2016 Graduate Student Research Symposium and Schofield Lecture
Preface

It is our great pleasure to welcome you to the 2016 Graduate Student Research Symposium (GSRS). This volume contains the abstracts for the 94 presentations at this year's symposium including 46 talks and 48 poster presentations. We would like to thank the graduate students and faculty members for their participation as well as the many sponsors of student research at the Ontario Veterinary College. The Organizing Committee hopes that you will find this symposium to be compelling and thought-provoking, and find that it increases awareness of research activity at the college and provides a valuable opportunity for students and faculty to discuss results and share ideas.

Sincerely,

The OVC Graduate Student Research Symposium Organizing Committee:

Emilija Penney, Pathobiology
Mikayla Ross, Pathobiology
Shaimaa Abdelmegid, Biomedical Sciences
Allison Tscherner, Biomedical Sciences
Kathy Matuszewska, Biomedical Sciences
Vashine Kamesan, Biomedical Sciences
Alexandra Rankovic, Clinical Studies
Bianca Di Sabatino, Clinical Studies
Rashi Asthana, Clinical Studies
Rachael Milwid, Population Medicine
Alexandra Sawatzky, Population Medicine
Nanette Lai, Population Medicine
Kaitlin Patterson, Population Medicine
Dr. Gordon Kirby, Associate Dean, Research and Innovation
Elizabeth Lowenger, Manager, Student Affairs
Barb Gaudette, Graduate Studies and Research Assistant
Anita Luu, Biomedical Sciences
Jonna Mazet, DVM, MPVM, PhD, is a Professor of Epidemiology and Disease Ecology and Executive Director of the One Health Institute in the UC Davis School of Veterinary Medicine, where she focuses on global health problem solving, especially for emerging infectious disease and conservation challenges. Dr. Mazet is active in international One Health research programs, most notably in relation to disease transmission among wildlife, domestic animals, and people and the ecological drivers of disease emergence. Currently, she is the Global Director of a $175 million viral emergence early warning project, named PREDICT, that has been developed with the US Agency for International Development’s (USAID) Emerging Pandemic Threats Program. She was elected to the US National Academy of Medicine in 2013 in recognition of her successful and innovative approach to emerging environmental and global health threats.

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2016 Schofield Memorial Lecture & Graduate Student Research Symposium

Wednesday, November 16
OVC Lifetime Learning Centre

Featuring:
Dr. Jonna Mazet, DVM, MPVM, PhD

Topic:
One Health Success: Moving toward a world free of pandemics

Schedule of Activities:

10:00 - 11:30 AM Poster Presentations 1707 B/C
12:30 - 3:50 PM Oral Presentations 1713/1715/1812
4:00 - 5:00 PM Schofield Memorial Lecture 1714
5:00 PM Awards Ceremony & Reception 1707A
### POSTER SESSION WITH GRADUATE STUDENTS, ROOM 1707 - 10:00 – 11:30

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EPIDEMIOLOGY OF TICKS COLLECTED FROM PET DOGS IN AN EMERGING LYME DISEASE AREA OF ONTARIO

James, C et al.

ACKNOWLEDGEMENTS
Characterization of the Fecal Microbiota of Commercial Mink

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There is increasing interest in characterizing the components of the gut microbiota to determine their impact on health, disease, and production. Our objectives were to characterize the fecal microbiota of mink and evaluate differences between females and kits as well as temporal stability. We also sought to determine whether dietary changes affect microbiota composition, as female mink diets change between summer and winter. Pooled fecal samples were collected from healthy females and weaned kits over 2 summers (females n=153; kits n=160), and healthy females in winter before breeding (n=30). Bacterial DNA was extracted and characterized using 16S next generation sequencing. More than 20 million sequences were identified from >1300 genera, only 3 of which were >5% of the total: Enterococcus (~6%), commensals of the mammalian gut; Lactobacillus (~11%), involved in lactic acid production; and Ignatzschineria (~14%), likely a contaminant from fly larvae. There were differences between years (p<0.001), likely due to variation in diet. The overall predominant phyla are Firmicutes and Proteobacteria, with median relative abundances of 58% and 31%, respectively. Differences were only identified between females and kits in uncommon phyla, unsurprising as the microbiota at weaning closely resembles that of adults in other species. The high relative abundance of Proteobacteria is unlike other mammals. This may be attributable to their diet in captivity, consisting largely of livestock byproducts with minimal food safety oversight. As some of these bacteria can cause morbidity and mortality in mink, these results may help to shape practices to optimize mink production.
Canine T-cell Lymphoma: Cytomorphological, Histomorphological, Immunohistochemical, and Flow Cytometric Analysis

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Lymphoma is common in dogs and frequently treated with chemotherapy. It is grouped into B- or T-cell using CD79a or CD21 (B) and CD3 (T) markers. T-cell lymphoma (TCL) is considered to have a worse prognosis than B-cell lymphoma, however, there are different types of TCL with variable biological behaviour. Few studies have examined whether prognosis differs between subtypes of TCL. The objective of this study was to determine the prognosis of patients with TCL characterized by cytopathology, histopathology, immunohistochemistry (IHC), and flow cytometry (FC). Lymph node aspirates and biopsies were collected and flow cytometrically analyzed for CD3, CD4, CD5, CD8, CD21, CD22, CD45, and MHC II antigens. Formalin-fixed paraffin-embedded sections were assessed for architecture and cytomorphology, and by IHC for expression and location of CD3, CD79a, and granzyme B. On frozen sections, CD4, CD8, and CD21 expression was determined. Tumors were classified following World Health Organization guidelines, and results compared to FC and cytopathology results, response to chemotherapy and survival. Based on FC, cases were CD8+ (n=4), CD4+ (n=5), and CD4-/CD8+ (n=4). Histomorphologically, tumors had a diffuse architecture with peripheral displacement and compression of follicles. Based on IHC, all tumors diffusely expressed CD3; four were granzyme-positive, and CD79a expression was restricted to follicles. In the frozen sections, CD4, CD8, and CD21 were expressed diffusely when present with varying levels of intensity. Based on preliminary analysis, patients with CD8+ T-cell lymphoma had a different response to chemotherapy than those with CD4+ lymphoma.
Production of stable cell lines expressing structural proteins of Newcastle disease virus

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Newcastle disease (ND) is a devastating disease of poultry caused by Newcastle disease virus (NDV). ND control is maintained through strict biosecurity measures and vaccination programs, however, vaccination does not elicit sterile immunity and its efficacy is decreased against virulent genotypes distant from those of vaccine strains. Production of poultry resistant to NDV infection could dramatically assist in controlling the disease, by mitigating the magnitude and the effects of ND outbreaks.

Animals that naturally express proteins from endogenous viruses (i.e., retroviruses) are resistant to homologous virus infection (or challenge). Similarly, mammalian cell lines persistently infected with certain viruses or expressing virus proteins show high degrees of resistance to homologous virus challenge (superinfection exclusion). In plants, this phenomenon has been exploited to create transgenic crops that are resistant to virus challenge by expression of virus structural proteins. This has been termed pathogen-derived resistance (PDR). Although extensively researched and applied in plant biology, PDR in animal species is poorly understood, and its applicability to control viral diseases of livestock has not been thoroughly investigated.

The overall goal of this study was to assess the feasibility of PDR against NDV in vitro, by assessing resistance to virus-induced cell death and virus growth kinetics in cell lines constitutively expressing NDV proteins.

Here, we present preliminary work done to produced stable cell lines expressing three NDV structural proteins (nucleoprotein [NP], phosphoprotein [P] and polymerase [L]). The open reading frames of the NP, P and L genes of NDV LaSota strain were cloned into lentivirus expression vectors as both translatable and untranslatable constructs, and packaged lentiviruses were used to transduce Vero cells to produce stable cell lines.
Mechanisms that Allow Oncolytic Viral Replication Inside a Tumour Despite Pre-existing Immunity Against a Virus-Encoded Antigen

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Oncolytic viruses (OVs) selectively target and destroy cancerous cells, while leaving normal cells unharmed. In addition to directly reducing tumour burden, OVs engineered as vaccine vectors can boost tumour-specific immune responses. Having confirmed that OVs are safer when used as booster vaccines, a mystery remained: How could OVs replicate and mediate oncolysis in hosts with pre-existing T cells specific for a tumour antigen that was also encoded by the virus? We identified two possible mechanisms: (1) Acute virus-induced lymphopenia that transiently reduced transgene-specific tumour-infiltrating lymphocytes (TILs), and/or (2) The immunosuppressive tumour microenvironment rendered TILs dysfunctional and inefficient at the clearance of viruses. We used an intradermal B16-F10 melanoma model in which mice were primed with an adenovirus expressing the melanoma antigen dopachrome tautomerase (DCT) and boosted with vesicular stomatitis virus also encoding DCT. Our results show that the numbers of DCT-specific T cells inside tumours were reduced at six hours post-boost, with a preferential reduction of those with a multi-functional phenotype. Assessments of transgene-specific CD8+ TILs revealed impaired degranulation, reduced production of multiple cytokines, and lower T-cell receptor avidity suggesting they were functionally impaired compared to blood- and spleen-derived T cells. In vivo cytotoxicity analysis also revealed reduced killing of target cells in the tumour. Although seemingly counterintuitive, this research identifies induction of OV-specific immunity as a strategy to enhance the safety of oncolytic virotherapy while retaining direct oncolytic activity.
Autophagy-Related Gene Expression during Feed Restriction in Rainbow Trout (Oncorhynchus mykiss)

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Autophagy is a highly conserved cellular mechanism that exists in organisms as diverse as yeast to mammals. Autophagy controls autophagosome function, including removal of damaged organelles and long-lived proteins. However, survival of intracellular microorganisms can also be altered. It is primarily an adaptive response to stressful conditions, such as starvation. Although autophagy has been extensively researched in yeast and humans, there are very few studies in fish. The purpose of the project will be to quantify a number of autophagy-related genes (Atg) in rainbow trout (Oncorhynchus that may serve as molecular biomarkers to further investigate autophagy in these two species. Sequences of potential Atg will be aligned between closely related species to identify conserved sequences suitable for PCR and quantitative polymerase chain reaction (qPCR). qPCR will be used to detect changes in Atg expression. The results will be compared to western blot for LC3II (microtubule-associated protein 1 light chain 3), an established technique for the detection of autophagy. Experimental models for rainbow trout will include fish undergoing feed restriction, and a gill and liver cell line subject to nutrient restriction. A range of tissue samples at various time points have been collected from the fish and cells for RNA extraction.
ADOPTION AND CONSISTENCY OF APPLICATION OF PRE-MILKING PREPARATION IN ONTARIO DAIRY HERDS

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Studies have found that consistency in milking methods and routines can vary substantially from farm to farm, and this can have an effect on udder health. The objective of this study was to assess the variation in adoption, and application consistency, of important milk harvest practices between and within farms over time. During the summer of 2013, 50 herds in southern Ontario were visited twice within a month during the summer time (June to September) during milking time, and a single person observed and time recorded pre-milking preparation procedures. A generalized mixed model was used to calculate variance partitions for pre-disinfectant contact time and preparation lag time, and determine the proportion of variation attributable to each random effect on the outcomes studied. Then, using logistic regression, two models were built to assess factors affecting adequate contact time and adequate prep lag time, respectively. Farm, visit number, and the person(s) milking were used as random effects in both models. In both models, farm-to-farm and cow-to-cow differences accounted for the largest part of the variability seen in both contact time (47% and 44% respectively) and prep lag time (40% and 36% respectively). For both outcomes, milkers seemed to be consistent in their routines over the two visits (9% and 3.1% of total variance for contact and prep lag time respectively). Overall, the majority of farms in the study complied with the recommendations for adequate milking practices but do not follow a consistent timed protocol. This study shows there are several potential sources of variation in the milking routine on a dairy farm.
Surface water quality and surveillance in Iqaluit, Nunavut

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Waterborne disease is a global public health priority. Endemic levels and outbreaks of enteric illness transmitted by contaminated water contribute to considerable morbidity, mortality, and economic costs in Canada. One of the highest incidences of self-reported enteric illness reported in the global literature is in Iqaluit, Nunavut. To understand potential waterborne disease transmission in Iqaluit, this project estimated the prevalence, identified risk factors, and examined molecular source attribution for Giardia and Cryptosporidium parasites in untreated surface water that community members collect for drinking. Water quality data were collected weekly from June to September, 2016, from two streams commonly used as sources of untreated drinking water. Samples were collected and processed in Iqaluit for pathogen isolation and quantification of indicator bacteria. Samples were tested weekly to provide information about the presence of these pathogens, and positive samples will be genetically characterized to provide information about the molecular epidemiology of these pathogens. Environmental risk factors were analyzed to identify potential associations between positive samples, weather conditions, and other water quality parameters. Preliminary results include 2.4% of samples testing positive for Cryptosporidium and 12.2% of samples testing positive for Giardia (n=41). These results suggest that rates of Cryptosporidium and Giardia in surface water are lower in Iqaluit compared to Southern Canadian regions. The research team will work with Northern partners to develop a culturally effective knowledge translation strategy to share our results with the community.
Why did the pig cross the road? Assessment of pig movement as contributor to disease spread based on contemporary Ontario data

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In recent years a number of novel infectious pathogens have emerged within North American swine populations including Ontario. Subsequently, due to the structure of the swine industry, some of these pathogens have spread throughout swine populations. Our goal was to use these relevant measures to understand potential for disease dissemination through pig movement. A dataset was obtained consisting of pig movement from 224 unique Ontario swine facilities between January and December of 2015. It was stratified by week and directed networks of animal movement were constructed. Descriptive network-level measures were then obtained including largest weak components, largest ingoing and outgoing contact chains (ICC and OCC). Additional node-level measures were also obtained. The maximum Weak Component Size ranged between 37 and 83 facilities, with an average of 54.4, (sd=9.4). Weak component could be used to assess potential for infection spread when direction of movement is ignored. Examples are emerging coronaviruses which result in high environmental contamination. ICC ranged between 5 and 53 facilities (mean=30.2, sd=6.2), and is a statistic that indicates the potential for contracting the infection through animal movement. Finisher facilities had the highest percent of occurrence (49%) within a maximum ICC in a given week. OCC ranged between 1 and 6 facilities (mean=4.2, sd=1.0). Through this pathway, it is expected that up to 6 facilities could be infected in a given week. These preliminary results illustrate detailed transmission potential based on relevant common network measures. Future work will examine differences across measures through subsequent weeks.
An Integrative Investigation of Inuit Mental Health and Climate in Nunatsiavut, Canada

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Inuit rely on the environment to support their livelihoods, culture, relationships and wellbeing. Changes in the physical environment can limit the ability of Inuit to engage in land-based practices such as hunting, trapping, and harvesting. As such, climatic changes have been linked to mental health challenges including increased family stress, substance abuse, suicide ideation, and concurrent reports of an increased use of mental health services. Therefore, the present study seeks to investigate how meteorology, season, and climate exposures impact Inuit mental wellness and the use of health services in Nunatsiavut.

