2009 Summer Leadership and Research Program Abstracts

The following abstracts were prepared by students participating in the 2009 Summer Leadership and Research Program at the Ontario Veterinary College.

Analysis of the molecular epidemiology of *bla*_{SHV} in *E. coli* and *Salmonella* from Canadian food animals

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Currently, third generation cephalosporins such as ceftriaxone and ceftiofur are used in human and animal medicine. The use of these antimicrobial agents exerts a selective pressure contributing to increased resistance to β -lactams in Gram-negative bacteria (1). Surveillance of β -lactam resistance in bacteria is necessary to detect emerging resistances which could interfere with the efficacy of these antibiotics in the treatment of human and animal infections. The primary mechanism of resistance to β -lactams is the production of β -lactamases which hydrolyze the β -lactam ring (2). In *Escherichia coli* and *Salmonella enterica* from Canadian food animals, *bla*_{CMY-2} has been the most prevalent gene encoding resistance to third generation cephalosporins (1). However, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) recently identified 18 *E. coli* and 2 *Salmonella* isolates, from abattoir cecal samples and retail meat, in which the *bla*_{SHV} gene encodes extended-spectrum β -lactamases (ESBLs). To confirm the *bla*_{SHV} gene is plasmid-encoded in these isolates, their plasmid DNA was extracted, transferred into a recipient strain by electroporation, and tested for *bla*_{SHV} using PCR. Preliminary results indicate that *bla*_{SHV} is plasmid-borne in the majority of the isolates, but chromosomally encoded in 2 of the *E. coli* isolates. Restriction analysis indicates that the plasmids encoding *bla*_{SHV} are genetically diverse, with some plasmid movement between and within *Salmonella* and *E. coli* populations. The overall diversity of the plasmids encoding *bla*_{SHV}, coupled with our finding of the gene in the chromosomal DNA of some isolates, suggests that the observed mobility of the gene is mediated by both transposons and mobile plasmids. Sequencing of the *bla*_{SHV} gene in each of the isolates, in addition to the area flanking the genes, will be completed to confirm this hypothesis. Susceptibility testing of transformants indicates that while there are variations in the resistance genes carried on the plasmid encoding *bla*_{SHV}, all carry resistance genes for tetracycline. This suggests that, besides a direct selection by

β -lactams, the use of tetracyclines in Canadian farms could be contributing to the recent emergence of *bla*_{SHV} as a resistance determinant in bacteria from Canadian food animals by co-selecting for ESBLs. Future work is necessary to further assess the factors selecting for *bla*_{SHV}, in order to implement appropriate policies to control its dissemination in the bacterial population of Canadian food animals, thereby preventing potential spread to humans through the food chain.

Evaluation of the Alberta Modified Atmosphere Chamber for humane depopulation of commercial poultry

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Determining humane, efficient, safe, and economically viable methods for depopulation of commercial poultry flocks is an important emerging issue. Various methods have been proposed to deal with mass depopulation of poultry for foreign animal disease outbreaks and for on-farm depopulation of spent laying hens. The Alberta Modified Atmosphere Chamber (AB-MAC) is a portable unit holding up to 650 birds that uses carbon dioxide (CO₂) to create hypercapnia, a condition leading rapidly to unconsciousness, cardiac arrhythmias, and brain death. The objectives of this study were to evaluate chamber operation, animal well-being, and personnel safety during use of AB-MAC for spent hen depopulation in a controlled field setting. Candidate gasses including CO₂, argon/CO₂ and nitrogen/CO₂ gas combinations were first evaluated by pumping them into the empty AB-MAC at consistent flow rates. CO₂ and O₂ concentrations were measured at various heights within the chamber to determine if any of these gasses or mixtures would result in appropriate CO₂ and O₂ levels leading in rapid unconsciousness and death, as determined from previous studies. Only 100% CO₂ was found to be suitable with this chamber. During in vivo studies, hens surgically instrumented for telemetry were lowered into the gas-primed chamber, and time to unconsciousness, onset of cardiac

arrhythmias, and brain death were recorded. Results from these trials indicate that the chamber can be used to rapidly stun and kill hens; however, current field loading rates likely exceed the time required for initial stunning, particularly at the middle and top of the chamber. These findings will be used to provide regulatory authorities and industry with recommendations for routine use of the AB-MAC.

