

A.L. Thomas-Bachli[1], D.L. Pearl[1], O. Berke[1], J. Parmley[2], I.K. Barker[3]

[1] Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada.
[2] Canadian Wildlife Health Cooperative, Guelph, Ontario, Canada
[3]Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada

Detection of WNv in Ontario has involved enhanced passive surveillance of dead wild corvids, and active surveillance of suspected vector mosquito species. The historical dead bird and mosquito surveillance data from the Ontario experience with WNv have not been compared in terms of speed to detection within a public health unit (PHU). The objectives of this study were to determine whether the timing of first WNv-positive test results from dead corvid and mosquito pool tests within PHUs were comparable, and to examine yearly trends and factors that may bias these surveillance modalities. Using survival analyses, we investigated the time, distribution, and yearly trends, sociodemographic and geographic factors associated with the first WNV-positive dead birds and mosquito pools detected within PHUs. Spatial scan statistics were employed for spatio-temporal cluster detection of first-positive dead birds, mosquito pools and human cases within public health units (PHUs) across Ontario. WNv activity within a PHU generally was detected earlier using dead birds, although there was variation related to year, region, and whether the PHU was predominantly rural or urban. Statistically significant ($p<0.05$) space-time clusters of PHUs with short time-to-detection of first WNv cases were found using all data streams during each year of study except dead bird data in 2005. There was geographic overlap between clusters of positive dead birds, mosquito pools and human cases during all years in which significant clusters were found. Time-to-first-positive dead bird and mosquito pool data may provide complementary information for public health surveillance of West Nile virus in Ontario.

Funding Source: OVC PhD Fellowship

Regulatory mechanisms controlling microRNA availability during bovine *in vitro* oocyte maturation

Tscherner, A. and LaMarre, J.

Department of Biomedical Sciences

Oocyte maturation comprises a series of events that precede ovulation *in vivo* during which the egg becomes competent for fertilization and builds a repertoire of mRNA and protein necessary for embryogenesis. Many aspects of the process can be modeled *in vitro*. Maturation events include the condensation of genetic material and a widespread decrease in transcriptional activity. This suggests that many gene expression changes in the maturing oocyte are regulated post-transcriptionally.

MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression. They are transcribed as precursors (pri-miRNAs) that are subsequently cleaved enzymatically into active forms that bind specific sites on target mRNAs. MiRNAs are abundant in oocytes and we hypothesize that pri-miRNA transcription and processing represent key events during maturation.

MiRNA-21 targets apoptosis-related mRNAs to promote cell survival and proliferation and is increased during *in vitro* oocyte maturation. We therefore investigated miR-21 biology in this context. Pri-miR-21 transcription was observed during the first 8-16 hours of maturation. Characterization of the pri-miR-21 transcript using 5' and 3' RACE revealed two isoforms, which were cloned and sequenced. Using bioinformatic approaches, we identified potential regulatory elements in the pri-miR-21 promoter. To explain the increase in mature miR-21, we then investigated the expression of the pri-miRNA processing enzyme Drosha. Western blot analysis revealed that Drosha levels increase late in maturation, coinciding with increased levels of mature miR-21. These findings suggest that regulated miRNA transcription and processing may guide oocyte maturation.

Funding Source: OMAFRA and NSERC

Angiotensin converting enzyme chronotherapy benefits cardiac structure and function in a murine heart attack model

E. V. Tsimakouridze, F. J. Alibhai, and T. A. Martino.

Introduction: The cardiovascular system exhibits diurnal rhythms which can be targeted to increase drug efficacy by optimizing timing of treatment. Angiotensin converting enzyme inhibitors (ACEi) are prescribed to heart attack patients. This study investigates diurnal efficacy of ACEi captopril using an established myocardial ischemia-reperfusion (IR) murine model.

Hypothesis: Administering captopril sleep vs. wake-time benefits cardiac structure and function post myocardial IR in mice.