Using an exploratory sequential mixed methods design, secondary analysis of in-depth interviews (n=116) with community members and local health professionals from across the region will be used to identify factors important to Inuit mental wellbeing and healthcare-seeking behaviour. In addition to providing an in-depth understanding of environmental-mental health relationships, locally-identified factors will inform quantitative regression models of associations between meteorological variables and the use of mental health services across the region. Outcome data will be extracted from community clinic records and de-identified for all mental health-related visits from 2009 to 2016. Qualitative and quantitative findings will be integrated to develop an informed understanding of the relationship between mental wellbeing and climate in the region. Findings will provide contextualized evidence for decision-makers, and will be essential for strengthening public health initiatives to support Inuit mental wellness in a time of rapid environmental changes.
In mammals, including bovines, mammary gland involution following lactation is triggered by milk accumulation, and is characterized by tissue remodeling and apoptosis, which occurs in parallel to transient disruption of epithelial junction integrity and apical-basal polarity. It is well documented that TGFβ promotes loss of apical basal polarity and apoptosis, and is significantly upregulated during the dry period. The general goal of this study is to investigate TGFβ's modulation of PI3K/Akt activity, and whether this plays a role in bovine mammary gland involution. To this end, we employed the normal bovine mammary gland cell line MAC-T, to address whether a putative TGFβ-PI3K/Akt signalling axis mediates apoptosis, and whether this associates with loss of apical-basal polarity. MAC-T cells were cultured in monolayer and treated with 5 ng/mL of TGFβ for 0, 0.5, 1, 2, 4, 8, 16, 24 and 48 hours. Immunoblotting was used to examine the expression of polarity markers Par6, E-cadherin and Claudin-7, TGFβ signaling mediators Smad2 and Akt, as well as downstream targets of Akt, the pro-apoptotic FoxO transcription factors. TGFβ1 treatment caused a transient increase (1 hour) followed by sustained reduction in Akt phosphorylation/activation; this did not translate into a significant effect in the expression of FoxO1 or FoxO3a transcription factors. Complimenting these findings, TGFβ1 treatment increased levels of Cleaved Caspase 3, a marker of apoptosis, at 48 hours of treatment, and reduced Claudin-7 expression. These results suggest that TGFβ1-induced apoptosis in MAC-T cells associates with a reduction of Akt activity and possibly a disruption of tight junctions.
Discovering Molecular Determinants of Resistance to Newcastle Disease Virus

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Newcastle disease (ND) is one of the most prevalent and severe avian diseases worldwide and is caused by virulent strains of Newcastle disease virus (NDV). Control of ND is carried out worldwide through large-scale vaccination programs. However, extensive vaccination over the past 50 years has failed to prevent the spread and evolution of this virus, due to the genetic variability among NDV strains vaccine mismatch and lack of sterile immunity in vaccinated animals. If birds that have a reduced rate of infection against NDV (i.e., resistant or partially resistant) are utilized, it may be possible to considerably decrease the rate of NDV spread and evolution, as well as to decrease the cost of ND control. One possibility to accomplish this is through the discovery of avian genes linked to NDV resistance, in order to implement breeding strategies of the poultry parent stock. Forward genetics strategies using insertional mutagenesis rely on the ability of engineered DNA elements to induce genome-wide integrations that can yield loss-of-function of random genes (gene-trap). Recently, the use of the nearly-haploid human stable cell line (HAP-1) in combination with a gene-trap approach has proven successful for discovering host genes essential for the entrance and replication of several intracellular pathogens, such as Influenza, Ebola, Marburg, Lassa, and Andes viruses. No insertional mutagenesis approaches have been performed to assess determinants of resistance to NDV. Here, we hypothesize that down-regulation (loss-of-function) of specific cellular genes can confer resistance to NDV infection in HAP-1 cells by interfering with the steps of the viral infection.
Next-Generation Parasitology: A Novel Assay for Determining Eimeria Species causing Coccidiosis in Ontario Sheep

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A survey conducted by the Ontario Animal Health Network recently identified coccidiosis in lambs as a top clinical issue; despite its perceived importance to producers and veterinarians, coccidiosis in sheep caused by Eimeria has not been studied systematically in Canada since 1985 (Dohoo et al., PMID: 3840053). There are ~11 named Eimeria spp. that infect sheep and at least two, E. crandallis and E. ovinoidalis, are considered pathogenic (Taylor and Catchpole, PMID: 8087156). The identification of individual species, particularly those that are most pathogenic, is crucial information for effective and targeted anticoccidial treatment. Less pathogenic species can be excreted at large numbers without causing clinical effects, therefore, it is difficult to determine the actual severity of infection using conventional oocysts enumeration methods (McDougald, PMID: 480272) that provide only an aggregate count. The objective of this project is to develop and validate an economical molecular assay for the identification of Eimeria species present in feces; following validation, field collections from commercial operations will provide samples to confirm the utility of the assay with clinical specimens. Combining PCR and next-generation sequencing will permit both parasite identification and relative abundance of individual Eimeria species in samples from sheep with mixed coccidial infections. Such enhanced diagnostic clarity can help in the development of management, feed and treatment options that have the greatest potential to decrease the impact of coccidiosis.
Bioactivity of Bovine Colostrum and Milk Exosomes from Low, Average, and High Immune Responders on Human Intestinal Epithelial Cells

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Bovine milk contains bioactive components with immune-regulatory potential in newborn calves and humans. Expression of milk bioactive components is fine-tuned by microRNA (miRNA). The packaging of miRNAs in exosomes confers their protection from degradation and potentially promotes uptake by recipient cells. While dairy cows classified as high immune responders (HIR$^{TM}$) have improved colostrum and milk quality compared to average (A) and low (L) responders, the bioactivity of colostrum and milk exosome-derived miRNA at the human intestinal epithelial barrier remains to be explored. Therefore, the purpose of this study is to evaluate the functional role of milk exosomes at the intestinal interface using cancerous human intestinal epithelial cells (Caco-2). Exosomes were isolated by differential ultracentrifugation from colostrum and milk of L, A, or H responders. Exosomes were viewed by electron microscopy and confirmed by immunogold labeling, ELISA ExoEL kit and Western blot analysis for the presence of common exosomal-proteins (CD9, CD63, CD81, and Hsp70). To assess bioactivity, exosomes are co-cultured with human intestinal epithelial cells (IECs). Fluorescent labeling of exosomes using PKH67 dye confirmed their uptake by IECs, suggesting that bovine colostrum and milk exosomes are bioavailable to humans. An MTT assay is conducted to determine if exosomes are cytotoxic or promote the viability of IECs. Finally, caspase-3 activity in IECs is measured to evaluate whether bovine milk exosomes can be used therapeutically to target and induce apoptosis in cancerous IECs. This study is helping determine the functional importance of bovine milk on gastrointestinal health of humans.
The connection between caribou conservation and indigenous livelihoods in Labrador, Canada.

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Indigenous peoples in Labrador have shared a deep relationship with caribou in the region for millennia, relying on these animals for food security, survival materials, and cultural identity. This relationship has been changing within recent years as the caribou herds in Labrador have experienced population collapses. Compounding this, in 2013, the Provincial Government issued a total hunting ban on the George River Caribou Herd, cutting off Indigenous peoples from this food source and disrupting a historic relationship. Understanding the Indigenous perspectives to caribou conservation is valuable in sustaining populations into the future.

The goals are to produce a research-based documentary film about the population decline of the caribou and the resulting impacts on Indigenous communities throughout Labrador; to produce shorter, region-specific videos that provide an in-depth understanding of each community's perspective relating to caribou; to conduct in-depth interviews with filmed and non-filmed stakeholders to understand the ways in which Indigenous peoples are connected to caribou physically, mentally, spiritually, socially, economically, and for food security.

We anticipate this project will highlight the importance of caribou for all aspects of culture and wellbeing for the Indigenous, stand as a testament of traditional knowledge and Indigenous science related to caribou, and will analyze how people see moving forward with caribou management and Indigenous culture into the future. Building on the video interviews, we plan to expand the scope of the film to work to include research, policy recommendations, and educational.
**Toll-like Receptor 5 and 21 Ligands as Adjuvants for Inactivated H9N2 Avian Influenza Vaccines**

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Low pathogenic avian influenza virus (AIV) infection in chickens can result in economic loss and act as a reservoir for potential infection in humans. Poultry vaccination is a tool which can be used to decrease infection and transmission of AIVs. Prior research has demonstrated that toll-like receptor ligands (TLR) can act as vaccine adjuvants and their addition to inactivated AIV vaccines enhances the immune response elicited in chickens. The objective of this study was to compare the adjuvant capability of TLR ligand 5 (flagellin) and 21 (CpG ODN 2007) alone and in combination when co-administered with an inactivated H9N2 whole virus vaccine in chickens. Specific pathogen free chickens (n=105) were administered an H9N2 vaccine containing either a single dose of CpG (2 Î¼g or 10 Î¼g), flagellin (0.4 Î¼g or 2 Î¼g) or a combination of both ligands. An additional group received AddavaxTM, a squalene-based adjuvant similar to commercially used poultry vaccine adjuvants. Chickens were vaccinated twice and blood samples were collected weekly following the primary vaccination. Hemagglutination inhibition (HI) and IgM and IgY ELISA assays were used to assess systemic antibody responses. Results obtained suggest that vaccines containing CpG induced significantly greater systemic antibody responses than vaccines containing flagellin or AddavaxTM. The combination of flagellin and CpG did not demonstrate an inhibitory, additive or synergistic effect on systemic antibody responses. These results emphasize CpG as a candidate vaccine adjuvant and suggest that CpG and flagellin combination does not promote greater systemic antibody responses.
Global climate change has made northern latitudes vulnerable to the potential establishment and spread of ticks that may carry a variety of zoonotic pathogens, including Powassan virus (POWV), Anaplasma phagocytophilum, Babesia microti, Borrelia burgdorferi, B. miyamotoi and Ehrlichia chaffeensis. The diversity of these pathogens and the extent of their geographic distribution within Ontario are poorly understood. The objectives of this study were to investigate the geographic distribution and prevalence of ticks and their potential tick-borne pathogens and to determine the most likely wildlife reservoir host species for POWV. During summer 2015-16, ticks, tissues and coagulated blood were collected from wildlife carcasses submitted to the Canadian Health Wildlife Cooperative. Blood and ticks were collected from live-trapped mammals and ticks were received from small animal veterinary clinics. Ticks were identified (life stage and species) and homogenized for PCR-testing for the above-listed tick-borne pathogens (ongoing). Homogenized tissues will also be tested for POWV antigen by RT-PCR and sera for anti-POWV antibodies by virus neutralization test. A total of 1,651 ticks of six species were collected, including 1,544 adults, 104 nymphs, and 3 larvae. Thus far, 10% (3/30) of Ix. scapularis tested positive for B. burgdorferi (source mammals were in Guelph and Lindhurst) and 3.3% (1/30) for A. phagocytophilum (Marysville). Furthermore, 182/723 tissue samples tested negative for POWV by RT-PCR. Results from this research will help identify the current distribution, wildlife host range, and prevalence of tick species and tick-borne pathogens in Ontario.
Maternal health care access in rural communities in southwestern Uganda: antenatal care attendance and delivery location among Indigenous Batwa and non-Indigenous Bakiga women

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Improved access to maternal health care services is key to progressing towards the Sustainable Development Goal of reducing the global maternal mortality ratio. Disparities in antenatal health care (ANC) services access need to be addressed in order reduce maternal morbidity and mortality and achieve this target. Despite the nationally-mandated four-visit ANC minimum set to reduce poor maternal health outcomes, Indigenous women in remote regions of Uganda could be at greater risk of poor antenatal health outcomes than non-Indigenous mothers. This study aims to examine and characterize ANC and delivery location in order to address disparities in antenatal and maternal health care access for both Batwa and Bakiga women. While Bwindi Community Hospital (BCH) in southwestern Uganda has specifically targeted the Indigenous Batwa women of the region, antenatal and delivery attendance was found to be low among Batwa women as compared to non-Indigenous Bakiga women. Antenatal and delivery attendance records were collected through collaboration with BCH, while guided, semi-structured community interviews and focus groups were conducted with local communities. Barriers to access such as distance to hospital, limited transportation options and inability to afford care are likely influencing factors of attendance. The barriers and promoters of identified to care will be evaluated through an explanatory mixed methods approach, using thematic analysis and descriptive statistics. Ideally, understanding the factors influencing ANC attendance and birth in this context will inform maternal health care policies in this region and within Uganda.
Antibody-based Protection against Ebola Virus Infection by Vectored Immunoprophylaxis

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Ebola viruses are among the most virulent infectious agents known, causing severe hemorrhagic fever in humans and nonhuman primates (NHPs) with fatality rates as high as 90%. Currently there are no licensed vaccines or treatments that prevent or cure infection with Ebola virus; however, monoclonal antibody (MAb)-based therapies have been shown to be efficient at reversing the progression of a lethal Ebola virus infection in NHPs. Passive treatment of Ebola virus infections with MAb cocktails is labour intensive, expensive and requires a cold chain to maintain the integrity of the product, which is challenging given the geographic location of Ebola virus outbreaks. Vectored immunoprophylaxis (VIP) involves a single injection of an adeno-associated virus (AAV) vector expressing pathogen-specific MAb genes leading to continuous and sustained secretion of antibodies into the blood stream and protection against infection. VIP circumvents many of the issues surrounding passive antibody treatment by utilizing the patient’s own cells to manufacture the MAbs. The aim of this project is to construct AAV vectors expressing neutralizing antibody genes against Ebola virus and to compare the efficacy of VIP to passive antibody treatment against Ebola virus infection in mice. The VIP platform can be applied to a wide variety of human infection diseases including other high-containment viruses or emerging pathogens requiring a rapid response.
Exploring the design features of automated feeding stalls and their effect on dairy calf behaviour

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There is evidence that the health and welfare of young dairy calves can be improved by increasing milk allowance and by providing milk through a teat. These aspects are easily incorporated into automatic milk feeding systems, which promote group housing that is important for social animals like cattle. Automatic milk feeders have the potential to be beneficial in many ways, however, little is known about how calves interact with them. We are investigating the effect of stall design features on calves learning to use the feeder. For the first experiment 114 Holstein calves are being enrolled at 4 days of age and will be introduced to a group pen and feeder. Two different stall designs are being tested: one with stall with gated (open) sides, and another with solid side walls. The hypothesis for this study is that solid stalls will result in a longer latency to approach and feed from the feeder. Main outcomes measured will compare first voluntary visit to the feeder, latency to first feeding and behaviour such as sniffing and licking of the feeder. This experiment is ongoing, but nearing completion. Preliminary results suggest there may be no difference in latency to first voluntary feeding time between stall designs, but that calves may be inclined to approach and explore the feeder with solid side walls sooner and spend more time exploring. Results from this experiment will be used to inform producers and those in the equipment manufacturing industry about how to make the transition to automatic feeders easier for young calves, by decreasing the amount of time for them to approach, enter and feed from the feeder.
Prevalence of Rotavirus, Astrovirus and Hepatitis E Viruses in Ontario Farmed Mink

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Currently, the role of viral infections in mink enteric disease is not well understood. There are no commercial diagnostic tests for routinely assessing their presence, and, specifically, astrovirus, rotavirus, and hepatitis E virus infections have not been monitored or reported in Canadian mink herds. These viruses can infect many species and have been associated with enteric disease in humans and in mink in Europe. Based on findings in other species, we hypothesized that low to moderate shedding of astrovirus and hepatitis E virus would occur in healthy females and kits, but that only diseased mink would shed rotavirus. The goal of this study was to determine the prevalence of these three viruses in Ontario mink herds. Pooled fecal samples were collected from 43 farms from clinically healthy adult females and kits (n=172). Viral RNA was extracted and assessed using optimized rt-PCR assays, and positive products were sequenced to validate chosen primers. Astrovirus was found in 23% (40/176) of all collected samples (27/86 kit and 13/86 female samples). Hepatitis E virus was detected in 15% (13/86) of the samples (11/86 kit and 2/86 female samples). A total of 5 astroviruses and hepatitis E virus co-infections were found (2 in female samples and 3 in kit samples). Rotavirus was not detected in any sample. Our results demonstrate a moderate level of subclinical astrovirus and hepatitis E virus infection in commercial mink. Further research on the role of these viruses on mink health, interactions with other viral infections, influence on the development of mink enteric disease, and risk level associated with zoonotic transmission are needed.
Benchmarking performance of 50 Ontario swine nurseries.

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The nursery phase of swine production presents a variety of challenges to the piglet (nutritional, environmental, physiological, and social). These stresses often result in reduced performance. Benchmarking is the comparison of performance against competitors or estimates of predicted performance. This process allows the identification of both targets and best practices, highlighting areas expected to improve from management interventions. The objectives of this study are to establish benchmark values for Ontario swine nursery performance (gain, feed efficiency, mortality), and to determine what risk factors for reduced performance present challenges for Ontario swine nursery producers. This study will examine 50 Ontario swine nurseries, sampling a subset of 20 pigs from one batch on entry and exit from the nursery phase of production. This subset will be used to calculate performance at the animal-level. Additionally, prospective herd production records will be gathered and a questionnaire will be administered to each producer to gather information about farm management and biosecurity strategies. These will be used to identify industry averages as well as possible risk or protective factors. Results from this study will provide producers with strategies that target the specific features of swine production in Ontario. Sharing of performance data among producers will allow creation of performance targets (benchmarking) specific to Ontario nurseries, aiding decision-making about management strategies or interventions.
Sex Disparity in Innate Immune Responses to Viral Infection: The Role of Type I Interferon Signaling

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Type I interferons (IFNs) protect cells against viral infection by inducing an anti-viral state in nearby cells and by activating other mechanisms of host defense. Blockade of IFN signaling promotes induction of a cytokine storm following viral infection in mice. We investigated the role of signaling through the type I IFN receptor (IFNAR) in preventing virus-induced cytokine storms in female and male mice infected with a highly attenuated recombinant vesicular stomatitis virus. Serum cytokine analysis at time points following viral infection showed that mice pre-treated with an IFNAR-blocking antibody produced higher levels of the proinflammatory cytokines interleukin (IL)-6, IL-12 and tumor necrosis factor-alpha than those with intact IFN signaling. Male mice pre-treated with anti-IFNAR showed an initial exaggeration of cytokine expression compared to males with intact IFNAR signaling at 5hrs post-infection (hpi), followed by reduction of cytokines to normal levels at 24hpi. However, female mice pre-treated with anti-IFNAR experienced even more exaggerated cytokine responses than female controls and all males at 5hpi. These responses then continued to climb or remain elevated at 24hpi, suggesting that females have an impaired ability to negatively regulate cytokine responses during IFNAR blockade. The basis for this sex-dependent susceptibility to cytokine storms is likely due to inherently different innate immune cell biology or signaling and may underlie the observed disparity in prevalence of immune-related diseases between males and females.
Investigating Small Ruminant Mortalities on Farm with an Online Support Tool

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Evaluation of disease-related status of a flock/herd is an important tool for improving animal health. Postmortems are rarely performed for fallen animals on small ruminant farms, meaning that disease information is often not available. This may be due to low animal value, cost of the procedure and poor understanding of the value of a diagnosis obtained from a veterinary-performed postmortem. Enhancing the ability of veterinarians to complete a well performed and timely postmortem on fallen animals will improve the ability of producers and veterinarians to develop a useful flock/herd health-management plan.