Effects of Ketoprofen (Anafen®) on ketosis and lying behaviour following left displaced abomasum surgery in Holstein dairy cows

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In Ontario, left displaced abomasum is typically corrected surgically. Practitioners rely on local anesthetics $\pm$ alpha-2 agonists to provide analgesia during surgery. However, it is not common practice to administer non-steroidal anti-inflammatory (NSAID) therapy for the alleviation of post-surgical pain. The objective was to determine the effect of Ketoprofen (Anafen®) administration on beta-hydroxybutyrate (BHBA) levels as well as on the lying behaviour for the 8-10 day period following surgery. Approximately 200 cases from four Southern Ontario clinics will be assigned to 1 of 2 treatment groups: (1) Anafen® (3mg/kg or 1.5mL/100lbs IM), (2) saline at an equivalent volume IM, pre- and 24hrs post-surgery. Whole blood samples, taken pre-surgery and at 2-4 and 8-10 days, were tested for BHBA levels using the Precision Xtra® handheld device. A HOBO® Pendant G Data Logger was placed at the time of surgery on the distal metatarsal to measure daily lying time as well as the lying side. Preliminary data are available from 76 animals. The BHBA levels at 2-4 and 8-10 days were significantly lower than at time of surgery for both treatment groups ($P < 0.05$). In this interim analysis there appear to be no differences in BHBA between the two treatment groups. There were no differences in the average amount of time per day spent lying versus standing. Although the data are sparse there may be differences in the patterns of lying on the right (surgical site) versus left side over time.

Determination of myocardial strain by speckle track echocardiography in Doberman Pinschers

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Background: Dilated cardiomyopathy (DCM) is a progressive, fatal disease with a incidence of up to 40% in Doberman Pinschers. Determining the usefulness and improving the accuracy of prognostic parameters is important in both identifying at-risk dogs before disease development, and in predicting individual disease progression rate. In particular, strain (St) and strain rate (SR)

have recently been shown to quantify myocardial function in dogs. Speckle tracking echocardiography (STE) is a recent development in imaging that may provide more accurate values than traditional echocardiography. This study proposed to evaluate St and SR measurements determined by STE in normal (NL) Dobermans and Dobermans with occult DCM (occDCM) and overt DCM (oveDCM). **Hypothesis:** It was predicted that STE St and SR parameters would differ between NL, occDCM and oveDCM dogs, and that differences within the groups would be associated with time to outcome for occDCM and oveDCM dogs. **Methods:** Criteria for classifying dogs as normal or DCM-affected were developed based on previous studies. As a retrospective study, echocardiographic measurements and case summaries were compiled for 112 client-owned Doberman Pinschers (NL: n=44; occDCM: n=39; oveDCM: n=29). EchoPAC Dimension (GE Healthcare) software was used to analyze STE St and SR for all dogs. Statistical analysis will be performed using JMP 7.0.1. Confounders will include medication dosages and durations, concurrent diseases and select biochemical indicators. **Results:** pending. **Conclusions:** pending.

A Meta-Analysis review of the efficacy of various common antimicrobials on *Actinobacillus pleuropneumonia*

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Actinobacillus pleuropneumonia (APP) causes Porcine Pleuropneumonia, a respiratory disease affecting continuous flow herds in the swine industry. A quantitative analysis was performed on 36 articles acquired from a systematic review to determine the efficacy of common antimicrobials in prevention of mortality caused by APP. The analysis included a meta-analysis, stratified meta-analysis and tests to determine heterogeneity, presence of influential studies and publication bias. Analysis included antimicrobials; ceftiofur, tulathromycin and oxytetracycline administered by injection, tiamulin administered by water, as well as tilmicosin and florfenicol administered by feed. Evidence of heterogeneity ($p < 0.05$) exists among ceftiofur results. Stratified investigation revealed study-level characteristics such as age of study animals, treatment dosage etc. contributed to the between study variance. Tau-squared values were highest for florfenicol (0.40), followed by oxytetracycline (0.35) and then ceftiofur (0.27). Ceftiofur had some degree of publication bias. Studies for ceftiofur and tulathromycin included influential treatment groups due to ineffective treatment intervals. Tilmicosin had one influential treatment group due to low baseline group risk. Oxytetracycline also included an influential treatment group from a study which involved comparison of oxytetracycline to long-acting oxytetracycline at a treatment interval where oxytetracycline was ineffective. On the basis of size of the relative risk for