Methods and Results: To determine whether wake or sleep-time captopril is most efficacious, mice were randomized to either: 1) wake-time captopril post-IR; 2) sleep-time captopril post-IR; 3) vehicle post-IR; 4) vehicle sham. Captopril was injected daily 1-5 wks post-IR. Morphometric analyses at 12 wks post-IR showed that sleep vs wake-time captopril significantly decreased heart weight: body weight (5.05±0.09 mg/g vs 5.75±0.25 mg/g). Echocardiography at 12 wks post-IR showed that sleep vs. wake-time captopril significantly improved left ventricular (LV) end diastolic diameter (4.56±0.05 mm vs 4.82±0.11 mm), LV end systolic diameter (3.16±0.04 mm vs 3.59±0.12 mm), ejection fraction (62.62±0.87% vs 56.86±1.45%) and fractional shortening (29.26±0.57% vs 25.67±0.83%). Hemodynamics at 12 wks post-IR showed that sleep vs. wake-time captopril significantly ($p<0.05$) improved mean arterial pressure (75.48±0.74 mmHg vs 69.68±1.75 mmHg) and stroke volume (19.08±0.58 ul vs 14.31±1.15 ul).

Conclusion: This study demonstrates that administering ACEi at sleep time benefits (reverses) cardiac remodeling post-IR. This novel strategy provides a new clinical approach to benefit patients with heart disease.

Funding Source: CIHR

Development of a Pig Grimace Scale for Evaluation of Pain and Analgesia Efficacy in Neonatal Pigs

Abbie Viscardi[1], Michelle Hunniford[1], Penny Lawlis[2], Matthew Leach[3], and Patricia V. Turner[1]

[1]Dept of Pathobiology, University of Guelph, Guelph, ON,
[2]Ontario Ministry of Agriculture and Food, Guelph, ON,
[3]School of Agriculture, Food, and Rural Development, University of Newcastle, Newcastle on Tyne, UK

In North America, a number of painful procedures are conducted on neonatal pigs, including iron injection, teeth clipping, ear notching/tagging, and castration. Given that over 200 million pigs are slaughtered in North America annually, at least 50% of which are male, developing and validating cost-effective procedures to minimize pain and distress associated with castration and processing will have a significant and positive impact on animal welfare. The objectives of this study were to develop and validate a Pig Grimace Scale (PGS) in association with behavioural scoring techniques to assess pain in castrated neonatal pigs and to assess the analgesic efficacy of meloxicam and EMLA (topical anesthetic cream) given prior to castration. Castration was performed on 4 litters of 5 day old pigs (n=21) with treatments randomized across litters: meloxicam + EMLA, meloxicam + non-medicated cream, saline + EMLA, saline + non-medicated cream, and no treatment (2-5 pigs/group). Pens were video recorded for 1h at 24h prior to castration, immediately after castration for 7h, and for 1h at 24h post-procedure. Thirty behaviours or postures were scored continuously for the first 10min at -24, 0, 1, 2, 4, 5, 6, 7, and 24h by an observer blinded to treatment. For PGS scoring, an observer blinded to treatment captured 627 facial images across the 9 timepoints. Facial action units and an associated scale were developed to include ear position, orbital tightening, and cheek bulge. Two individuals blinded to treatment scored each photo separately. Baseline PGS scores from -24h pigs were subtracted from scores obtained post-castration. Data was analyzed using a GLM ANOVA with Bonferroni post hoc tests. Pigs demonstrated significant behavioural changes up to 7h post-castration and the use of meloxicam and EMLA were not associated with a reduction in painful behaviours or postures. No litter-associated differences were noted in behavioural or PGS data so data was combined across litters. There were no treatment differences in PGS scores; PGS scores at 0, 3, 4, and 5h were significantly higher than those at 7h post-castration ($p<0.05$). These findings indicate that the PGS may have utility for evaluating pain in neonatal pigs.

Funding Source: CCSAW

Molecular and statistical analysis of *Campylobacter* spp. carriage and antimicrobial resistance in mammalian wildlife and livestock species from Ontario farms (2010)

Viswanathan M., Pearl D., Taboada E., Parmley E.J., and Jardine C.