As part of this project, a distance support system for veterinarians has been developed to improve performance of on-farm postmortems. The goals are: to improve the postmortem procedure, including suitability of the samples submitted to the laboratory; and to ensure completeness and accuracy of the case history, thus increasing the chance of diagnostic success. This will enable increased surveillance of diseases. Further aims include the improvement of knowledge transfer between veterinarians and small ruminant producers. Quantitative and qualitative research methods will be used to generate understanding of stakeholders' experiences, and perceived motivations and barriers with the system.

Veterinarians often struggle to provide veterinary services perceived to be of value to small ruminant producers. As such, mixed research methods will also be used to assess which processes are perceived to be of value, and what motivations and barriers may exist: specifically, to performing postmortems of adult animals, and generally, to adoption of flock/herd health programs.
The Burden of Cryptosporidium and Giardia in Clams in Iqaluit, Nunavut

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Recent research has uncovered the highest rates of self-reported enteric illness (i.e., diarrhea and vomiting) reported in the world to be in the Canadian Arctic. Store-bought retail foods and locally-harvested country foods are potential sources of pathogens which can cause foodborne enteric illness. However, the foodborne pathogens which cause enteric illness have not been thoroughly studied in the Canadian Arctic. This project aims to reduce this gap in knowledge by gathering information on pathogens in country food in Iqaluit, Nunavut.

Through consultation with community members, local government representatives, and health professionals in Iqaluit, Cryptosporidium and Giardia were identified as pathogens of interest in clams, a common country food. In September 2016, clams (n=398) were gathered from local harvesters in Iqaluit. Hemolymph (circulatory fluid) and digestive tissues have been sampled from these clams, and are currently awaiting testing for Cryptosporidium and Giardia.

This project aims to investigate clams as a potential source of foodborne enteric pathogens in Iqaluit. The results of this project will increase understanding of the high incidence of enteric illness in the Canadian Arctic, and provide information for public health programs and initiatives to reduce the high rate of enteric illness in the Canadian Arctic.
Feline Tissue and Cell Expression of SAMHD1 and Role in FIV Restriction

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Background: Sterile alpha motif (SAM) and histidine-aspartate domain 1 is an enzyme with viral restriction functions. The deoxynucleoside triphosphate triphosphohydrolase and 3'-5' exonuclease activity of SAMHD1 restricts retroviruses replication.

Aim(s) of the work: The aims of this study were to determine the presence and location of SAMHD1 in feline tissues and T-cell line using immunohistochemistry and Western blotting.

Methods: The SAMHD1 expression was evaluated by Tissue Microarray (TMA)-based immunohistochemistry using monoclonal antibodies combined with proportional and intensity scoring system. TMAs were constructed by acquiring 1.5 mm cylindrical biopsies from 16 different formalin-fixed paraffin-embedded tissues of 3 healthy male cats. SAMHD1 subcellular localization (western blotting) were evaluated in feline T-cell line (FeTJ) as well.

Results: By histomorphological criteria, SAMHD1 expression was identified in: macrophages and immature hematopoietic cells of bone marrow, lymphocytes of secondary lymphoid organs, hepatocytes, exocrine acini of pancreas, adrenal gland cells (more intense in zona granulosa), pneumocytes, epithelium of salivary gland ducts, exocrine acini of pancreas, kidney tubules, spermatogenic cells, Sertoli cells, Leydig cells, epithelium of the epididymis, keratinizing stratified epithelium of the skin and tongue, hair follicle cells, epithelial cells of the mucosa of the esophagus, stomach, small intestine, colon, trachea and bladder. Immunocytochemical staining identifies numerous nuclear and cytoplasmic SAMHD1 positive cells in the feline T-cell line FeTJ.

Discussion/Conclusions: This study is the first systematic description of SAMHD1 expression profile in domestic cats and indicates high expression of SAMHD1 in the retrovirus entry sites and sites of replication.
Pilot Studies to Investigate the Effect of Aerosolized Heat-killed Bacteria on Calves

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Bovine respiratory disease (BRD) is the most common disease of beef cattle and is estimated to cost the North American beef industry $5 billion annually. It causes animal suffering and is the major reason for antimicrobial use in beef cattle. BRD in beef cattle occurs at a specific time, on arrival to the feedlot when stresses and viral infections, caused by weaning and commingling, suppress the natural defenses of the respiratory system and allow infection of the lung with bacteria. The objective of this work is to develop a method to stimulate the innate immune system and correct this immunosuppression to prevent BRD. Initial pilot studies were conducted to determine the effects of innate immune stimulation. Innate immune responses were stimulated in cattle by aerosol delivery of heat-killed bacteria. Various doses and timing of aerosol exposure to heat-killed bacteria were assessed. Clinical and hematological parameters (including haptoglobin, fibrinogen and complete blood counts) were measured prior to, and following administration of the immunostimulant to identify adverse or systemic effects. Measures of the immune response were compared between calves that received an immunostimulant and control calves, including neutrophil recruitment based on analysis of bronchoalveolar fluid, histology of respiratory tissues, gene expression of tracheal antimicrobial peptide and other innate immune proteins, as well as any pathologic findings. The results of this study will be used to determine a dose of heat-killed bacteria that will be used in future studies that will evaluate the effectiveness of innate immune stimulation in preventing bacterial pneumonia in cattle.
A protocol for a systematic literature review: Comparing the impact of seasonal and meteorological parameters on acute respiratory infections in Indigenous and non-Indigenous peoples

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Background: Acute respiratory infections (ARI) are a leading cause of morbidity and mortality globally, and are often linked to seasonal and/or meteorological conditions. Globally, Indigenous peoples may experience a different burden of ARI compared to non-Indigenous peoples. This protocol outlines our process for conducting a systematic review to investigate whether associations between ARI and seasonal or meteorological parameters differ between Indigenous and non-Indigenous groups residing in the same geographical region.

Methodology: A search string will be used to search PubMed, CAB Abstracts/ CAB Direct, and Science Citation Index aggregator databases. Articles will be screened using inclusion/ exclusion criteria applied first at the title and abstract level, and then at the full article level by two independent reviewers. Articles maintained after full article screening will undergo risk of bias assessment and data will be extracted. Heterogeneity tests, meta-analysis, and forest and funnel plots will be used to synthesize the results of eligible studies.

Discussion: This protocol paper describes our systematic review methods to identify and analyze relevant ARI, season, and meteorological literature with robust reporting. The results are intended to improve our understanding of potential associations between seasonal and meteorological parameters and ARI and, if identified, whether this association varies by place, population, or other characteristics.
Investigation of the Immune Response of Sheep to Gastrointestinal Nematodes under Ontario Grazing Conditions

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Infection with gastrointestinal nematode parasites (GINs) and in particular Haemonchus contortus has a serious impact on sheep health and productivity in Ontario. The resistance of H. contortus to anthelmintics is widespread. Alternative control strategies are needed; one such strategy is to select animals for superior ability to mount an immune response to GINs. This project will describe how Ontario sheep develop immunity to GIN infections by following a group of ewe lambs over an 18 month period, including two grazing seasons and one lambing and lactation event. Immunity will be measured directly using the CarLA® Saliva Test (AgResearch Ltd.), which measures GIN-specific IgA antibody in saliva, and indirectly with the change in fecal egg count (FEC). The level of GIN infection will be monitored using FEC and changes in hematologic and clinical parameters. Also, lambs from the University of Guelph Ponsonby Research flock, pre-selected for stress or cell- and antibody-mediated immune responses are being co-grazed with these lambs to determine if stress and innate immune phenotypes influence immunity to GINs. This information will be used in future studies to determine the heritability of these immune traits and their potential role in genetic selection.
The effect of the neurosteroid allopregnanolone on alpha4beta2 nicotinic acetylcholine receptor function

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The corticolimbic system of the brain consists of the hippocampal formation (HF), prefrontal cortex and amygdala. Its proper development depends on the interaction between the neurotransmitter acetylcholine (ACh) and nicotinic acetylcholine receptors (nAChRs) located on neurons. The corticolimbic system is important for resiliency in stressful situations. In response to stress, one neuroprotective mechanism is the de novo production of endogenous anxiolytics such as allopregnanolone (3α-5α-THP). It is well known that 3α-5α-THP provides anxiolytic action by potentiating gamma-aminobutyric acid (GABA) type A receptor function. However, studies in reduced preparations suggest that it also negatively modulates the primary subtype of nAChR, known as the α4β2* nAChR. In this experiment, we sought to understand the effect of 3α-5α-THP on α4β2* nAChRs within living neurons of the medial prefrontal cortex.

Electrophysiology experiments were performed using male and female CD1 strain mice at postnatal days 14 to 21. Our findings suggest that acute exposure of 3α-5α-THP inhibits α4β2* nAChRs of medial prefrontal cortex layer VI neurons. There was a significant effect of 3α-5α-THP exposure on the magnitude of inward currents elicited by 1 mM ACh in female but not male mice. Determining the effects of 3α-5α-THP on α4β2* nAChR function within living pyramidal neurons of the corticolimbic system is significant because this will advance our understanding of the impact that endogenous anxiolytics have on the function of these brain regions.
Identification of fear behaviours shown by puppies in response to novel objects

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Fear behaviors in puppies have not been properly identified, and it is unknown whether they are similar to behaviors seen in older dogs. We assessed which puppy behaviors are associated with fear of a novel object. Puppies (<6 months; N=25) were introduced into a 3.5 meter long run and trained to approach the far end to obtain a food reward. After training, each puppy completed four trials with noisy or unpredictable novel objects and four with no objects (control). All sessions were video-recorded, and behavior during each trial (N=193) was scored using Noldus Observer 12. Trials were categorized as "fearful" if the puppy did not approach the end of the run, or the latency to approach was greater than the mean+2 SD of the control trials. Linear and logistic mixed models, with puppy as a random effect, were used to model behavior durations and presence (yes/no). The following behaviors occurred more in "fearful" trials in comparison to control trials: lowered posture (p<0.001), lowered tail (3.40; p=0.001), freezing (p<0.001), retreating (p<0.001), flinching (p<0.001), paw lift (p=0.006) and barking (p=0.002). Sniffing (p<0.001), locomotion (p<0.001) and panting (p<0.001) occurred less in "fearful" trials. No significant difference was found for ear position and tail wagging. There was a tendency for lip licking to be higher in the fearful trials (p=0.061). Yawning, shaking, elimination, whining and growling occurred too infrequently for analysis. These results indicate that postural, retreating and barking behaviors are the most reliable indicators of fear in puppies in situations where they are able to control their approach to objects that elicit fear.
Cell identity, proliferation, tri-lineage potency and cytogenetic assessment of equine umbilical cord blood mesenchymal stem cells

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The aim of this study was to expand and characterize cryopreserved mesenchymal stem cells (MSCs) from horse umbilical cord blood (UCB) with regards to immune-phenotype, proliferation capacity, tri-lineage differentiation and genome stability. UCB-MSC immuno-phenotypic analysis revealed a cell identity consistent with a MSC phenotype by high expression of the CD90, CD44, and very low expression of the CD4, CD8, MHC-I and MHC-II antigens. Tri-lineage potency was confirmed by successful differentiation towards the osteogenic, chondrogenic and adipogenic cell fates in vitro. A large number of MSCs did not have normal equine karyotype (2n=64); 20% of polyploidy and 70% of aneuploidies were observed. These findings suggest that equine UCB-MSCs are readily obtainable and highly proliferative cells. However, further studies are needed to investigate their chromosomal abnormalities and determine isolation protocols that yield cells with a normal karyotype.
Macroscopic and proteomic aspects of regeneration in the Caribbean Star Coral, Montastraea cavernosa

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Absolute coral cover in the Caribbean has decreased more than 70% since the 1970s. Given this extreme decline, it is critical to determine the factors that most strongly affect coral regeneration in this region as well as the proteins that are active during the regenerative process. Transects in marine regions of interest in the coastal waters of Grenada and Carriacou were photographed weekly during fall 2014. Images were captured every 0.5 m along 0.5 m x 30 m long belt transects near targeted coral heads both inside and outside marine protected areas. Twelve mm diameter x 2 mm deep circular lesions were made in 124 healthy M. cavernosa colonies at 10-12 m depths. Water samples were collected weekly for organic nutrient component analysis. Analysis of benthic diversity as a proxy for ecosystem heath, organic nutrient levels, and the presence of marine protected areas were examined for their potential impact on M. cavernosa tissue regeneration rates. Using these predictors, separate mathematical models for gross tissue regeneration were developed for Grenada and Carriacou. During fall 2015, identical circular lesions were made in 30 healthy M. cavernosa colonies at 10-12 m depths at sites in Carriacou selected from the previous year. Lesions were re-sampled bi-weekly during the regeneration process to collect tissues for proteomic analysis. Differential expression of proteins associated with regeneration was assessed in regenerating and non-regenerating tissues using mass spectrometry.
Cardiomyocyte proliferation in the lizard heart: exploring criteria for spontaneous self-repair

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The capacity for cardiac self-repair varies across vertebrates. Mammals demonstrate little to no self-repair following injury, while teleost fish and urodeles have the ability to completely restore structure and function following a direct injury to the heart. Several features common to all species capable of cardiac regeneration have been identified. These include: (1) a trabeculated myocardium; (2) postnatal cardiomyocyte proliferation; and (3) an endogenous reservoir of cardiac stem/progenitor cells (SPCs). Here we investigate the presence of these features in an emerging model of tissue replacement, the leopard gecko (Eublepharis macularius). Similar to other reptiles, we found that the ventricle of the gecko is characterized by a trabeculated, spongy lumen. Unlike a compact myocardium seen in mammals, trabeculation provides an increased surface area for diffusion and hence a decreased reliance on coronary circulation. Using bromodeoxyuridine (BrdU), proliferating cell nuclear antigen (PCNA) and phosphorylated histone H3 (pHH3), we identified constitutively proliferating cardiac cells in the (postnatal) gecko heart, including a large proportion (~10%) of PCNA+ cardiomyocytes. Finally, we demonstrate the presence of a slow-cycling reserve of cells, some of which co-localize with the stem/progenitor cell marker c-kit. These findings provide compelling evidence that the gecko is an excellent candidate to study cardiac self-repair and regeneration.
An Epidemiological Survey Describing Dog Bite Incidents in Rural and Urban Ontario Households

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Dog bites are an important and often serious public health issue. One of the more important concerns surrounding the issue of dog bites is the transmission of rabies, particularly given changes to the regulation of this federally reportable disease in Canada. The objectives of this study were to describe dog bites in rural and urban residents in Ontario and quantify rabies vaccination status of household dogs within these comparative communities. To meet our objectives, we carried out a cross-sectional observational study utilizing an on-line survey of 2006 individuals; 1004 urban and 1002 rural (i.e. populations with less than 1000 residents). Overall, 975 (approximately 49%) of the respondents' households owned one or more dogs. For a one-year period (September 2013- September 2014), approximately 10% of households had at least one family member bitten by a dog. The data were also categorized based on who was bitten (age, sex), type of dog (age, sex, breed, and rabies vaccination status), circumstances surrounding the bite (e.g., play, provoked), the anatomical location of bite, victims' healthcare-seeking behaviour following the bite, and whether it was by an individual's own dog or from a dog outside of the home. Additionally, regardless of dog-owning status, data were collected describing individuals' contact with dogs outside of the home, and reasons for receiving pre-exposure rabies immunization. These data will provide valuable information for targeted public health programs related to dog bite prevention and control strategies in rural and urban Ontario.
Veterinary Students' Perceptions and Knowledge Structures of Companion Animal Nutrition: a mixed-methods study

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There is now much evidence supporting the importance of effective nutrition education for veterinary students, with the rising prevalence of pet obesity. As part of the veterinary curriculum, students receive formal nutrition education, yet uptake of this knowledge can be influenced by their own pre-existing perceptions and opinions as pet owners. The purpose of this study was to explore existing knowledge structures and perceptions of pet nutrition held by veterinary students upon entering veterinary school and investigate if previous exposure to veterinary care shapes those views. Four in-depth onsite focus group discussions were conducted (n=19); two with first-year veterinary students and two with pre-veterinary students interested in attending veterinary school. Following transcription verbatim, systematic coding and analyses using Inductive Thematic Analysis, the prevailing themes included: (i) accessibility to information, (ii) time and money constraints, (iii) mistrust in pet food industry, (iv) social interactions shape knowledge/expectations and (v) use of nutrition to assess the pet's quality of life. Results suggested that several factors (e.g. family upbringing, societal trends, media) are influencing, positively or negatively, the perceptions and thoughts of veterinary students with respect to nutrition; and that students utilize different sources of information to learn about their own nutrition versus their pet's nutrition. These discussions serve as the first phase of an exploratory sequential mixed-methods study, informing the design of a questionnaire tool that will be used to compare perceptions across Canadian and US veterinary schools.
Modulation of cell migration by the TGFβ-TAZ signaling axis in canine osteosarcoma cell lines

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Osteosarcoma (OSA) accounts for 85% of all primary bone tumors in canines. Despite aggressive treatment modalities, 90% of canine patients develop metastasis within one-year of diagnosis, which is the ultimate cause of patient mortality. Previous literature has demonstrated that transforming growth factor beta (TGFβ) and transcriptional coactivator with a PDZ-binding motif (TAZ) both independently and cooperatively promote cancer metastasis. However, this relationship has not been explored in canine OSA. To explore the function of TGFβ-TAZ signaling in promoting cell migration, in vitro techniques involving small interfering RNA (siRNA) specific to TAZ and TGFβ treatment were used. The impact of TAZ knockdown and TGFβ treatment on cell migration was evaluated using the scratch-wound and transwell assay in three canine OSA cell lines: two derived from primary tumors, and one derived from a metastatic lesion. A two-way ANOVA and post-hoc Bonferroni t-test was used to determine significant differences in wound closure and cell migration. The metastatic cell line displayed similar results between both assays, as it was found that TAZ-knockdown significantly impaired wound closure 8 hours and 16 hours post wounding (p = 0.001) and the number of migrated cells (p = 0.018) when compared to the control group. As these results were not observed in the two primary OSA cell lines, it suggests that TAZ may play a more crucial role in mediating cell migration during latter stages of OSA. Further analyses utilizing additional primary and metastatic OSA cell lines and an environment that better recapitulates the tumor microenvironment are required to elucidate this relationship.
A Survey of Disease-Causing Agents in Wild Turkeys (Meleagris gallopavo) in Ontario, Canada.