each antimicrobial and examination of additional components of the analysis, it is determined that ceftiofur (RR=0.35), tulathromycin (RR=0.33), oxytetracycline (RR=0.29), tiamulin (RR=0.32), tilmicosin (RR=0.20) and florfenicol (RR=0.14) are all efficacious in prevention of mortality caused by APP. Results should also be used in combination with economic analysis so that producers know which antimicrobial is most cost effective.

Characterization of gender-related differences in mesenteric vessels of male and female rats using computer-assisted video microscopy

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We investigated gender-related differences in vascular reactivity in the mesenteric veins of normal male and female rats using exogenous application of vasoactive agents: potassium chloride (KCl: 20-120mM), endothelin-1 (ET-1: 1×10^{-11} – 3×10^{-8} M), and sarafotoxin 6c (S6c: 3×10^{-11} – 1×10^{-8} M). Changes in diameter of mesenteric veins (150-300 μ M diameter) were measured *in vitro* using computer-assisted video microscopy. Contractile responses to KCl were observed to be greater in females than in males; but, significant difference was not achieved. Contractile responses to ET-1 and S6c were not different between males and females. Contributions by endothelial-mediated dilators were eliminated via pretreatment of mesenteric veins with nitric oxide synthase inhibitor, nitro-L-arginine (NLA: 100 μ M), and cyclo-oxygenase inhibitor, indomethacin (indo: 1 μ M). Blockade of endothelial-mediated dilator release did not significantly change ET-1 contractile responses when compared to control responses; but did significantly contribute to S6c contractile responses. Furthermore, in the presence of NLA and indomethacin, female rats were observed to be more sensitive to S6c in comparison to males. In summary, no significant gender-related differences in vascular reactivity in the mesenteric veins of normal rats were detected in this study.

***In vitro* characterization of osteosarcomal development**

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Osteosarcoma (OSA) is the most common type of bone tumor in dogs. It is a mesenchymal tumour thought to arise from transformed osteoblasts or pre-osteoblasts. Once diagnosed with this disease, the prognosis is generally poor because metastases are frequently already present. Metastases are commonly found in the lungs and bone, but

can also be found in kidneys, liver and other organs. The aim

of this study was to establish a range of cell lines to represent different stages of OSA (primary tumour and metastatic tumours). The establishment of cell lines will serve as a resource for studies investigating gene expression in OSA and will also facilitate early pre-clinical drug trials for novel therapeutic in OSA. Our approach to establish cell lines was to obtain very fresh clinical tumour samples and derive cells from the explants. Tumor material was retrieved from four dogs referred to the Ontario Veterinary College Small Animal Hospital for either limb amputation or euthanasia and necropsy. So far cells have been successfully grown from explants of a primary OSA tumour from a Greyhound and a metastatic tumor found in retroperitoneal fat of a Rottweiler. More cases are anticipated in the future to add to this collection. Once enough cells are grown from each explants, several clones will be derived and characterized for their *in vitro* behaviour and genetic characteristics.