Department of Population Medicine

This study aimed to assess risk factors for carriage of *Campylobacter* and antimicrobial resistance (AMR) of *Campylobacter* among livestock and mammalian wildlife on farms in Ontario, and to determine if *Campylobacter* subtypes are exchanged between wildlife and livestock based on molecular subtyping results. Using data collected from a cross-sectional study of 25 farms, associations between *Campylobacter* and AMR *Campylobacter* carriage and the following explanatory variables were assessed using mixed logistic regression models: animal, farm type, livestock or wildlife sample, and *Campylobacter* species; included was a random intercept to account for the farm site of collection. Subtyping was done using *Campylobacter*-specific 40 gene comparative fingerprinting assay. Livestock isolates were significantly more likely to exhibit AMR to ≥1 antimicrobials compared to wildlife isolates. *C. jejuni* was significantly more likely to exhibit AMR to ≥1 antimicrobial compared to *C. coli*. Resistant *C. jejuni* isolates were resistant to only tetracycline while *C. coli* exhibited multi-drug resistance patterns, ≥2 antimicrobials. Livestock samples were significantly more likely to test positive for *Campylobacter* than wildlife samples. Swine had significantly increased odds of testing positive for *Campylobacter* compared to dairy cattle. The odds of shedding *C. jejuni* was significantly greater in beef cattle compared to dairy cattle and raccoons. Only one subtype of the 50 subtypes of *Campylobacter* identified was found in both wildlife and livestock. We concluded that the sharing of *Campylobacter* between livestock and wildlife was uncommon based on identical subtype similarity and AMR patterns.

Funding Source: Ontario Veterinary Scholarship

Prioritizing community well-being in Guelph, Ontario

Lauren E. Wallar and Andrew Papadopoulos

Department of Population Medicine

Community well-being represents a state in which all community members achieve the highest quality of life. It can be measured at the municipal level using a set of indicators across 8 domains. In order to meet well-being needs, public health must marshal their limited resources in order to deliver select programs and services. Knowledge of community well-being priorities would improve resource allocation; however, priority measurement can be costly and time-consuming. To address this, Wellington-Dufferin-Guelph Public Health collaborated with the University of Guelph to conduct a well-being prioritization exercise. The objective of this collaborative project was to determine the relative importance of 25 well-being indicators by socioeconomic status in the City of Guelph. A maximum difference scaling survey instrument was created in which respondents were repeatedly asked to choose the “most important” and “least important” indicator from different indicator subsets. The survey instrument also collected postal code and demographic information. Surveys were distributed to respondents on-line and in geographically diverse locations in Guelph. Preliminary responses were analyzed using Hierarchical Bayes to determine importance scores for each well-being indicator. Final results are anticipated to elucidate community well-being priorities by socioeconomic status, and ultimately aid local public health planning and delivery of targeted community programs and services.

Funding Source: 2014-15 Ontario Graduate Scholarship, 2012-15 Ontario Veterinary College Scholarship, and 2013-17 Sawtooth Software academic grant

Ear notching of mouse pups does not require analgesia as assessed by the Mouse Grimace Scale and behavioral scoring

Walsh, J, Cheng, P, Viscardi, A, Niel, L, Litman, M, Turner, P,

Dept of Pathobiology[1] and Population Medicine[2], Office of Research Services[3], University of Guelph, Guelph, ON

Our objectives were to evaluate and validate the Mouse Grimace Scale (MGS) and behavioral scoring as tools to assess pain in preweaned mice, and to assess the efficacy of 10 mg/kg carprofen in drinking water. Ear notching was performed on 110 (14 litters) 19-28 d.o. mice during routine colony management. Six litters received 10 mg/kg/day carprofen in drinking water beginning 24h before ear notching, 6 negative control litters received no analgesic in the water, and 2 untreated control litters received no manipulations or treated water. Litters were video recorded immediately after ear notching for 5h at the same time each day. For behavioural data, 21 behavioral categories were scored continuously for the first 10min at 0, 0.5, 2, and 4h by an observer blinded to treatment. For MGS scoring, 363 facial images were captured at 0, 2, and 4h post-ear notching and scored by two observers. MGS scores from untreated control pups were subtracted from scores from carprofen-treated or untreated pups. Data was analyzed using a linear mixed model ANOVA with post hoc Bonferroni tests. No litter-associated differences were noted in behavioral or MGS data and data was combined across litters. No differences were noted in behaviours of carprofen-treated vs untreated mice except at 4h, when untreated mice spent more time nursing ($p<0.03$). There were no treatment differences in MGS scores. This indicates that ear-notching is not a highly painful or distressing procedure for mouse pups and that the MGS has utility for evaluating pain in preweaned mice. Provision of carprofen in water at approximately 10 mg/kg to mouse pups provided no additional benefit.