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Numerous disease-producing agents pose a threat to the health of both wild and domestic animals. Since their reintroduction to Ontario in 1984, wild turkeys (Meleagris gallopavo) have increased in number and geographic range, yet information on diseases affecting them is lacking. A retrospective analysis of wild turkey diseases diagnosed over a 20-year period at the Canadian Wildlife Health Cooperative was performed and provides a long-term outlook of detected diseases. Additionally, pathogens that cause disease outbreaks or decrease the fitness of commercial turkeys (e.g., Mycoplasma spp., Eimeria spp., avian pox virus, and avian influenza viruses) were targeted for testing in 152 hunter-harvested wild turkeys in Ontario to determine their frequency and spatial distribution. The majority (98.7%) of individuals tested positive for Mycoplasma spp. (of 6 identified species), 76.0% samples tested positive for Eimeria spp., 2 had avian pox skin lesions, and 152 wild-turkeys tested negative for avian influenza viruses. Additional pathogen assessment will include Salmonella, E. coli, Campylobacter spp., Histomonas meleagridis, and lymphoproliferative disease virus. Data generated from this research will establish baseline disease prevalence for future monitoring of disease emergence in wild turkeys and will assess potential pathways for disease transmission between domestic and wild turkeys. It will support emergency management preparedness and the formation of disease prevention strategies for the Ontario poultry sector, and contribute to conservation and management strategies to help ensure sustainable populations of wild turkeys in Ontario.
Managing veterinary euthanasia and client grief support

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For more than 65% of North American’s today, companion animals are regarded as integral members of the family and often given child or sibling status [1-2]. Therefore, the death of a companion animal can result in emotional grief similar to that of the loss of a family member [3]. Dissimilar to a human family member however, the loss of a companion animal is often not acknowledged as a significant source of grief [4]. In some cases, pet owners experience an increased grief reaction which is more prolonged and complicated [3]. Past research has shown that how well the veterinary team manages a euthanasia can either alleviate or aggravate client grief [5]. To date, research exploring companion animal euthanasia protocols and management of client grief is negligible. Thus, the aim of this study is to gain a better understanding of the protocols and support practices currently in place in companion animal clinics through both qualitative and quantitative measure.

At this stage of this project, focus group and individual interviews with ten Guelph area clinics have been conducted and verbatim transcripts analyzed. The outcomes of this analysis resulted in three main themes, i) Initiation and Preparation, ii) Personalization iii) Personnal and Professional Standards and three addition themes which related to the concept of out-of-clinic euthanasia i) In-Home Euthanasia ii) Convenience Euthanasia and, iii) Human Medicine Comparisons.

Data from the focus groups are now informing the design of a questionnaire which will be distributed to all companion animal veterinary professionals in Canada. In addition, future objectives include the exploration of the perceptions and experien
Use Of Proximity Logging Technology To Quantify Equine Contact Patterns Within Ontario Equine Facilities

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Equine contact patterns are complex and can demonstrate variability within and between facilities. However, contact pattern data within an equine facility has yet to be explicitly characterized. The characterization of such contact data is essential to the understanding and accurate description of equine contact networks. This research will use logging technology to collect the data necessary for the quantification of equine contact networks.

The objective of this study is to describe and quantify the contact patterns of horses and people within equine training facilities. Ontario equine facilities of various disciplines and sizes will be recruited to participate. Questionnaires will be administered to facility managers to obtain facility-level information such as signalment and routine daily activities. Participating horses will be fit with a proximity logger onto their halter. These Internet of Things based loggers will record each time individuals come within 2m of one another, the duration of the contact, and which tags (individuals) were in contact. Data will be collected at each facility for a one week period. This contact data will be used to analyze and describe the contact network of individuals within and between facilities and disciplines.

The collected data will be used to inform dynamic disease transmission models. These models use computer simulations to evaluate the way various pathogens may behave if they were introduced to different types and sizes of facilities, as well as to assess the impact of prevention and control strategies. Accurate equine contact pattern data is needed to properly inform these dynamic models.
Increasing the Magnitude of Tumour-Specific T Cell Responses by Spreading a Vaccine Dose Across Multiple Injection Sites

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Vaccination is a common immunotherapy to generate potent humoral and cell-mediated immunity. Cancer patients require rapid induction of robust tumour-specific effector T-cell responses to halt neoplastic growth and hopefully eliminate malignant cells. Immunological tolerance imposes strong selection pressures against autoreactive T cells. Therefore, T cells that can respond to vaccines targeting self-tumour-associated antigens (TAA) are usually at low frequency. In clinical settings, vaccines are often administered as single bolus injections. We reasoned that administration of a cancer vaccine at multiple injection sites would increase the amount of secondary lymphoid tissue involved in antigen presentation, thereby maximizing the engagement of low-frequency TAA-specific T cells. To test this, C57BL/6 mice were vaccinated with a replication-deficient human serotype 5 adenovirus expressing the TAA dopachrome tautomerase (Ad5-DCT). 1x10^8 plaque-forming units of Ad5-DCT was injected intramuscularly into one (hamstring or triceps brachii), two (both hamstrings or both triceps brachii) or four (both hamstrings and both triceps brachii) sites. The magnitude of the CD8+ T cell response against the immunodominant epitope of DCT (DCT180-188) increased with the number of injection sites. Enhanced responses associated with multiple vaccination sites were evident at the peak of the primary response as well as post-contraction. These results suggest it may be beneficial to administer cancer vaccines to patients at more than one site. Whether this has implications for vaccines beyond those targeting self-antigens remains to be tested.
**Time course of Salmonella shedding and antibody response in naturally-infected pigs through the grower-finisher stage until slaughter**

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A longitudinal trial was conducted to determine the course of Salmonella shedding and antibody response in naturally-infected grower-finisher pigs. Feeder pigs (n=45) about 10 weeks of age from a farm with a history of salmonellosis were housed at the Ponsonby research facility. Weekly fecal samples and tissue samples at slaughter were cultured for Salmonella. Serum samples were tested for presence of Salmonella antibody by ELISA. Data were analyzed using a multilevel mixed-effects logistic regression model. Salmonella was cultured from fecal samples 4 times from 41 of 45 pigs (91%) and cultured from more than 5 samples from 4 (9%) pigs. The estimated median of Salmonella shedding duration was 3-4 weeks but some pigs shed Salmonella for up to 8 weeks. Salmonella isolates (n=89) belonged to serogroups C1 (44%), E4 (29%), B1 (19%) and others (8%). During the study period, 70.3% (26/37) of pigs were reinfected with Salmonella belonging to a different serogroup. A subset of isolates (n=29), recovered from 18 pigs at different occasions, belonged to serotypes S. Typhimurium (28%), S. Livingstone (21%), S. Infantis (14%), S. Montevideo (7%), S. Benfica (3%), S. Amsterdam (3%), S. Senftenberg (17%), and S. I:Rough-O (7%). At slaughter, Salmonella was isolated from 7 pigs of which, 5 (71%) had not tested positive for at least 7 weeks prior to slaughter. Antibody response peaked 4 weeks after the peak of Salmonella shedding; Salmonella shedding decreased as antibody response increased (P<0.05). These findings indicate that pigs exposed to Salmonella at an early age may shed Salmonella for weeks in the grower-finisher stage and may be reinfected with different serotypes.
Identifying research priorities in Indigenous Maternal Health: Protocol for a Scoping Literature Review

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Maternal mortality is inequitably distributed; most deaths occur in developing countries. The major causes of maternal mortality are hemorrhaging, eclampsia, unsafe abortions, infection, and obstructed labor/delivery complications; however, these are preventable with sufficient access to health care. Maternal health care in developing countries is characterized by low prevalence of hospital deliveries, births with skilled attendants, antenatal care, and access to contraception and family planning services. Furthermore, Indigenous women encounter additional health inequity as a result of historical patterns of neglect by governments, colonialist legacies, land dispossession, lack of access to resources and services, discrimination, and extreme poverty. This protocol provides transparency of methods and process for conducting a scoping review to identify and synthesize the extent, range, and nature of literature on Indigenous maternal health and determinants. A systematic search of peer-reviewed electronic databases and gray literature will be conducted. Two stages of screening will be conducted by team members; on title and abstract, followed by full text review. The results will be summarized in tables, graphs and maps. Additionally, qualitative and thematic analyses will be used to develop the discussion section of the paper. This protocol will support the development of the first review to summarize the published literature on Indigenous maternal health. The results of the scoping review will identify important gaps in the literature, best practices in Indigenous maternal health research and future research priorities in the field.
A case-control study: an investigation of the early phase of the porcine epidemic diarrhea (PED) outbreak in Canada 2014

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Porcine epidemic diarrhea (PED) is an emerging disease that has significantly impacted the swine industry. PED was first reported in Canada in January 2014 on an Ontario farm. Initial investigations identified a single feed company (FC1) as the likely source of infection. In February 2014, FC1 voluntarily recalled nursery feed products containing porcine plasma. The porcine plasma from FC1 was capable of reproducing the infection under experimental conditions. The study objective was to evaluate the role of feed after controlling for potential confounders in the early phase of the PED outbreak.

A total of 23 Canadian herds (n=9 case herds; n=14 control herds) were included in the case control study. A case was defined as any swine herd with confirmed rt-PCR positive results for PEDv, with typical clinical signs at the herd level between January 22nd and March 1st 2014. Control herds were randomly selected and matched on herd size, herd type, and time of PED onset in case herds. The association between the number of pig/people movements onto and off farms, the number of feed deliveries received, whether a herd received any feed from FC1, and herd biosecurity measures and PED were evaluated. More case herds received feed from FC1 (n=8) than control herds (n=3). The odds of PED outbreak are increased by 1.5 times with every shipment of feed from FC1 (P<0.05). This confirms the role of feed as a risk factor for PEDv during the early phase of the Canadian outbreak. In contrast, no difference in the number of live pigs delivered onto sites, semen deliveries, and frequency of deadstock pickup between the case and control herds could be detected.
Extending the nighttime rest period with short-wavelength light delays 24h rhythms in activity and cardiac circadian gene expression

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Rationale: Circadian rhythms in the cardiovascular system are regulated by a molecular circadian mechanism and are responsive to changes in the light/dark environment. Short-wavelength light from electronic devices has garnered recent attention for the capacity to shift internal circadian timing, but whether short-wavelength light can shift rhythms in the heart is unknown. Hypothesis: We hypothesize that extending the rest phase with short-wavelength light will delay rhythms in both 24h activity and cardiac gene expression. Methods and Results: First, to show that short-wavelength (blue) light can shift 24h activity, we housed C57Bl/6 mice in a 12h light:4h blue:8h dark cycle or a control cycle of 12h light:12h dark. Exposure to blue light caused a 4h delay in the onset of activity, compared to the control light cycle. In contrast, mice on a 12h light:12h red (long-wavelength) cycle showed no change in activity profiles, supporting a specific role for short-wavelength light. Second, to show that blue light can shift circadian gene expression in the heart, we used RT-PCR to examine 24h mRNA profiles of the core circadian mechanism genes Bmal1, Clock, Per2, Rev-Erb, and Nfil3. All targets exhibited significant rhythms (JTK p-value<0.05), however, blue light induced a 2h delay in the expression of Bmal1, Clock, Per2, Rev-Erb, and Nfil3 in the heart compared to the control light cycle. Conclusions: These data demonstrate that short-wavelength light can delay circadian gene expression rhythms in the heart. We are now investigating whether short-wavelength light alters expression of other gene targets involved in key cellular processes in the heart using microarray analysis.
Coccidiosis is an intestinal disease that causes large economic costs to poultry production. There is a lack of knowledge in the presence of the organisms that cause the disease in Ontario broiler chicken farms. There are several species of the Eimeria genus that cause the disease. Current practices to control Eimeria include either in-feed prophylactic medication or live Eimeria vaccination. Observing fecal material for oocysts (i.e. OPG) is a reliable method for determining infection level. 28 commercial broiler farms were sampled during the summer of 2016 to determine oocyst concentration in either medicated or vaccinated facilities. Chicken performance data was collected regarding growth, feed consumption, and mortality to associate OPG counts. Field sampling will be repeated in the winter to understand the seasonal variation of the disease. Visual and morphometric observations of oocysts are not sufficient to identify the species of Eimeria. Therefore, oocysts obtained will be identified by extraction and amplification of the COI mtDNA. By using novel sequencing technology and template sequences of the species, mixed species infections will be identified for the proportion of each species present. Field isolates will be tested for anticoccidal resistance the Eimeria have developed to common medications. Recent legislation changes have allowed chemical anticoccidials in antibiotic-free production, so these compounds will be the focus. By using vaccine strains of the parasites, it is possible to reduce this resistance, but the rate of reduction is unknown. Therefore, farm isolates that have used varying lengths of vaccination will also be investigated for resistance.
Mathematical modeling of potential disease spread within a network of horses attending an equestrian event

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Horses attending equestrian events create a contact network where opportunities exist for disease introduction and spread. The effect of this contact structure on disease transmission in the Ontario equine population can be evaluated using mathematical models to provide insight into population-level interventions that may not be practical to implement without stronger evidence. The objective of this research was to examine how the contact structure of horses attending an equestrian show influences the spread of equine influenza within the network. Horse show participants completed a questionnaire about their horses and horses stabled at their home facility to construct a network, which was represented using an agent-based model. The model was run for 1000 iterations where one infected horse was randomly introduced at the beginning of each iteration. Outcomes of interest included: (1) the overall attack rate, (2) average outbreak duration, and (3) frequency of between-facility transmission events. The network consisted of 765 horses (55 horses attending the show and 710 secondary contacts) at 38 home facilities. In the absence of interventions other than pre-existing vaccination, the average attack rate was 5.5% (range: 0.1 to 21.3%) and the average outbreak duration was 19 days (range: 7 to 35 days). In 93% of simulation runs, the outbreak was contained to one facility; 7% of the simulation runs resulted in outbreaks that involved between 2 and 6 facilities. The model will be used to evaluate different interventions to determine effective strategies to prevent and control an outbreak, improving the ability to design disease management plans.
Evaluation of Two Opioids for Reducing Pain in Piglets Undergoing Surgical Castration

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In North America, over 100 million piglets are subject to painful procedures each year, including tail docking and surgical castration. While these practices are known to cause significant pain and distress, they are often done without the use of appropriate analgesics or anesthetics. The objective of this preliminary study was to assess the efficacy of two opioids (0.2 mg/kg butorphanol and 0.04 mg/kg buprenorphine) in reducing pain in castrated piglets, using validated behavioural scoring techniques, vocalization assessment and a novel Piglet Grimace Scale (PGS). Two litters of 5 day-old male piglets (n=12) were treated IM with butorphanol, buprenorphine or saline 20 min prior to surgical castration. Piglets were video recorded 1h pre-procedure, immediately post-castration for 8h and for another 1h, 24h post-procedure (total of 10h). Behaviours and postures were scored continuously for the first 15min of every hour of data collected by 2 observers blinded as to time and treatment. Data was analyzed using a mixed model ANOVA with repeated measures and a post-hoc Tukey test. All piglets were significantly more inactive 2-4h post-castration (p<0.0001) and no saline-treated piglets were observed playing. Buprenorphine-treated piglets were significantly more active than saline-treated piglets (p=0.015). Grogginess and sporadic vomiting were side effects noted in butorphanol-treated piglets. These early findings indicate that buprenorphine is the most appropriate opioid for use in piglets undergoing surgical castration. Future trials are required to determine whether buprenorphine can significantly reduce castration pain in piglets.
Vitamin D status and acute phase protein concentrations in canine cancer patients

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In humans, low blood 25-hydroxyvitamin D (25(OH)D) levels are linked with increased acute phase protein (APP) concentrations in several diseases. These findings have been observed separately in canine cancer patients, but links between the two have not been studied. Our objective was to determine and assess the relationship between blood 25(OH)D and APP levels in healthy dogs and dogs with cancer.