Amanation and evaluation of markers for assessing early embryonic health

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Early embryogenesis, as a critical period during development, is a great determinant of embryonic health and reproductive efficiency. However, early embryonic mortality (EEM) in humans has been estimated to be anywhere between 30 to 62% and can account for up to 75 to 80% of all embryonic and fetal deaths. The cause of this embryonic loss has been attributed to the lack of specific cues from specific gene in a spatiotemporal manner. Previous studies from our laboratory have isolated p66Shc, TERT, Caspase-3 and H2A.X as candidate genes of interest for assessing embryonic quality. Therefore, the objective of the present study was to ascertain if p66Shc, TERT, Caspase-3 and H2A.X can be good predictors of embryonic health. *In vitro* bovine embryos produced using standard *in vitro* embryo production techniques were harvested at timed stages of development and categorized into healthy or unhealthy embryos (showing fragmentation, vacuolization or fading) based on their morphological appearance. The embryos were fixed in 2% paraformaldehyde and stained for the proteins of interest using standard immunofluorescent staining protocol. Our study showed that Caspase-3 and p66Shc co-localization suggested lower survivability while Caspase-3 staining during early stages of development was unreliable as a marker. TERT localization was highly differential across preimplantation embryo stages while p- γ -H2A.X foci, present at the double stranded DNA break sites showed a direct correlation between its expression and unhealthy embryos. Future studies in this area include quantitative analysis of proteins and live cell imaging of these marker molecules.

Expression of EqIFN- β 1, EqIFN- β 2 and MX1 in normal and compromised early pregnancy in mares

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Early embryonic loss causes significant economic loss in the equine breeding industry. In early pregnancy in mares, the mobile conceptus undergoes fixation after Day 15 but the pregnancy recognition signals involved are unknown. Pregnancy recognition in ruminants involves interferon tau (IFN- α), which horses do not have. However, two interferon delta genes (EqIFN- β 1 and EqIFN- β 2) are expressed in the endometrium and trophoblast during early pregnancy in horses. In these studies, we examined how IFN- α is involved in early pregnancy in the mare, by analyzing endometrial expression of MX1, an IFN responsive gene, and both IFN- α genes. Conceptuses and endometrial biopsies were collected from pregnant mares on Days 15 to 18 after insemination. Three or four days prior to collection, half the mares were treated with synthetic prostaglandin to induce luteolysis and pregnancy failure. Samples from the current and recent breeding seasons were stored in liquid nitrogen before RNA extraction. Gene expression was analysed by qualitative and semi-quantitative RT-PCR, relative to β -actin. Endometrial expression of both IFN- α genes and MX1 was evident during early equine pregnancy. However there were no significant changes during Days 15 to 18 in normal pregnancy, and expression was similar in normal or compromised pregnancies. These studies show that IFN induced MX1 expression in the endometrium during early pregnancy in mares is much lower than has been reported in ruminants. The results therefore suggest that in mares interferons lack a major signalling role in pregnancy recognition or in luteolysis-associated pregnancy failure, leaving their role during early pregnancy unknown.

The role of wives in the Veterinary profession during the mid twentieth centuryKatie Anderson¹, Elizabeth Stone², Catharine Wilson¹¹*Department of History, College of Arts and* ²*Dean, Ontario Veterinary College*

During the mid twentieth century, few professional occupations, including the veterinary profession, considered women as appropriate candidates for employment. However, while few women prior to 1980 successfully enrolled into veterinary college to become certified practitioners, wives of veterinarians made significant contributions to the profession during this period. The purpose of this investigation is to reveal, define, and recognize the role of wives of veterinarians during mid twentieth century in Canada and the United States. Oral history interviews conducted throughout Southern Ontario have begun to reveal that the contributions

by wives of veterinarians, whose husbands graduated from the Ontario Veterinary College between 1940 and 1980, can broadly be categorized into two areas. Wives supported their husbands and the profession based on their husbands' career choices, therefore wives of veterinarians in small or large animal practices often assisted within the clinic, primarily if it operated from the home. Specifically this included assisting with surgery, book keeping, receptionist duties, and purchasing and stocking of supplies. However, for veterinarians who chose academic occupations, many wives organized themselves into official auxiliary groups to the veterinary profession at the local, regional, national, and international level. These groups supported the profession through public relations, raising money for scholarships, and promoting fellowship within the veterinary profession. Future work on this project intends to compare and contrast between the wives of OVC graduates from the 1940s, 1950s, 1960s, and 1970s, to examine how an increase in opportunities for women impacted the contributions made by wives of veterinarians.