Funding Source: Funding was generously provided by the AALAS Foundation, Foundation and Center for Alternatives to Animal Testing, John's Hopkins University and UFAW.

Dietary vitamin D intake and vitamin D status in canine cancer patients

Weidner N[1], Woods JP[1], Conlon P[2], Meckling KA[3], Atkinson JL[4], Bayle J[5], Verbrugghe A[1]

[1]Department of Clinical Studies, University of Guelph, ON, Canada [2]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, ON, Canada [3]Department of Human Health and Nutritional Sciences, College of Biological Sciences, University of Guelph, ON, Canada [4]Department of Animal and Poultry Science, Ontario Agricultural College, University of Guelph, ON, Canada, [5]Royal Canin Research Center, Aimargues, France

Background: Low vitamin D status is linked to increased risk of cancer in humans. This association is starting to be explored in canines. We examined the link between cancer, and dietary vitamin D intake and the vitamin D status of canines. Canines with cancer were hypothesized to have lower plasma 25-hydroxy-vitamin D (25(OH)D) when compared to healthy canines due to their lower dietary vitamin D intake.

Methods: Canines with osteosarcoma (n=18), lymphoma (n=22) and mast cell tumors (n=21) were enrolled, as well as healthy canines (n=20). Owners provided dietary information and a sample of the dog's food for calculation of each dog's dietary vitamin D intake, based on vitamin D information provided by the pet food manufacturer and vitamin D_3 analysis of the pet food sample. Blood samples were analyzed for plasma 25(OH)D, ionized calcium, parathyroid hormone and parathyroid hormone-related protein. Statistical analysis was done with SAS 9.3.

Results: Median plasma 25(OH)D concentration was significantly higher in healthy dogs (126.38 nmol/L) than in those with osteosarcoma (93.591 nmol/L, p = 0.0056) and lymphoma (96.74 nmol/L, p =0.0088), but not mast cell tumours (106.947 nmol/L, p=0.0990). There was an independent effect of cancer (p=0.0203), dietary vitamin D intake (p=0.0093) and plasma ionized calcium (p=0.0313) on plasma 25(OH)D concentrations.

Conclusions: The independent effect of cancer suggests that dietary vitamin D intake is not responsible for observed differences in plasma 25(OH)D status. Further research is needed to investigate whether decreased plasma 25(OH)D concentrations are a factor in cancer development, or a consequence of cancer.

Funding sources: OVC Pet Trust, AAVN/Waltham Research Grant and Royal Canin

The Individual and Combined Effects of Long-chain n-3 Polyunsaturated Fatty Acids and Low-dose Aspirin on Platelet Function in Healthy Dogs

Westgarth S, Blois SL, Wood RD, Ma DW, Verbrugghe A, Defarges A, Lang S

Department of Clinical Studies

Background: Thromboembolic events are a common complication of many diseases in veterinary patients, and anti-platelet therapies can be ineffective in their prevention. n-3 polyunsaturated fatty acids (n-3 PUFA) can inhibit platelet function in people, and enhance the effect of anti-platelet therapy. The efficacy of n-3 PUFA on platelet function in healthy dogs is unknown.

Objectives: To evaluate the effects of low-dose aspirin (ASA; 1 mg/kg/day) alone, n-3 PUFA (100 mg/kg/day) alone, and the synergistic effects of both treatments on platelet function and activation in healthy dogs.

Animals: 18 healthy owned dogs.

Methods: Platelet function was measured using platelet function analyzer (PFA) closure time and whole blood aggregometry. Flow cytometry was used to measure platelet activation and platelet leukocyte aggregates. Above tests were evaluated at baseline, during therapy with ASA and n-3 PUFA alone, and during combination therapy to assess effects on platelet function.

Results: n-3 PUFA alone did not cause a significant change in platelet function tests. ASA alone decreased platelet function as measured by PFA-epinephrine following one week of therapy ($p<0.0001$). ASA plus n-3 PUFA decreased platelet function significantly more versus ASA alone when measured by whole blood aggregometry (agonists: arachidonic acid $p=0.008$; collagen $p=0.006$). No change in platelet activation was detected via flow cytometry after any therapies.