Dogs with lymphoma (n=34), osteosarcoma (n=21), and mast cell tumors (MCT) (n=26), and healthy dogs (n=25), were enrolled. Blood samples were collected before treatment and analyzed for plasma 25(OH)D and plasma C-reactive protein (CRP), haptoglobin (Hp), alpha-1-acid glycoprotein (AAG) and serum amyloid A (SAA). Variance analyses followed by appropriate post-hoc analysis were performed. Pearson correlation coefficients were also calculated.

Lymphoma patients showed higher CRP, Hp, SAA, AAG concentrations and lower plasma 25(OH)D concentrations than healthy dogs (p<0.001, p<0.001, p=0.010, p=0.004, p=0.035, respectively). Dogs with osteosarcoma had elevated blood CRP, Hp, and SAA concentrations (p<0.001, p=0.005, p=0.010) and MCT dogs had increased Hp concentrations (p=0.020) compared to healthy dogs. There was a moderate, inverse relationship between plasma 25(OH)D and AAG concentrations (r=-0.48, p=0.004) in lymphoma patients, and positive relationships between 25(OH)D and CRP (r=0.41, p=0.040), and 25(OH)D and SAA in MCT patients (r=0.43, p=0.027).

25(OH)D and APP concentrations are consistent with previous findings. Certain APP concentrations in cancer dogs were above reference range, so future research studying the effect of treatment on these variables is warranted.
Comparison of high flow nasal cannula oxygen administration to traditional nasal cannula oxygen therapy in healthy dogs

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Introduction: Respiratory distress is a common presenting complaint for dogs admitted to an emergency department. Standard oxygen therapy by traditional nasal cannula (TNC) may not relieve the sensation of difficulty breathing. High flow nasal cannula (HFNC) is a novel means of non-invasive ventilation that provides heated, humidified oxygen at higher flow rates. The objectives of this study were to determine the feasibility, degree of respiratory support and safety of HFNC, in sedated and awake healthy dogs, when compared to TNC.

Materials & Methods: In a randomized crossover study using eight healthy dogs, TNC was assessed at 0.1, 0.2 and 0.4 L/kg/min and HFNC was assessed at 0.4, 1, 2, 2.5 L/kg/min. Variables measured included: inspiratory/expiratory airway pressures, respiratory rate, FiO2, ETO2, PaO2, PaCO2, FiCO2, ETCO2, and vital parameters. Sedation status, tolerance and respiratory scores, as well as complications were also recorded.

Results: Continuous positive airway pressure (CPAP) was achieved using HFNC at 1 and 2 L/kg/min. At 2.5 L/kg/min tolerance scores were high and pressure support was not improved. The FiO2 using TNC 0.1 L/kg/min was 27% and was not different from room air. The FiO2 at 0.4 L/kg/min for HFNC and TNC was 72%. The FiO2 at all higher HFNC flow doses was 95%. The only noted complication was aerophagia, and there was a mild increase in PaCO2 only when subjects were sedated (P=0.0063).

Discussion: Use of HFNC in dogs is feasible and safe, delivers predictable oxygen support and offers the advantage of CPAP. Flow rates of 0.4â€“2 L/kg/min are recommended. For use of TNC, consider starting at flow doses above 0.1 L/kg/min.
COMPARISON OF PLATELET STORAGE LESIONS BETWEEN LEUKOREDUCTED AND NON-LEUKOREDUCTED CANINE PLATELET CONCENTRATES

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Background and objectives: Platelet storage lesions (PSL) are the sum of deleterious changes that platelets undergo during storage, affecting platelet concentrate viability for transfusion. Removal of leukocytes has been proven to reduce febrile post-transfusion reactions and decrease platelet premature activation and apoptosis. The present study objectives were to determine if leukoreduced concentrates have fewer and delayed onset of in vitro storage lesions compared with non-leukoreduced concentrates and to determine which variable best predicts in vitro platelet viability.

Methods: Platelet concentrates from twelve dogs were stored at room temperature for 7 days. Each sample was equally distributed into leukoreduced (LR), leukoreduced control (LR-C), non-leukoreduced (NLR) and non-leukoreduced control (NLR-C) concentrates. Platelet count, platelet indices, platelet morphology, pH, glucose, HCO3, pO2, pCO2, lactate, platelet aggregation, and P-selectin and Annexin V expression were assessed on days 1, 3, 5 and 7. All samples were sent for aerobic bacterial culture on day 7.

Results: All bacterial cultures were negative and platelet counts did not change significantly over time. There was an earlier onset of decreased pH in NLR samples compared with LR but both decreased over time. All the other variables showed greater negative changes in NLR samples compared with LR samples except for platelet morphology and platelet aggregation.

Conclusion: The changes over time were greater in NLR concentrates than in LR samples. These findings may correlate with a longer shelf life and better in vivo recovery and function of platelets in LR samples compared to NLR samples.
Endometrial nitric oxide synthase activity in mares susceptible or resistant to persistent breeding-induced endometritis and the effect of a specific iNOS inhibitor

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Emerging research suggests that the nitric oxide system may play a role in the development of persistent breeding-induced endometritis (PBIE) in the mare. Differences in uterine nitric oxide (NO) levels between mares susceptible or resistant to PBIE and a dose-dependent inhibitory effect of NO on uterine contractility have been demonstrated. The objectives of this study were to investigate the difference in total endometrial nitric oxide synthase (NOS) activity between susceptible and resistant mares and the effect of a specific inducible nitric oxide synthase (iNOS) inhibitor on total endometrial NOS activity. Six susceptible and six resistant mares were selected based on preset criteria and the results of an intrauterine challenge with killed spermatozoa during estrus. Endometrial biopsy samples were collected 24 h post challenge and cultured at 37°C for 24 h in L-arginine supplemented minimum essential medium with or without the specific iNOS inhibitor 1400W dihydrochloride (1 mM). The medium and the cultured endometrial tissue were collected after 24 h of culture and assayed for NO and total protein, respectively. Total NO content of the medium, normalized to endometrial tissue wet weight or total protein, was used as a measure of total endometrial NOS activity. Non-parametric tests were applied for statistical analysis. Susceptible mares had significantly greater endometrial NOS activity than resistant mares. The iNOS inhibitor treatment significantly reduced endometrial NOS activity in both susceptible and resistant mares. These findings provide a basis for clinical testing of NOS inhibitors as preventative or therapeutic options for PBIE in mares.
Comparison of Multiplate, Platelet Function Analyzer-200, and Plateletworks in healthy dogs treated with aspirin and clopidogrel

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Background: Platelet function testing may be warranted to assess response to aspirin and clopidogrel.

Hypothesis/Objectives: To evaluate the effects of aspirin, clopidogrel, or combination therapy using three platelet function tests: Multiplate Analyzer (MP), Platelet Function Analyzer-200 (PFA), and Plateletworks (PW).

Animals: Six healthy research Beagles.

Methods: Randomized double-blind placebo controlled study (crossover design). Dogs were given aspirin 1 mg/kg, clopidogrel 2 mg/kg, or combination therapy for 1 week each, with a washout period of 2 weeks. Platelet function was assessed on days 0 and 7 of each phase using MP (adenosine diphosphate [ADP], arachidonic acid [AA], collagen [COL] agonists), PFA (P2Y, COL-ADP [CADP], COL-Epinephrine [CEPI] cartridges), and PW (ADP, AA, COL agonists). Platelet counts were obtained with impedance and optical counters.

Results: For MP, mean aggregation was decreased for COL and AA with combination therapy, and for ADP with all treatments. For PFA, mean CT was increased for the CEPI cartridge with aspirin; and for the P2Y and CADP cartridges with clopidogrel or combination therapy, but PFA P2Y was superior to PFA CADP for detecting clopidogrel effect in all dogs. For PW, mean aggregation was inhibited for AA with all treatments; for ADP with clopidogrel or combination therapy; and for COL with clopidogrel. Only PW with AA detected all drug effects in all dogs. PW results with the two hematology counters showed almost perfect agreement.

Conclusion and clinical importance: All platelet function tests detected treatment effects in some dogs and may have utility for monitoring therapy.
Progenitor and non-progenitor functions of canine adipose-derived and bone marrow derived mesenchymal stromal cells

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Mesenchymal stromal cells (MSC) hold promise for both cell replacement and immune modulation strategies owing to their progenitor and non-progenitor functions, respectively. Characterization of MSC from different sources is an important and necessary step before clinical use of these cells is widely adopted. Little is known about the biology and function of canine MSC compared to their mouse or human counterparts. This knowledge-gap impedes development of canine evidence-based MSC technologies.

We hypothesized that canine adipose tissue (AT) and bone marrow (BM) MSC (derived from the same dogs) will have similar differentiation and immune modulatory profiles. Our objectives were to evaluate progenitor and non-progenitor functions as well as other characteristics of AT- and BM-MSC including 1) proliferation rate, 2) cell surface marker expression, 3) DNA methylation levels, 4) potential for trilineage differentiation towards osteogenic, adipogenic, and chondrogenic cell fates, and 5) immunomodulatory potency in vitro.

No significant differences were found between AT- and BM-MSC with regard to their immunophenotype, progenitor, and non-progenitor functions. Both MSC populations showed strong adipogenic and osteogenic potential and poor chondrogenic potential. Both significantly suppressed stimulated peripheral blood mononuclear cells. The most significant differences found were the higher isolation success and proliferation rate of AT-MSC, which could be realized as notable benefits of their use over BM-MSC.
Gut microbiota and the initiation of antiviral immunity against avian influenza virus (H9N2) infection in chickens

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Many of the roles of commensal microbes against bacterial pathogens such as the production of biosurfactants, production of different metabolites with antimicrobial properties, and promotion of the maturation of secondary lymphoid organs have been characterized. However, little is known about the relationship between viral pathogens and commensal microbiota. In addition to replication in the upper respiratory system, low pathogenic influenza viruses are known to replicate in the gut. Two studies were conducted to assess the role of commensal microbiota against low pathogenic H9N2 virus infection in chickens. Two groups, control and H9N2-infected, were used in the first experiment, and Next-Generation sequencing (Illumina Miseq®) was used to study the composition of gut microbiota. Infection of chickens with H9N2 resulted in dysbiosis of gut microbiota starting at day 3 post infection, and phyla firmicutes and proteobacteria were dominant in the control and H9N2-infected groups, respectively. This dysbiosis was also associated with increased virus shedding. A second experiment was conducted where chickens were depleted of their gut microbiota with a cocktail of antibiotics and infected with H9N2. Infection with H9N2 resulted in a significant (P<0.05) increase in the shedding of the virus in depleted chickens compared to non-depleted chickens. Furthermore, depleted chickens were compromised for their type I interferon (IFN-alpha/beta) responses both in the gut and respiratory system. In conclusion, H9N2 infection in chickens results in disruption of the gut microbiota, and gut microbiota are necessary for the initiation of innate anti-viral immune response in chickens.
Impact of implementing an antimicrobial-use algorithm on treatment and mortality rates of diarrheic calves.

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The objective of this study was to evaluate the impact of implementing an antimicrobial-use algorithm on AB use treatment and mortality rates of diarrheic calves.

Four commercial dairy farms (F1, F2, F3, F4) were enrolled. Farm records were obtained to collect data about the incidence of diarrhea, AB treatment and mortality rates for the preceding 18 months. Then, an antimicrobial-use algorithm for farm personnel based on the presence of three clinical signs (diarrhea, fever and decreased milk intake) was developed to direct AB therapy in diarrheic calves (<30 day-olds) and the same farm data were collected from the same records systems for 12, 7, 7 and 4 months for F1, F2, F3, and F4, respectively.

Treatment records of 1208 (F1), 1051 (F2), 190 (F3) and 117 (F4) calves were available for the pre-intervention period. The incidence of diarrhea was 78, 91, 57% and 60%, AB was administered to 93, 94, 36% and 55% of diarrheic calves and mortality rates attributed to diarrhea were 1.4, 3.4, 2.1 and 0% in F1, F2, F3, and F4, respectively. After the algorithm implementation, data were available for 438 (F1), 660 (F2), 141 (F3) and 40 (F4) calves. Diarrhea developed in 77, 90, 68 and 87% calves on the four farms, but treatment rates were significantly lower; 28, 13, 1 and 17% (P < 0.0001), with no impact on mortality (1.4, 0.3, 0.7 and 2.5 %, respectively, P > 0.05).

The use of a simple and practical antimicrobial-use algorithm can provide a significant reduction in the use of antimicrobials, decreasing treatment costs and improving antimicrobial stewardship with no negative impacts on calf health.
Validating feline behavioural and physiological parameters in response to handling during a mock veterinary examination

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Many cat owners do not seek adequate veterinary care, due to perceived feline stress during veterinary visits. The current study aimed to determine which feline responses to handling differentiate between passive restraint (n=22), and full body restraint (n=25), during mock veterinary examinations. Based on current restraint stress literature, we made the a priori assumption that full body restraint is more negative for cats than passive restraint. Cats were initially assessed as either friendly or unfriendly during interactions with a stranger, and this was included as a covariate in analyses. Cats were then restrained according to treatment, and assessed for behavioural (tail lashing, lip licks/minute, negative vocalizations, ear position) and physiological (heart rate, respiratory rate, relative pupil size) responses during examination.

During full body restraint, cats showed an average (SE) of 40.3 (1.79) breaths per minute, an average (CI) of 2.30 (1.82, 2.81) lip licks/minute and were more likely to hold their ears in a back or flat position during the first 15 seconds of handling (p<0.0001; Mantel-Haenszel chi-square), compared to cats handled with the passive restraint method. Analysis of relative pupil size showed an interaction between treatment and friendliness; unfriendly cats handled using full body restraint showed a larger average (SE) pupil ratio of 0.66 (0.042) compared to unfriendly cats handled with the passive restraint. The current results indicate that the feline responses which are valid indicators of negative responses to handling include respiratory rate, number of lip licks/minute, ear position, and pupil dilation ratio.
The Cytoprotective Role of Cytochrome P450 2A5 against Oxidative Stress in Mouse Liver

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Cytochrome P450 (CYPs) are a superfamily of enzymes involved in the metabolism of endogenous substrates and xenobiotics. Isoform CYP2A5 is uniquely induced upon exposure to various hepatotoxins and during pathophysiological conditions, while levels of most CYPs are unchanged or down-regulated. Previous studies have shown that CYP2A5 induction is mediated by the Nrf2-antioxidant response pathway, a major mechanism in cellular defence against oxidative stress. We hypothesize that CYP2A5 has a cytoprotective role against oxidative stress by managing reactive oxygen species (ROS). Primary mouse hepatocytes were isolated from wild type (WT) and Cyp2a5-null (NULL) C57BL/6 mice by retrograde collagenase perfusion. Hepatocytes were treated with CYP2A5 inducers: pyrazole, bilirubin, menadione, lead chloride or 0.1% DMSO as a vehicle. Cellular damage in WT and NULL mice were compared using lactate dehydrogenase assay as a biomarker for cytotoxicity. Protein expression of apoptotic markers, cytochrome c and caspase-9 was examined by immunoblotting. ROS production in WT and NULL mice were compared using DCFDA ROS detection assay. Cytotoxicity, ROS levels and apoptotic markers, cytochrome c and caspase 9 were significantly higher in NULL mice compared to WT mice in all treatment groups. Pre-treatment with antioxidants reduced cytotoxicity and ROS production in both WT and NULL mice. In summary, CYP2A5 plays a role in preventing apoptosis and regulating ROS production, although the exact mechanism by which CYP2A5 protects against oxidative stress still remains unclear. Future studies will determine if CYP2A5 reduces ROS levels through a mechanism involving free-radical quenching.
In vitro evaluation of a simulated capnoperitoneum on proliferation of canine transitional cell carcinoma

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Purpose: There is controversial data regarding tumor dissemination and growth after laparoscopic tumor resection in human in vitro studies. No effects have been documented in companion animals. Therefore, the purpose is to determine cell viability and proliferation of canine transitional cell carcinoma (TCC) following simulated CO2 pneumoperitoneum in vitro at various pressures.

Methods: Canine TCC and MDCK cells were exposed to 100% CO2 (21°C) at 0, 5, 10 and 15 mmHg for 2 hours using an insufflator at 37°C. Culture media pH was measured. Viability and proliferation were assessed using Resazurin and Trypan Blue exclusion dye assays, respectively. 3-way ANOVA and one-way ANOVA were used for statistical analysis with a p<0.05 were considered significant.

Results: pH was significantly decreased immediately after (p<0.0001) CO2 exposure but returned to normal 1-hour post exposure. There were no significant effects on proliferation in any of the experiments (p=0.9472). Viability is significantly (p<0.0001) affected by different pressures of CO2 insufflation in both healthy and carcinoma cells. Although the trend is not linear, 5 and 10 mmHg had a more significant effect than 15 mmHg on viability when compared to control and 0 mmHg in both carcinoma and healthy cells.