Behavioural changes of dairy cows during drying-off using abrupt cessation of milking

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Drying-off high-producing dairy cows with abrupt cessation of milking could be a welfare concern. From May to July 2009, a total of 44 cows were evaluated for behavioural changes during the drying-off period. The objectives of this study were to document changes in standing and lying activity during drying-off, and to evaluate the associations between parity (lactation 1 relative to lactation 2 and above) and production level (> 24 kg/d compared to < 24 kg/d) and changes in these activities. Cows from one commercial free-stall herd (Herd 1) and two tie-stall dairy research facilities (Herds 2) were fitted with HOBOS[®] data loggers to record lying and standing activity from two days preceding to six days following drying-off. High production cows showed a reduction in lying time from d 0 (drying-off) to d 1, with d 1 lying time significantly different between production levels ($p < 0.05$). High production cows also exhibited a significant decrease in the number of standing bouts per day, compared to low production cows ($p < 0.05$) for d 0, 1 and 2. Similarly, lactation 1 cows showed a reduction in lying time from d 0 to d 1, followed by increased activity at d 2. Lactation 1 cows also showed a decrease in standing bouts from d 0 to d 1. However, a limited number of lactation 1 cows were available for this preliminary study. In conclusion, these preliminary data suggests that abrupt cessation of milking may affect standing and lying behaviour, and that these changes may be influenced by production level and parity. Continued enrolment and further analysis of this data set is warranted.

Prognostic indicators following trauma in dogs

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Objective: To determine whether the type of trauma, injuries sustained, physical examination findings, or laboratory values recorded on presentation to a veterinary teaching hospital following trauma are associated with outcome.

Procedure: A retrospective study reviewing the medical records of traumatized dogs presenting within 24 hours of injury between January 2007 and December 2008. Signalment, vital signs, laboratory values, and outcome were recorded. The ability of each parameter to predict death was evaluated using AUC analysis. **Results:** Ninety canine trauma cases were identified including 82% blunt (n=74) and 18% penetrating (n=16) traumas. The median (range) duration of ICU and hospital stay was 3 (1-14) days. The mortality rate was 16% (n=14) of which 14% died (n=2) and 86% were euthanized due to a grave prognosis (n=12). The following parameters were associated with mortality at a level achieving statistical significance ($p < 0.05$): Modified Glasgow Coma Scale score, ionized calcium, albumin, lactate, heart rate, transfusion requirement, creatinine, Acute Trauma Triage score, packed cell volume, systolic blood pressure, bicarbonate, potassium, and the presence of abdominal trauma. Ionized calcium was of equivalent predictive value to lactate and heart rate for assessing mortality risk. Temperature, respiratory rate, mean arterial pressure, presence of arrhythmias, partial pressure of venous oxygen, carbon dioxide, pH, sodium, glucose, total solids, activated clotting time, and type of trauma were not associated with mortality in this group of dogs. **Conclusion:** Many clinical and laboratory findings following trauma may be used to predict outcome. These prognostic indicators may assist clinical assessment and warrant further investigation.

The association between selenium administration and selenium status in neonatal calves

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Selenium is an important nutritional element for health and performance and is commonly supplemented in the ration, by injection prior to calving, or systemic administration to the newborn calf. Large variation of selenium levels in neonatal calves exists between herds, with many producers not meeting recommended levels. The study objectives were to quantify the variation of serum selenium of neonatal heifer calves, determine the selenium levels in close-up dry cow total mixed rations (TMR) between herds, survey selenium supplementation methods on Ontario farms, and to correlate serum selenium levels in neonatal calves with TMR selenium levels and selenium supplementation methods. 272 heifer calves, 1-7 days of age, were enrolled from 32 commercial dairy farms in Ontario, from May-July of 2009. Blood

samples and a sample of the close-up dry cow TMR were collected and analyzed for serum selenium concentration and selenium levels, respectively. A herd level questionnaire was used to determine the selenium supplementation methods used on participating farms. Considerable variation was present in both the mean serum selenium and feed selenium levels between herds. Most commonly, selenium is supplemented in the cow's diet. However, the most effective, but least common method was to inject calves at birth. The correlation between mean serum and feed selenium levels proved to be weak, likely due to the large amount of variables involved with providing selenium to neonatal calves. 11% of enrolled calves had deficient selenium levels, 79% had marginal selenium levels and 10% had the recommended levels of selenium. The proportion of selenium deficient calves confirms that selenium deficiency is still a problem needing attention.