Conclusions: ASA plus n-3 PUFA appear to have a synergistic effect on inhibition of platelet function in healthy dogs. This combination therapy may increase efficacy of anti-platelet therapy in dogs at risk of thromboembolic complications.

Funding Source: OVC Pet Trust

Equine allogeneic umbilical cord blood mesenchymal stromal cells reduce acute synovial fluid nucleated cell count and induce low-grade sustained inflammation when evaluated in a LPS induced synovitis model

Lynn B Williams[*], Judith B Koenig[*], Belinda Black[*], Thomas WG Gibson[*], Shayan Sharif[†], Thomas G Koch[‡§]

Departments of Clinical Studies[*], Pathobiology[†], and Biomedical sciences[‡]; University of Guelph, Guelph, Ontario, Canada. Department of Clinical Studies[§], Orthopaedic Research Lab; Aarhus University, Aarhus, Denmark

Clinical improvement has been reported following intra-articular (IA) injection of mesenchymal stromal cells (MSC) in several species. This has led to use of IA MSC in equine practice with little understanding of the mechanisms which perceived improvement occurs. We hypothesized that IA injection of allogeneic CB-MSC would decrease the inflammatory response associated with LPS injection.

Two feasibility studies evaluated IA injection of LPS or allogeneic CB-MSC alone into the tibiotarsal joint. Acute middle carpal joint synovitis was then induced bilaterally using LPS in six horses and allogeneic CB-MSC were injected into one middle carpal joint. Lameness, routine synovial fluid analysis, and synovial fluid (SF) biomarkers were evaluated.

LPS injection alone resulted in transient lameness and marked signs of inflammation. Injection of 30-million CB-MSC resulted in mild synovitis that resolved without treatment. Mild lameness was observed in two animals and severe lameness was observed in the remaining animal in MSC-treated limbs 24h post-injection.

Injection of LPS and CB-MSC resulted in significant reduction in SF total nucleated, neutrophil, and mononuclear cell numbers compared to LPS-only treated joints at 8 hours post-injection and mild, sustained inflammation at 48 and 72 hours. No differences were detected in other parameters. Lameness in CB-MSC treated limbs was observed at 8h, which resolved by 24h.

Allogeneic CB-MSC significantly reduced SF cell populations in an acute synovitis model. Further evaluation of dose, timing of treatment, and additional effects of treatment are needed to evaluate CB-MSC as an efficacious therapeutic option in equine sports medicine.

Funding Source: Equine Guelph

Comparative Analysis of the Genomes of the Fish Pathogen *Flavobacterium psychrophilum*

Anson K.K. Wu, Andrew M. Kropinski, Daniel Ashlock, Janet I. MacInnes

Department of Pathobiology, Ontario Veterinary College

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease and rainbow trout fry mortality syndrome in salmonid fish, cyprinids, and eels and is associated with significant losses in the aquaculture industry. The virulence factors and molecular mechanisms of pathogenesis of *F. psychrophilum* are poorly understood and as there is no vaccine available and antibiotic resistance is increasing, control of this pathogen is difficult. At the present time the only complete genome sequence available is that of a European strain. To better understand the agents of BCWD in North America we sequenced the genome of *F. psychrophilum* ATCC 49418^{T} , the virulent type strain isolated in Washington state, two virulent Ontario strains, FPG101 and FPG 10, and an avirulent Ontario strain, IWL08. The genome of the ATCC49418 strain is 2,715,909 bp with a G+C content of 32.75%. It contains 6 rRNA operons, 49 tRNA genes, and is predicted to encode 2,329 proteins. No pathogenicity islands were present; however, some putative and previously characterized virulence factors were found including 4 metalloprotease genes, 2 new hemolysin genes, 6 iron transport genes, and 11 similar, but not identical, outer membrane protein genes. In silico analysis using progressive Mauve revealed major differences amongst the strains from the three countries, which is consistent with the notion that they have adapted to specific regions. This analysis is vital for the understanding the virulence mechanisms of *F. psychrophilum* and in identifying good targets for the development of an efficacious vaccine.

Funding Sources: NSERC