Conclusions: CO2 pneumoperitoneum at various pressures causes a significant change in the mitochondrial activity of healthy and carcinoma cells without affecting their proliferation rate. Due to the varied physiological effects of the abdominal microenvironment, this work warrants further in vitro and in vivo studies. Promising research in dogs can be translated into humans to improve outlook for both species.
Combining PCR and Next-Generation Sequencing to explore the diversity of coccidia (Eimeria spp.) infecting turkeys globally

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Coccidia (Eimeria spp.) are single-celled organisms causing an extremely common and costly parasitic disease found in most commercial poultry operations. Hardy environmental forms of these parasites (sporulated oocysts) contaminate feed, dust, and water in the poultry house and can be ingested by the birds. Development of the parasites in poultry causes damage to the intestinal tract that greatly reduces growth or egg production with considerable cost to farmers. Eimeria spp. diversity in turkeys has not been explored as thoroughly as for coccidia that infects chickens. Turkey fecal samples will be screened for coccidia using standard fecal flotations and the genotypic diversity of parasites in the positive samples will be determined using a combined PCR-NGS assay targeting one or more genetic loci (essentially determining global coccidial biodiversity in the field samples). PCR will be performed using universal COI or 18S rDNA primers and amplicons will be sequenced using NGS to quantify and enumerate the parasites present. Thus, NGS will allow for the detection of minor (infrequent) and perhaps previously unidentified species in samples with multiple parasites. Cross immunity studies will establish if protection against all identified Eimeria species infecting turkeys is generated by the available live coccidiosis vaccines for turkeys. Cross-immunity will be measured in poults immunized against one Eimeria sp. followed by individual challenge infections with each Eimeria species that can infect turkeys. The results of this work will help to improve the efficacy and use of live coccidiosis vaccines in turkeys to aid in control of the disease in commercial turkey operations.
Inhibition of AKT-1 for the treatment of human non-small cell lung cancer (NSCLC)

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AKT is a serine-threonine kinase implicated in tumorigenesis as a central regulator of cellular growth, proliferation, survival, metabolism, and migration. Activated AKT is overexpressed in approximately 50-70% of NSCLC tumors and has exhibited an association with poor prognosis as well as chemotherapeutic resistance to platinum-based therapy. AKT inhibitors such as MK-2206 are currently undergoing clinical investigation for the treatment of human NSCLC however, these agents broadly target all three (1-3) AKT isoforms. Recent evidence suggests opposing roles of the AKT isoforms in lung tumorigenesis where loss of AKT-1 inhibits while the loss of AKT-2 enhances lung tumor development in transgenic mouse models. Therefore, it is hypothesized that preferentially inhibiting AKT-1 will warrant a more effective therapeutic strategy compared to broad AKT inhibition. WST-1 cell viability assays have revealed that a selective AKT-1 inhibitor A-674563 is a more potent regulator of survival in NSCLC cell lines compared to a pan-AKT inhibitor MK-2206. In addition, flow cytometry and western blot analysis have uncovered divergent downstream effects of the two drugs which may explain the therapeutic advantages of the AKT-1 specific inhibitor. Overall, our results have also suggested that off-target inhibition of CDK2 by the AKT-1 inhibitor A-674563 is at least partially responsible for the increased efficacy of the AKT-1 inhibitor. Future research will focus on comparing the effects of the drugs on the localization of the AKT isoforms. In addition, we will investigate the toxicity of these inhibitors on normal human small airway epithelial cells (HSAECs).
Harnessing Deep Sequencing to Improve Molecular Clonality Testing for Canine B-Cell Lymphoma

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Though canine lymphoma is incurable, prompt chemotherapy can extend the mean survival time from 4-6 weeks to several months. Acquiring an accurate diagnosis is hence essential, and can be facilitated with molecular clonality assays. The assays detect neoplastic proliferations by assessing genetic diversity of lymphocytes through amplification of the hypervariable immunoglobulin heavy chain (IGH) gene. Current assays are prone to false negative results because they do not detect all gene segments that rearrange to form the mature IGH gene, nor do they consider frequent gene mutations. The aim of this study is to develop an improved B-cell lymphoma clonality assay that considers IGH gene usage and mutational patterns. Deep sequencing was performed on 10 target-enriched IGH cDNA samples (5 tissues from 2 normal dogs each) on the Illumina MiSeq platform using paired-end reads. Of 86 germline IGH variable genes (VH), 85 were represented in the sequenced reads, while all 6 germlines IGH joining genes (JH) were identified. Considerable variation in gene rearrangement frequencies was found, ranging from 9.9% to 0.3% of reads for VH and 59.5% to 1.1% for JH. A highly multiplexed primer set was designed based on a detailed analysis of mutation patterns at putative primer sites. The new primer set will be tested against existing assays using a series of neoplastic and non-neoplastic lymphoid proliferations. The new assay is expected to have a higher sensitivity than currently available test and allow for a definitive diagnosis in more cases, resulting in timelier treatment and improved patient care. It can also be used to monitor minimal residual disease during and after treatment.
There is a strong need for animal shelters to determine strategies to decrease the incidence of healthy animals being euthanized, as there still exists shelters which euthanize animals due to a lack of space. Several shelters throughout Canada and the United States have implemented the Capacity for Care program in an attempt to decrease length of stay of cats within the shelter and improve their overall welfare while in the shelter. The current study uses passively collected data from the Guelph Humane Society between 2011 and 2016. A Cox Proportional Hazard regression model was used to determine factors affecting a cat's time-to-adoption. Breed and sex were found to significantly affect a cat's time-to-adoption at the Guelph Humane Society. In addition, cats were 24% more likely to be adopted after the implementation of the Capacity for Care program compared to before. This was the first study to use long-term data to identify the Capacity for Care program as a risk factor for a shorter time-to-adoption.
Investigation of the prevalence and distribution of Echinococcus multilocularis in coyotes and foxes in southern Ontario

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Alveolar echinococcosis (AE), a typically fatal disease in humans and dogs when untreated, is caused by the intermediate stage of the zoonotic tapeworm Echinococcus multilocularis. The parasite typically uses wild canids (e.g., coyotes and foxes) as definitive hosts. Prior to 2012, E. multilocularis had not been diagnosed in Ontario. Since then, five cases of AE have been reported in dogs, and two in lemurs in southern Ontario, six of which had no travel history. AE in dogs is a rare diagnosis in North America and is thought to result from the ingestion of a large number of eggs from wild canid feces.

As a part of a 2-year (2015-2017) study to determine the prevalence and distribution of E. multilocularis in southern Ontario, 204 wild canids (182 coyotes and 22 foxes) were collected from November 2015 to August 2016 from across the region. Fecal samples were collected during post-mortem and analyzed via a semi-automated magnetic capture probe DNA extraction and real-time hydrolysis probe PCR method for the presence of E. multilocularis DNA. Overall, 16% (95% confidence interval (CI) 11.4%, 22.7%) of coyotes and 14% (95% CI 2.9%, 34.9%) of foxes from western, central, and eastern regions of southern Ontario tested positive. A statistically significant spatial cluster (i.e., an infection “hot spot”) consisting of 67 wild canid carcasses, including 27 of the 33 cases (RR=10.3; p=0.001), located in the western and central regions of southern Ontario was identified using the spatial scan statistic. This is the first report of E. multilocularis infection in wild canids in Ontario and strongly suggests its establishment in a wildlife cycle in the region.
Evidence for neurogenesis in a novel amniote model, the leopard gecko

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Endogenous neural stem/progenitor cells (NSPCs) residing within neurogenic niches have been identified across vertebrate species. In adult mammals, NSPCs are located in discrete brain regions, minimally contribute to homeostatic neurogenesis, and are incapable of replacing neurons following injury. In contrast, among species capable of brain regeneration, NSPCs are broadly distributed and frequently contribute to homeostatic neurogenesis. Here we explore neurogenesis in an emerging amniote model of central nervous system regeneration, the leopard gecko. We have identified a population of NSPCs residing in the ventricular zone (VZ) of the lateral ventricle. Strikingly, VZ cells ubiquitously express the NSPC markers SOX2 and Musashi-1. They also have a morphology and protein expression profile (Vimentin and GFAP immunopositive) reminiscent of embryonic radial glial cells, a cell population known to guide neuronal migration. The VZ is mitotically active (as evidenced by immunostaining for proliferating cell nuclear antigen and phosphorylated histone H3) and is located immediately adjacent to a migratory region expressing neuroblast marker PSA-NCAM. The presence of constitutively active NSPCs in the VZ offers compelling evidence for the neurogenic and possibly regenerative capacity of the leopard gecko brain.
USE OF EX VIVO LUNG SLICE TISSUES TO MONITOR INFECTIVITY OF RESPIRATORY VIRUSES UNABLE TO REPLICATE IN VITRO

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Jaagsiekte Sheep Retrovirus (JSRV) and Enzootic Nasal Tumor Virus type 1 (ENTV-1) are important betaretroviruses of small ruminants. JSRV and ENTV-1 induce tumors in the lower respiratory tract and nasal turbinates, respectively. Despite this difference in tropism, these viruses share 89% nucleotide identity and the same cellular receptor for entry, hyaluronoglucosaminidase 2 (Hyal2). The envelope (Env) glycoprotein of both JSRV and ENTV-1 has been shown to function as a rapidly transforming oncogene. Notably, many of the signaling pathways activated in lung and other cancers in humans are also active during Env-mediated oncogenesis. Furthermore, since they exploit cellular functions for their replication, both viruses can be used to further our understanding of pathogenesis and may reveal novel mechanisms of oncogenesis or therapeutic targets relevant to human cancer. While there is currently no cell line that can support virus infection, Cousens et al. recently reported using ovine lung slices as a tool to study JSRV infection in vitro. We aim to adopt this technology to elucidate tropism determinants and to unravel JSRV and ENTV-1 pathogenesis. We have optimized the growth and maintenance of ovine lung slices and have shown they are susceptible to lentiviral vector infection. We are currently optimizing transduction conditions with JSRV, ENTV-1, and JSRV-ENTV-1 hybrid viruses in order to identify regions of their genomes responsible for the restricted tropism. Overall, we hope this work will expand our knowledge of betaretroviral pathogenesis and cellular transformation, and provide an elegant in vitro model for evaluating novel viral vectors for lung gene delivery.
Equine cord blood-derived mesenchymal stromal cells for the derivation of biphasic osteochondral constructs

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Horses often sustain traumatic cartilage injuries during training. Cartilage defects can be treated with osteochondral autograft transfers, however, this technique is associated with limited numbers of grafts and donor site morbidity. Tissue engineering shows promise in removing these challenges. Mesenchymal stromal cells (MSCs) can be induced to undergo chondrogenesis, and are an ideal candidate for cartilage regeneration.

We hypothesize that we can induce neocartilage from cord blood-derived MSCs (CB-MSCs) on top of a bone substrate. Our objective is to generate biphasic osteochondral plugs using equine CB-MSCs and a calcium polyphosphate (CPP) bone substitute.

Three CB-MSC cultures were expanded and directly seeded onto CPP substrates. We evaluated the use of a ROCK inhibitor to reduce cell contraction on the CPP, then examined the tissues produced by various cell seeding densities, culture duration (3 week or 6 week), and variations in TGFB3 supplementation in the 6 week cultures. We evaluated the tissues for histology, immunohistology, biochemical content, and mRNA expression.

ROCK inhibitor was found to be essential for MSC adherence to the CPP bone substrate, and seeding density of 1.5x106 cells per CPP produced thick, hyaline-like cartilage tissue after 3 weeks. There was no significant increase in GAG/DNA or collagen/DNA content among 3 and 6 week cultures or upon varying TGFB3 exposure. No significant differences were found in mRNA expression.

This study demonstrates that hyaline-like cartilage tissue can be generated by seeding MSCs directly onto a bone substrate. Further work is needed to optimize culture conditions prior to testing them in vivo.
The Role of IRF7 and NF-ÎºB Pathways in the Induction of Antiviral Responses in Chicken Tracheal Epithelial Cells following Exposure to TLR3 and 4 Ligands

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The chicken upper respiratory tract is the portal of entry for respiratory pathogens including avian influenza virus (AIV). Specifically, airway epithelial cells are the primary target for the virus. Host innate responses in the respiratory system provide the first line of defense against AIV. Host cells detect the presence of viral components by means of germline-encoded pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). There is a lack of evidence supporting the induction of antiviral responses in the chicken trachea following TLR activation and underlying mechanisms. In this study, we hypothesized that TLR ligands induce antiviral responses in chicken tracheal epithelial cells which can influence the function of other cells, such as macrophages. Primary chicken tracheal epithelial cells (cTECs) were cultured and stimulated by polyI:C and LPS from Escherichia coli 026:B6, TLR3, and 4 ligands, respectively. Then, cells were infected with AIV. We also used BX795 and celastrol, inhibitors of IRF7 and NF-ÎºB pathways, respectively, in order to gain insight into the mechanisms involved in the induced antiviral responses. In addition, we explored the effect of cTECs treated with TLR ligands on the migration of chicken macrophages, using chemotaxis cell migration assay. Our results demonstrated that treatment of cTECs with TLR ligands reduced the replication of AIV in cTECs. In addition, our results confirmed the importance of IRF7 and NF-ÎºB pathways in the induction of antiviral responses in cTECs. We confirmed functional activity of supernatants of cTECs treated with TLR ligands.
Barriers to knowledge translation. A survey of swine veterinarians.

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In human medicine a well-developed infrastructure supports knowledge translation (KT) between those who apply it and those who generate it. A parallel but modest KT infrastructure in veterinary medicine is being built. To manage arguably the biggest One Health challenges of this generation - antimicrobial resistance and emerging infectious disease - efficient and effective transfer of knowledge across and between multiple disciplines will be essential.

Yet barriers to knowledge translation in veterinary medicine have been sparsely studied. Food animal veterinarians have distinct KT priorities with emphasis being disproportionately on infectious disease including primary and secondary interventions versus tertiary veterinary care. We are not aware of any other surveys exploring specifically food animal veterinarian barriers to KT.

Working collaboratively with the American Association of Swine Veterinarians, we surveyed members to benchmark priority infectious disease interests, and for the first time explore how time, skill, process, and access act as KT barriers for swine veterinarians. Results showed substantial barriers exist with respect to time, access, and confidence limitations to evaluate individual research papers and to keep current. Interests are well consolidated, ~ 60% have an hour or less per week and express moderate or greater stress and lack efficiency with keeping current. Almost half said they could not explain confounding bias, 37% have access to only 2 journals, and 25% report no confidence to evaluate statistical methods used. Findings suggest great opportunity for research syntheses including systematic reviews to address KT barriers
The Effect of Abiotic and Biotic Factors on the Establishment of Ixodes scapularis in Ontario, Canada

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In eastern North America, the hard tick Ixodes scapularis is the vector for the causative agent of Lyme disease, Borrelia burgdorferi. Within the last two decades, the spread of I. scapularis has accelerated northward and this spread is hypothesized to continue, in part due to climate change. Other ecological factors also influence I. scapularis and may play a role in population establishment. The objective of this study was to understand what abiotic and biotic factors are significant in the establishment of I. scapularis in Ontario. Tick dragging was conducted at 154 sites in Ontario during the months of May to October 2014 and 2015. Ecological and georeferenced data was collected for each site. Multivariable mixed logistic regression models were created to assess the impact of the ecological factors on the presence of I. scapularis. Cumulative annual degree days and the depth of the litter layer were positively associated with the presence of I. scapularis, as well as the density of the understory, the presence of shrubs and the interaction of these two ecological factors. Elevation was negatively associated. These findings enhance our understanding of the factors contributing to I. scapularis population establishment and can be used to enhance current predictive models and risks maps for I. scapularis.
NetF-producing Clostridium perfringens: Clonality and Plasmid Pathogenicity Loci Analysis

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The beta-pore-forming toxin NetF-producing strains of type A Clostridium perfringens are important causes of foal necrotizing enteritis and canine hemorrhagic enteritis. Strains producing NetF also produce a toxin protein homologue NetE, encoded on the same large tcp-conjugative plasmid, as well as CPE enterotoxin and CPB2 on a second plasmid, and sometimes the putative toxin NetG on another separate large conjugative plasmid. Comparative analysis showed that netF/netE and netG are located in unique pathogenicity loci on plasmids. Previous genome sequences of two netF-positive C. perfringens showed that they both shared three similar plasmids, including the NetF/NetE and CPE/CPB2 toxins-encoding plasmids mentioned above and a putative bacteriocin-encoding plasmid. The main purpose of this study was to determine whether all NetF-producing strains share this common plasmid profile and whether their distinct NetF and CPE pathogenicity loci are conserved. To answer this question, 15 equine and 15 canine netF-positive isolates of C. perfringens were sequenced using Illumina Hiseq2000 technology. In addition, the clonal relationships among the NetF-producing strains were evaluated by core genome multilocus sequence typing (cgMLST). The data showed that all NetF-producing strains have the common three plasmid profile recognized earlier and that the pathogenicity loci on the plasmids are conserved. cgMLST analysis showed that NetF-producing C. perfringens strains belong to two phylogenetically distinct clonal complexes. The pNetG plasmid was absent from isolates of one of the clonal complexes, and there were very minor differences between the NetF/NetE and CPE/CPB2 plasmids between the two clonal complexes.
Exploring Inuit perspectives on developing values-based environment and health metrics for surveillance in Rigolet, Nunatsiavut, Labrador