Mutational analysis of the transforming domain of the enzootic nasal tumour virus envelope protein

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Enzootic nasal adenocarcinoma (ENA) is a contagious neoplastic disease affecting the upper respiratory system of sheep and goats. ENA is characterized by the transformation of nasal secretory epithelial cells leading to tumour formation in the ethmoid turbinates. Experimental transmission of ENA using nasal fluid from naturally occurring cases has been described, illustrating the contagious nature of ENA. A retrovirus known as enzootic nasal tumor virus (ENTV) is consistently found in the nasal tumours of animals with ENA, and the envelope protein of ENTV (Env) has been demonstrated to have transforming activity. The Env protein is thought to cause a disruption in the normal cellular signalling of the host cell, leading to uncontrolled cellular proliferation and ultimately tumour formation. Mutational analysis of the cytoplasmic tail of Env was conducted to elucidate the contribution of individual amino acids to the tumorigenic properties of this oncogene. The cytoplasmic tail of the ENTV Env contains three conserved tyrosine-based YxxØ motifs; where Y stands for tyrosine, X for any amino acid, and Ø for a bulky hydrophobic residue. This tyrosine-based motif has been implicated in a number of different cellular processes including endocytosis, intracellular trafficking, basolateral sorting, and signal transduction. To determine whether these YxxØ motifs are important for transformation, the three amino acids in the Ø positions, as well as two others thought to be directly involved in signal transduction, were mutated to alanine and to isoleucine. When the amino acid at the Ø position was mutated to the small, less reactive amino acid, alanine, transformation was significantly inhibited. Conversely, mutation to a bulkier hydrophobic amino acid increased transformation. These results suggest that the Ø positions of

the ENTV Env seem to function as binding sites for intracellular molecules that induce endocytosis and/or impact localization and trafficking of the Env protein and that the bulkier the amino acid the more active the motif.

Central expression of CRHR and BDNF in SD rats housed in different environmental conditions

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Adverse stress in rats results in modulation of the HPA axis, including alterations in corticotrophin-releasing hormone (CRH) expression, as well as its receptors (CRHR). This has implications for all types of research as well as raising concerns regarding animal welfare. Suboptimal rodent housing has received increasing scrutiny as it may be a source of chronic stress. Short term studies suggest that rats have a preference for group housing; however, this has not been studied long term, which may be more relevant to typical experimental housing conditions. Indicators of acute stress in rats include increased levels of CRHR within the pituitary gland. In this study, male and female SD rats were housed in several paradigms: alone (n=8), in pairs (n=8) or in isosexual groups of 4 (n=4) with additional cage enrichment for a 5-month duration. At study conclusion, brains were harvested and pituitary glands were isolated and placed in RNase Later for subsequent mRNA evaluation. For CRHR analyses, the anterior pituitary glands were homogenized and RNA extracted for cDNA synthesis via RT-PCR, using 1 µg of total RNA. Relative quantitative analyses were conducted using real-time GAPDH mRNA expression as the reference gene. Significant differences in CRH receptor expression were observed between paired and group-housed female rats, single and paired male rats, paired and group-housed rats (except for CRHR2ins), and single and group-housed rats (for CRHR1). These data suggest that female rats are optimally housed in groups of 4, while pair housing is preferable for male rats.