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Inuit across the Canadian North are dealing with various environmental issues as a result of climate change and resource development that have direct and indirect impacts on health. Research that seeks to effectively address health impacts of environmental changes in the North must take Inuit values and experiences into consideration. The main goal of this research project was to explore Inuit perspectives to help guide the development of a community-driven environment-health surveillance program in Rigolet, Nunatsiavut, Labrador. The objectives were to: understand how Inuit in Rigolet identify and prioritize environment and health metrics; explore underlying values that affect the relative importance of these environment and health metrics; and synthesize perspectives of community members and government stakeholders on how to incorporate values-based environment and health metrics into a sustainable surveillance program. In-depth, semi-structured interviews were conducted with Inuit in Rigolet and regional government stakeholders to explore what environment and health metrics are important, and why these metrics are important to monitor. All interviewees emphasized the importance of environment-health connections and felt that synergies between Inuit and scientific knowledge should be identified to benefit both community and environmental health. Building from this foundation, an integrated environment-health surveillance program will be developed that is aligned with community goals in Rigolet. This research demonstrates the utility of qualitative methods to conduct research on environment-health metrics rooted in Inuit values for contextually-sensitive surveillance.
Inuit Childbirth in Canada: An Exploration of Place, Culture, and Health

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Inuit experience the highest infant mortality rate in Canada, as well as significantly higher rates of preterm births, stillbirths, and maternal health issues, as compared to the non-Inuit population. These differences are attributed, in part, to the lack of availability and type of obstetrical care women receive. Most women are required to fly out of their communities for birth (obstetric evacuation, OE), and may have to remain in southern hospitals for weeks or months. A literature review was conducted to examine the range, extent, and nature of peer-reviewed literature about OE in Northern Canada. The literature documents women reporting feeling isolated, lacking prenatal support, experiencing language barriers, and receiving obstetrical care from a Western model that is incongruous with Indigenous teachings on holistic well-being. Fathers and older siblings find it challenging to connect emotionally with a newborn after missing the birth, and for communities, OE represents the loss of Inuit self-governance. Importantly, place-attachment, "one's psychological, emotional, and spiritual connection to the land, and to the kin- and community-based relationships that are sustained in a particular place", is central to the well-being of Indigenous peoples, and connected to all facets of health. As such, OE may have especially serious impacts on the overall health and well-being of an Inuit mother and child, but also that of her family and community. This presentation will provide an overview of what we know so far, as documented in the peer-reviewed literature, about the connections between place, culture, and health in relation to Inuit experiences of obstetric evacuation.
Guidelines for proper milk harvest and mastitis prevention have been widely studied and publicized in the dairy industry, yet a portion of the producer population is still not following or adopting the best practice. This project was part of the first National Dairy Study (NDS) conducted in Canada, and funded by Dairy Farmers of Canada through the Dairy Research Cluster 2 program. The objective of this part of the study was to describe the current adoption of recommended milking practices on dairy farms across all of Canada's 10 provinces.

During the spring of 2015 every licensed dairy producer in Canada was invited to complete a comprehensive questionnaire that addressed a range of health and management issues. The questionnaire was available in both English and French, could be accessed electronically, by paper copy, or could be completed by phone interview. The questions addressing udder health were adapted from a bilingual questionnaire previously validated by Dufour et al. (2010).

Of nearly 12,000 Canadian dairy producers 1,342 completed the survey. The adoption of recommended milking practices varies across farms and provinces, with markedly different adoption rates for different practices. Some of the practices, most notably the use of a post milking teat disinfectant, were reported to be widely adopted. Other practices, including cleaning gloves with disinfectant between cows and use of a pre milking teat disinfectant are much less widely in use. What remains to be explained are the reasons behind choices producers make, and why certain practices are used more widely than others.
Extended-spectrum Cephalosporin Resistance in Escherichia coli from Alberta Beef Cattle

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It has been suggested that there is a low prevalence of resistance towards extended-spectrum cephalosporins (ESCs) in Escherichia coli isolated from Canadian beef cattle. However, using enrichment techniques, we were able to detect resistance determinants in approximately 90% of fecal samples collected from Alberta beef cattle, prompting a further investigation into the occurrence and distribution of these genes within the Alberta beef cattle population. The objective of this study was to assess the prevalence and diversity of ESC resistance determinants in E.coli from Alberta beef cattle feces.

Fecal samples from Alberta beef cattle were enriched using EC broth with cefotaxime and underwent DNA extraction and purification. The DNA extracts were screened by real-time PCR for the following major ESC resistance gene families: blaCMY, blaCTX-M, blaOXA-48, blaNDM, blaKPC, blaSHV, and blaTEM. Isolates obtained from these enrichment samples were also screened for the same gene families using conventional PCR.

Results from the direct detection in the enrichment samples supported our previous findings that suggested resistance determinants can be commonly recovered from Alberta beef cattle feces: 82.6% of samples tested positive for blaCMY, 58.7% for blaCTX-M, 17.3% for blaSHV, and 2.1% for blaTEM only. All the samples tested negative for blaOXA-48, blaNDM, or blaKPC. Resistant determinants were confirmed to be present in 88% of isolates; further characterization is ongoing. These results show that ESC resistance is present in the Alberta beef cattle population and warrants further studies on the source of these resistance determinants and their implications for public health.
OPTIMIZATION OF TOTAL RNA & miRNA ISOLATION FROM NATIVE EQUINE CARTILAGE

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MicroRNAs (miRNA) are a class of small single-stranded non-coding RNA molecules approximately 18-24 nucleotides in length that regulate gene expression. miRNAs regulate post-transcriptional gene expression by complementary binding to mRNA transcripts and act by repressing translation or degrading the transcript altogether. Currently, there have been no published findings regarding miRNAs involved in gene expression profile from native equine cartilage tissue. This is largely in part due to the difficulties in working with equine articular cartilage. Isolating RNA from articular cartilage is complicated by factors such as an extremely dense extracellular matrix, low cellular content, and a high amount of negatively charged molecules. Currently, standard practice is to utilize chondrocyte-derived cell lines to determine gene expression from equine cartilage, however we hypothesize that this process may induce gene expression changes due to the nature of the harsh degradation process. In order to determine the gene expression profile, we attempted to isolate RNA from native equine cartilage. Our preliminary focus was to optimize a protocol to efficiently isolate RNA in large quantities with high purity. Thus, we tested 3 variables in our attempt to optimize a protocol, which were: tissue disruption method, lysis buffer incubation, and extraction kit used.

5 fetlock and 5 stifle joints were harvested from healthy equine samples. Subsequently, these samples were subjected to tissue disruption by either large ball or small ball homogenization, after which they were either incubated in lysis buffer for 24 hours or directly taken to isolation. RNA isolation was conducted using
Prevalence and Reproductive Consequences of Chromosomal Abnormalities in Canadian Swine Herds

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Objectives: To investigate CAT parameters as biomarkers for hypercoagulability and possible prognostic indicators in patients with IMHA.

For the past five years the King lab has carried out a screening program for chromosomal abnormalities in breeding boars from commercial herds in Canada. Over 2500 boars have been karyotyped, and 26 new translocations have been identified in 59 carriers. The frequency of translocation in our screening population is approximately 1%. Screening the parents of carriers has allowed the origin of some translocations to be established, indicating that 7 translocations originated from the dam, and 1 originated from the sire. We consider 7 to be de novo, while the remaining 11 are of unknown origin. Amongst autosome-autosome translocations, we have identified 23 reciprocal translocations, one inversion, and one Robertsonian translocation. A rare t(Y,13) sex chromosome-autosome translocation was also identified only the third of this type ever found in pigs. In-farm reproductive records indicate that translocation carriers are less fertile, having an average of 4 less piglets per litter. With DNA from translocation carriers, and their parents, we have established a DNA bank with which we will apply SNP array, and whole genome sequencing. These tools will be used to identify the molecular characteristics of translocations at the nucleotide level, allowing the identification of molecular events and genetic variations that may be associated with chromosome structural abnormalities. At present this constitutes the largest cytogenetic screening effort in Canadian swine herds. We hope to gain insight into the formation of translocations, and possible risk factors for developing a translocation, allowing the improvement of screening, and breeding practices.
Assessment of canine and feline body composition by veterinary healthcare teams in Ontario, Canada.

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Introduction

Research in the United Kingdom found that veterinarians rarely assess body composition in dogs, despite the simplicity of body condition scoring (BCS) and body weight (BW) measurements. Both World Small Animal Veterinary Association (WSAVA) and American Animal Hospital Association (AAHA) nutritional assessment guidelines recommend using BCS, BW and muscle condition scoring (MCS) for every patient. The objective of this study was to determine how Ontario veterinary healthcare teams (VHTs) assess body composition in companion animal patients.

Materials & Methods

An online survey was distributed to Ontario VHTs via email, social media and veterinary association websites. Survey questions included; how often body composition is assessed, methods used, determining ideal BW for weight management plans, and demographic data. Descriptive statistics and logistic regression were used for analysis.

Results

Out of 177 responses completed by Ontario VHTs, only 66.7% of respondents noted they always assess body composition, with the remaining 33.3% selecting often, sometimes, rarely or never. BCS (99.4%) and BW (99.4%) are used most often, with MCS (33.9%) used less frequently. However, respondents (94.4%) would use an alternative, more accurate method, if available.

Discussions & Conclusions

WSAVA and AAHA recommend that body composition is assessed in every examination; however, only 66.7% of respondents always assess body composition. Although BCS, MCS, and BW are easy and practical to use, practitioners would prefer a more accurate method. Until such method is available, practitioners should implement the WSAVA and AAHA nutritional guidelines in practice.
Annual variation in susceptibility of generic Escherichia coli isolates to ceftiofur from retail chicken meat surveillance in Canada

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Minimum inhibitory concentration (MIC) data are often reported as resistant (R) or susceptible (S) using established breakpoints, but this may mask subtle temporal changes in MIC. We compared results of regression models using MIC and breakpoint susceptibility data for analysis of annual variation in susceptibility of generic Escherichia coli to ceftiofur from retail chicken meat surveillance. Annual variation in susceptibility was evaluated using multivariable linear, tobit, logistic, multinomial logistic and ordinal logistic regression with and without random effects. MIC or breakpoint susceptibility data were used as outcome variables for the appropriate statistical models. Sampling year was modeled as a categorical predictor variable. Region was modeled as a fixed categorical predictor variable to account for clustering. Multilevel models included random effects to account for clustering by retail establishment. As appropriate for each statistical model, assumptions and goodness-of-fit were evaluated. For both the fixed and random effects models, only the logistic and multinomial logistic regression models fit the data or met model assumptions. The logistic regression models identified significant annual and regional variation in susceptibility. The multinomial logistic regression models identified annual and regional variation that varied between MIC categories relative to the base category. This study demonstrated that evaluation of annual variation in surveillance data should take advantage of the greater information available in the MIC distribution by using multinomial logistic regression models, rather than using logistic regression models with R/S data.
Assessment of the welfare and physiological response of commercial meat rabbits to carbon dioxide

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The commercial meat rabbit industry is in need of validated on-farm euthanasia methods for sick and injured animals, as well as approval emergency depopulation methods. Carbon dioxide (CO2) has never been assessed for these purposes in rabbits. The welfare and physiologic response of 81 rabbits across three age categories (pre-weaned, growers, and adults) was compared between two different chamber fill rates: a gradual-fill rate resulting in 10%-16% CO2 in the chamber in the first minute, and a fast-fill rate of 30%-40% CO2 in the chamber in the first minute. Rabbits were exposed to CO2 in a clear chamber with a hand input that allowed for the assessment of reflexes. Rabbits remained in the chamber for 6.5-7 min and were evaluated upon removal for sustained insensibility and death. The fast-fill rate resulted in a significantly shorter period of time to loss of consciousness (p<0.001) and death (p<0.001) than the gradual-fill rate. The average CO2 chamber concentration and time to loss of consciousness was 20.7% and 99s for gradual-fill, and 16.5% and 40s for fast-fill. Chewing motions (n=27) were significantly more common during gradual-fill (p=0.018). CO2 exposure caused a change in respiration rate observed as increased breathing (n=59), gasping (n=34) and open mouth breathing (n=80). Vocalizations were rare at either fill rate (n=6). Neither of these behaviours or other observed behaviours significantly differed between treatment groups. It was concluded that there are minimal differences between the fill rates examined. The fast-fill rate did result in a quicker onset of insensibility allowing less time for rabbits to respond to potential aversive properties of CO2.
Epidemiology of zoonotic pathogens and antimicrobial resistant bacteria in Canada geese from southern Ontario

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Antimicrobial resistant bacteria and zoonotic pathogens have previously been isolated from Canada geese. Because Canada geese are mobile, there is a concern that these birds may transmit antimicrobial resistant bacteria and zoonotic pathogens from one geographic location to another. Our objective was to determine the prevalence and patterns of carriage of three enteric bacteria, Salmonella, Campylobacter, and antimicrobial-resistant E. coli in Canada goose fecal samples collected from three sources in southern Ontario. Samples were obtained from the following sources: hunter-caught birds, diagnostic specimens submitted to the Canadian Wildlife Health Cooperative, and fresh fecal samples from live birds. The impact of the following risk factors on the prevalence of Salmonella, Campylobacter, and antimicrobial-resistant E. coli was examined using exact logistic regression: season, source, and bird demographics (sex and age). The prevalence of Salmonella, Campylobacter, and E. coli were 7.80%, 0%, and 73.54%, respectively. Among E. coli isolates, 6.25% had reduced susceptibility to at least one antimicrobial, and 4.46% were multidrug resistant. Nineteen unique E. coli resistance patterns were identified with some including resistance to antimicrobials of highest importance in human medicine. The prevalence of Campylobacter and E. coli single and multidrug resistance varied significantly by season and source, but not with bird demographics. Isolating Campylobacter and antimicrobial-resistant E. coli from Canada geese suggests that these birds may play a role in disseminating these organisms within the environment, and may pose a threat to human and
Developmental ethanol exposure leads to long-term alterations in layer VI medial prefrontal cortex neuron morphology in male mice.

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Exposure to ethanol during development can lead to a wide variety of teratogenic effects, which are diagnosed in humans as Fetal Alcohol Spectrum Disorders (FASD). Although attention deficits are one of the most common and persistent neurobehavioural components of FASD, the mechanisms underlying this outcome are not known. Previous work has shown that developmental ethanol exposure produces attention deficits in mice and dysregulates neuron function within layer VI of the medial prefrontal cortex (mPFC), a brain region critical for normal attention performance. This work also suggested that the morphology of layer VI neurons may be altered by developmental ethanol exposure. In this current study, we analyzed adult neuron morphology of both long and short neurons in layer VI of the mPFC in mice that had been exposed to ethanol or sucrose control during development, using a modified Golgi-Cox staining technique. Sholl ring analysis and branch structure analysis were completed on these neurons. Together, layer VI neurons of mice exposed to ethanol during development had less apical dendrite branching and matter compared with neurons of mice exposed to sucrose during development. Specifically, long layer VI neurons were thinner and longer, and short neurons had a less complex branching pattern in mice exposed to ethanol during development. These morphological changes may impact synaptic inputs to these neurons likely affecting signaling within prefrontal circuitry. This work confirms that developmental ethanol exposure has persistent effects on the morphology of neurons within the mPFC, which likely alters their role to support communication within mPFC attentional networks.
Treatment with Toll-like Receptor 3 Ligand Reduces Marek’s Disease Virus Infection in Chicken Embryo Fibroblast Cells

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Evolutionarily conserved pathogen recognition receptors, including Toll-like receptors (TLRs), recognize pathogen-associated molecular patterns (PAMPs) that are present in microbes. PAMPs induce several pathways downstream of TLRs which lead to induction of anti-viral responses. The objective of the present study was to investigate the effect of various PAMPs (as TLR ligands) on Marek’s disease virus (MDV) infection in chicken embryo fibroblast cells (CEFs). To that end, CEFs were pre-treated with Pam3CSK4, Poly(IC), lipopolysaccharide (LPS) and CpG ODN as TLR2, TLR3, TLR4 and TLR21 ligands, respectively. CEF cells were then infected with MDV at 24 hours post-stimulation (hps) with TLR ligands. The results indicated that pre-treatment with Poly(IC), Pam3CSK4, LPS and CpG ODN reduced MDV infection by 81%, 35%, 26% and 23%, respectively, at 96-hour post-infection (hpi). Transcriptional analysis of gene expression in CEF cells indicates that Poly(IC) treatment significantly increased the expression of type I interferons, interferon regulatory factor 7 (IRF7), interferon-induced protein with tetratricopeptide repeats 5 (IFIT5) and Mx1. Further, treatment with Pam3CSK4 significantly increased the expression of type I interferons, interleukin (IL)-1β, IFIT5 and Mx1. In addition, treatment with LPS significantly increased the expression of IL-1β, IFIT5, and Mx1. In conclusion, Pam3CSK4, Poly(IC) and LPS demonstrated the ability to inhibit MDV infection in CEF cells. Further studies are needed to explore the mechanism by which PAMPs reduce MDV infection in CEFs that may be helpful in controlling the replication of MDV in the chicken.
The Use of Calibrated Automated Thrombogram to Evaluate Thrombin Generation in Dogs with Immune-Mediated Hemolytic Anemia

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Background: A current challenge in patients with immune-mediated hemolytic anemia (IMHA) is the early identification and treatment of thromboembolic complications. Up to 60% of the mortality in IMHA is associated with thrombosis or disseminated intravascular coagulation. No conventional hemostasis test is able to accurately determine which patients are at risk of thromboembolic complications, and viscoelastic testing has recently been proven to be influenced by anemia, so is not reliable in IMHA patients. Calibrated automated thrombogram (CAT) is a thrombin generation assay that has been validated in clinically healthy dogs. Thrombin generation measured by CAT has never been reported in dogs with IMHA but may be a useful method for early detection of thromboembolic complications.

Objectives: To investigate CAT parameters as biomarkers for hypercoagulability and possible prognostic indicators in patients with IMHA.

Animals: 30 patients from the OVC-HSC with confirmed IMHA, prospectively enrolled.

Methods: Complete blood count, coagulation profile, and CAT were performed at the time of diagnosis and then every 48h for a total of three samples. Bilirubin, hematocrit, platelet and white blood cell counts were also measured and correlated with survival and discharge. Results were compared to 40 healthy dogs from a previous study.

Results: Lag time was prolonged for patients with IMHA compared to healthy dogs at day 0 (D0) and D2. Peak was higher in dogs with IMHA at D0, D2, and D4; and time to peak was higher on D0 compared to healthy dogs but not statistically different on D2 or D4. There was no statistical difference in the endogenous thrombin potential between IMHA pat
Temporal and spatial trends of calls concerning companion animals, to a North American poison control centre (2005-2014).

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The American Society for Prevention of Cruelty to Animals (ASPCA) operates an animal poison control centre (APCC) hotline that records data on companion animal exposure to potential toxic or poisonous substances. The purpose of our study was to use call centre data from the ASPC PCC hotline to determine the temporal and spatial distribution of calls concerning accidental ingestion of poisons in companion animals and assess whether the rate of calls to the hotline were associated with species, geographical and seasonal factors. We used data from this hotline from January 1, 2005 to December 31, 2014 inclusive, to construct multilevel Poisson regression models, to estimate the association between the above factors and the rate of calls to the hotline. Using scan statistics, based on a Poisson model, we identified temporal, spatial and spatiotemporal clusters of high rates of calls to the hotline, for each study year, for cats and dogs. This research will provide the ASPCA with important information concerning the distribution of calls to their APCC hotline and factors that may bias the reporting and surveillance of poisoning events in companion animals. These factors may subsequently be used to adjust for important covariates when using data from this hotline to identify large scale animal poisoning events.
Environmental factors that influence the ecology of zoonotic pathogens in wild urban exploiter species - a review and narrative synthesis

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Knowledge of pathogen ecology, including the impacts of environmental factors on pathogen and host dynamics, is essential for determining the risk that zoonotic pathogens pose to people. This review synthesizes scientific data on environmental factors that influence the ecology and epidemiology of zoonotic microparasites (bacteria, viruses, and protozoa) in globally-invasive urban exploiter wildlife speciesâ€”rock doves (Columba livia domestica), European starlings (Sturnus vulgaris), house sparrows (Passer domesticus), Norway rats (Rattus norvegicus), black rats (R. rattus) and house mice (Mus musculus). Pathogen ecology, including prevalence and pathogen characteristics, are influenced by geographic location, habitat, and season/weather. For example, the prevalence of zoonotic pathogens in mice and rats varies markedly over short geographic distances but tends to be highest in ports, disadvantaged (e.g., low-income) and residential areas. Future research should use epidemiological approaches, including random sampling and robust statistical analyses, to evaluate a range of biotic and abiotic environmental factors at spatial scales suitable for host home range sizes. Moving beyond descriptive studies to uncover the causal factors contributing to uneven pathogen distribution among wildlife hosts in urban environments may lead to better-targeted surveillance and intervention strategies. Application of this knowledge to urban maintenance and planning may reduce the potential impacts of urban wildlife-associated zoonotic diseases on people.
Functional Differences of Monocyte-Derived Macrophages in Cattle Classified Based on Adaptive Immune Responses

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The High Immune Response (HIR™) technology is an immunogenetic method to measure adaptive immune responses in dairy cattle and categorize them based on their IR genetic merit. Comparing key pathways of IR between high and low responder phenotypes is expected to shed light on the major factors in the immune system that shift the responses to either of these phenotypes. In this study, two fundamental functions of monocyte-derived macrophages (MDM) in response to three pathogens, S. aureus (SA), E. coli (EC) and M. avium subspecies paratuberculosis (MAP), were compared between 4 high and 4 low responder cows. Phagocytic activity of MDMs were tested following challenge with fluorescent-labelled bacteria. Nitric oxide (NO) production as an indicator of bactericidal activity was measured at 48 hours after challenging the MDMs with heat-inactivated bacteria. The results of this study showed MDMs from cows with the high responder phenotype phagocytize significantly more bacterial particles compared to MDMs from low responders (p< 0.05 for all treatments). The production of NO was higher in the high responder group after challenge with all three pathogens. Nevertheless, it was not significant in MAP treatment and a trend toward statistical significance was observed in response to EC and SA (p<0.1). Resistance or susceptibility to a pathogen is the outcome of a finely tuned and robust immune response. The results of this study, at least for two fundamental functions of macrophages, align with the hypothesis that stronger adaptive immune responses are initiated by stronger innate responses.
The innate immune system is an important first line of defense against infectious diseases, and mutations in genes of the innate immune system can impair the ability of animals to respond to pathogens. Expression quantitative trait loci (eQTL) are regions of the genome that contain variants that affect the expression of a given gene. In previous studies on the porcine innate immunome, we showed that 112 innate immune genes have a variable hepatic expression and that certain mutations are more common in diseased versus healthy pigs. In this study, we used targeted next-generation sequencing to comprehensively identify mutations in the variable expressed porcine innate immune genes and their surrounding regulatory DNA. We then performed eQTL analysis to identify variants that impacted the expression of those innate immune genes. DNA was obtained from the liver of 87 healthy, market weight pigs being processed at a large Ontario abattoir. The coding regions, introns, and up to 50 kb of upstream and 3 kb of a downstream sequence of the 112 variable innate immune genes were targeted for next-generation sequencing. Target sequence enrichment was performed using custom probes from Roche Nimblegen and the library was sequenced over 2 lanes of an Illumina HiSeq. The targeted 6.4 Mb of DNA was sequenced to an average depth of 14x per pig. After applying quality control filters, 41 894 short genetic variants were identified. The association between the variants and gene expression was analyzed using Matrix eQTL. Overall, 320 significant eQTLs spanning 18 innate immune genes were identified. These loci provide useful information for the development of future breeding strategies.
A survey of management practices and producers' perceptions regarding manual and automated milk feeding systems for dairy calves

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Dairy calves are commonly housed individually and fed by manual milk feeding (MMF) methods, with buckets or bottles. Automated milk feeders (AMF) allow for more natural milk feeding frequency and volume, with calves usually housed in groups. A national online survey was developed to determine management practices for the care of milk-fed calves in Canada, and the factors that influence use of MMF or the switch to AMF. A total of 670 responses were received (5.7% of all dairy farms in Canada). Of respondents, 16% used AMF and 84% used MMF. Having a herd size > 80 milking cows was associated with having an AMF among tie-stall farms (odds ratio [OR] = 3.8). For loose-housed farms (i.e., free-stall or bedded-pack), herd size > 80 milking cows (OR = 3.5), having an AMS (OR = 3.1), and use of cow brushes (OR = 3.1) were associated with having an AMF. Automated milk-fed calves were typically housed in groups of 10 to 15, while nearly 75% of the farms with MMF housed calves individually. Daily milk allowance per calf by week was higher (wk 1: P < 0.01; wk 2 to wk 4: P < 0.001) on AMF farms compared to MMF. The 4 most important producer-identified factors that motivated producers to switch to automation were to raise better calves, offer more milk to calves, reduce labor, and improve working conditions. For MMF farms, the investment in equipment and in group housing facilities, and farm size were the primary reasons given for maintaining manual feeding methods. To conclude, AMF farms were larger, provided more milk to calves, and use more automation. The adoption of AMF is potentially allowing for improved animal welfare through social of calves and increased access to more milk.
Evaluating the knowledge, attitudes, and behaviours toward radiation safety in the veterinary field and the impact of specific training methods on improving current practices

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Radiation safety practices in Ontario veterinary clinics have not been previously reported and are presumed to be highly variable. Radiation exposure is an important occupational health risk facing veterinary team members. Teaching radiation safety in veterinary school could be an influential way to impact radiation safety practices, however, there have been no studies describing the most effective way to teach students about radiation safety. The current study aims to understand DVM students’ attitudes about radiation safety when they enter the program and to determine if these change following radiation safety training. The first year veterinary students were surveyed at two time points; once before and once after a radiation safety lecture. The survey was conducted online via Qualtrics and was comprised of 32 questions. These were divided into categories aimed to establish 1) students' background in practice prior to entering the program, 2) students' experiences with making radiographs in practice, 3) students' understanding of basic radiation principles. The preliminary results will be presented. This work forms a portion of a larger DVSc project in which third year veterinary students and general practitioner surveys are also planned. From this work, it is anticipated that baseline data regarding current radiation safety practices will be established and that a greater understanding of how radiation safety training impacts veterinary students' attitudes will be gained. This will contribute to the long-term goal of improving teaching methods around radiation safety at OVC and encouraging appropriate radiation safety practices in Ontario veterinary clinics.
Characterization of neural stem/progenitor cells in the body spinal cord of the leopard gecko, Eublepharis macularius

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As for many lizards, the leopard gecko is able to drop its tail as an anti-predation strategy and subsequently regenerate a replacement appendage complete with a functional spinal cord. Research to date has shown that within the tail spinal cord, neural stem/progenitor cells (NSPCs) are present, activated following injury, and appear to contribute to the regenerate spinal cord. Interestingly, less is known about the regenerative potential of the body spinal cord. Our results indicate that NSPCs are present in the ependymal layer of the body spinal cord in the leopard gecko. Using a bromodeoxyuridine (BrdU) pulse-chase experiment we demonstrated that a sub-population of cells in the ependymal layer are label-retaining (slow-cycling) following a 20-week chase. Next, we documented the expression of NSPC markers SOX2 and vimentin among cells of the ependymal layer. In addition, we found that ependymal layer cells constitutively proliferate, as evidenced by their expression of proliferating cell nuclear antigen (PCNA). Intriguingly, a sub-population of ependymal layer cells express pan-neuronal marker HuC/D and may represent a unique sub-population within this layer. Taken together, these data suggest that the regenerative capacity of lizards extends beyond the tail.
Antimicrobial resistance to extended-spectrum cephalosporins in Enterobacteriaceae from dogs in Southern Ontario, Canada

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Resistance to the critically important extended-spectrum cephalosporins (ESCs) in Enterobacteriaceae from animals needs to be further investigated. Besides Escherichia coli and Salmonella enterica, the role of other members of Enterobacteriaceae in the epidemiology of ESC resistance is almost unknown. This project aims to determine the diversity and spread of ESC resistance genes in Enterobacteriaceae species from dogs in Southern Ontario, and to compare these genes between fecal and clinical isolates. From November 2015 to November 2016, clinical isolates are being collected from diagnostic laboratories in Ontario. In addition, ESC-resistant Enterobacteriaceae were isolated from fecal samples collected from healthy dogs in 2016. All ESC-resistant fecal isolates from enrichment (n=86) and clinical isolates (n=223) were confirmed for ESC resistance by Kirby-Bauer disk diffusion. The fecal isolates were screened by PCR for the ESC resistance genes blaCMY, blaCTX-M, and blaSHV. The frequency of ESC-resistant fecal Enterobacteriaceae carriage in healthy dogs was 28.6 ± 8.3% and the frequency of a random E. coli isolate being ESC-resistant in each sample was 3.2%. The blaCMY and blaCTX-M genes were identified in 62.8% (n=54) and 22.1% (n=19) of 86 resistant isolates respectively, with the majority of them being E. coli. Preliminary DNA sequencing results show that the CTX-M variants are the same as those found in poultry and humans in Canada. In clinical isolates, 20 out of 223 (9.0%) were ESC-resistant. These results suggest that there is relatively frequent carriage of ESC-resistant Enterobacteriaceae in dogs in Ontario, including a significant proportion of CTX-M producers.
Evaluation of the Impact of a Dental Prophylaxis on the Oral Microbiota of Canines

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The oral cavity houses a rich and complex bacterial population that likely plays important roles in health and disease. With the adoption of next-generation sequencing, a complete understanding of the canine oral microbiome has emerged. The longer-term effects of dental cleaning on the oral microbiota of dogs have not been studied previously. This study evaluated the impact of dental prophylaxis on the oral microbiota of dogs. 30 healthy dogs from a research colony had plaque and oral swab samples taken just prior to, and one week after a dental cleaning. A subset of 10 dogs was also sampled at two weeks and five weeks post-dental cleaning. A total of 5,984,826 sequences were obtained from 80 plaque samples. The phyla Spirochaetes, Proteobacteria, and Firmicutes predominated at all time points, while Treponema was the most commonly identified genus. Jaccard and Yue & Clayton community indices demonstrate that bacterial membership and structure cluster based on time of collection, and by five weeks after cleaning, it returns to its initial state. LEfSe identified 129 genera that were significantly enriched among the 30 dogs. From 80 oral swab samples, 10,252,679 sequences were obtained. Proteobacteria was the most abundant phyla and maintained a relative abundance above 50% at all time points. Psychrobacter had a 20% relative abundance prior to prophylaxis, while Pseudomonas was most abundant at the one and two weeks time points. Neither genus was present by the end of the study. Population and community analyses indicate the oral microbiota trends back towards a pre-dental state. The results indicate a diverse, but relatively stable oral microbiota in dogs.
A descriptive analysis of MBTI personality types, demographics, and career interests in veterinary students at the Ontario Veterinary College

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With growing awareness that veterinary professionals and students face higher risks of mental health issues compared to the general population, evaluating factors associated with mental health such as personality has become increasingly important. The Myers-Briggs Type Indicator (MBTI) is the most widely used personality instrument in the world and is used by many educational institutions. The MBTI indicates an individual's preferences on four dichotomous scales, including how they process information and make decisions. To date, only one published study has explored the MBTI distribution of veterinary students; the authors reported significant associations between MBTI type and gender, as well as MBTI type and grade point average (GPA).

Our present research investigates associations between MBTI and gender, GPA, veterinary student career interests, and mental health. Eleven years of MBTI, demographic, and lifestyle data from first-year veterinary students have been collected at the Ontario Veterinary College (OVC). Results from regression analyses exploring associations between MBTI, gender, and first year veterinary career interests will be presented. Future analyses will explore associations between MBTI and mental health and GPA data. Preliminary results indicate significant associations of MBTI with both gender and career interests at entry to the program. MBTI distributions of OVC veterinary students also differ significantly from the general Canadian population. The results of this study will help provide insight into how best to improve the learning environments of veterinary students and improve academic support systems throughout their veterinary program.
Epidemiology of ticks collected from pet dogs in an emerging Lyme disease area of Ontario

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In Ontario, Ixodes scapularis is the only known vector for Borrelia burgdorferi, and the geographical range of this tick is expanding. Both humans and their pet dogs are at risk for tick bites and both are susceptible to tick-borne diseases such as Lyme disease and anaplasmosis. The aims of our study were to 1) identify the tick species carried by pet dogs, 2) determine the prevalence of Borrelia burgdorferi and Anaplasma phagocytophilum in I. scapularis collected, and 3) identify risk factors for I. scapularis carriage by dogs.

Working with 20 veterinary practices in eastern Ontario from April to December 2015, we collected 863 ticks from 543 pet dogs, as well as questionnaire data concerning pet demographics and management factors. Seven species of ticks were identified; 97.6% of dogs were carrying at least one of three tick species: I. scapularis, Dermacentor variabilis, or Ixodes cookei. Using PCR, B. burgdorferi and Anaplasma phagocytophilum were detected in 11.4% and 0.7% of I. scapularis. Overall 7.5% of dogs used in the analysis were carrying an I. scapularis tick positive for B. burgdorferi, while only 0.4% of dogs had a tick positive for A. phagocytophilum.

Using mixed-effects logistic regression, I. scapularis carriage relative to other tick species differed significantly by season, geographical region, size and sex of the dog, and with other environmental exposures. These results suggest that the epidemiology of I. scapularis carriage in dogs relative to other tick species is different and that tick prevention may require species-specific strategies.
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