Occurrence and antimicrobial resistance of *M. haemolytica* isolated from Alberta feedlot cattle over a two year period

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Mannheimia haemolytica constitutes part of the normal commensal flora of the upper respiratory tract of beef cattle, but can cause disease such as shipping fever in immune-deficient animals. This veterinary pathogen has generally been susceptible to most antimicrobials, but acquisition of antimicrobial resistance (AMR) would increase the cattle industry's healthcare-related burden as well as pose a potential risk to public health through resistance gene transfer to human pathogens. As such, a three year multi-

partner surveillance pilot program has been implemented in four southern Alberta feedlots to monitor AMR in a number of bacterial species of interest, including *M. haemolytica*. The objective is to phenotypically describe AMR of public health interest in *M. haemolytica* from feedlot cattle during two years (2007-2008) of the program. Standard culture methods were employed for the isolation of *M. haemolytica* from deep-guarded nasopharyngeal swabs used to collect samples from animals upon arrival and thirty days pre-slaughter. One isolate per positive sample was tested for susceptibility to a panel of fifteen antimicrobials of public health interest using the Sensititre broth microdilution system (TREK Diagnostics, Cleveland, OH). Overall, AMR was found to be low, with <3% of isolates demonstrating resistance towards any single antimicrobial tested with established or provisional breakpoints. Continued AMR surveillance in *M. haemolytica* will aid in the detection of emerging AMR problems from both a human and animal perspective, and may assist in the development or modification of antimicrobial use protocols in food animal production and veterinary medicine designed to mitigate the development and spread of AMR.

Role of thyroid hormones in early embryonic development

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Assisted reproductive technologies rely heavily on successful development of the pre-implantation embryo. Currently *in vitro* embryo production yields a blastocyst rate of a mere 30-40%. Many efforts are consecrated on improving these rates by replicating *in vivo* physiological conditions during culture. Thyroid hormones are ubiquitous in the body and play important roles in various cell functions. Our lab has identified thyroid hormones in the bovine female reproductive tract, suggesting that addition of these hormones to the media could potentially play a role in early embryonic growth. In order to test this hypothesis, thyroid hormones (either T3, T4, both or neither as a control) were added to the culture media at a concentration of 50ng/ mL. No significant difference was noted between the groups in terms of cleavage rates or blastocysts rates. However, preliminary data suggests improved quality of embryos treated with T3 compared to other groups i.e. the percentage of higher quality blastocysts out of total in the T3 treated group was higher than the remaining groups. In order to elucidate the mechanism of thyroid hormone action, polymerase chain reaction (PCR) was performed to determine gene expression for thyroid hormone receptors and deiodinases. Embryos treated with thyroid hormones express thyroid hormone receptors, unlike untreated embryos which do not. Initial results for deiodinase PCR show that deiodinase enzymes are not present in the embryo, suggesting an inability to assimilate T4 into T3. In

conclusion, this study demonstrates that supplementation of thyroid hormone to the culture media of preimplantation embryos helps in getting better quality blastocysts, however further studies are required to ascertain their mechanism of action. Research support: NSERC, Ontario Veterinary College

Does embryonic estrogens affect the mare's endometrium?

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Early pregnancy losses in mares are costly and frustrating for horse owners and breeders. The maintenance of pregnancy depends on dialogue between the mare and her conceptus (embryo + membranes). The conceptus produces steroid hormones, including estradiol (E2), which probably participate in the dialogue. The ability to combat loss requires an understanding of the dialogue. Knowing that expression of interferon δ (IFN- δ) in endometrium is pregnancy-specific, we hypothesize that E2 secreted by the conceptus has a direct effect on endometrium, demonstrable in vitro through analysis of IFN- δ expression. Five mares were used on 8 occasions to collect endometrial biopsy samples and jugular blood on days 11 - 14 after ovulation which was diagnosed by transrectal ultrasonography. Biopsy samples were cultured for 24 h in the presence or the absence of E2 (5 or 50 ng/ml) by the method of Meadows et al. (Biol Reprod Mono 1, 171-180, 1995). Tissue was harvested at 4 h and 24 h for mRNA was extraction using the Qiagen RNeasy mini kit and cDNA was synthesized with the Invitrogen ThermoScript™ RT-PCR System. Semi-quantitative real-time PCR analysis was performed in duplicate for: IFN- δ 1, IFN- δ 2, myovirus resistance-1 (an IFN-stimulated gene). Gene expressions were obtained for the 50ng E2 series (full analysis pending; 5ng E2 series not yet run). Although the testing of the hypothesis is not yet complete, the system of endometrial culture followed by analysis of gene expression shows promise as a means of investigating the putative influence of conceptus-produced steroids on endometrial function.

Clinical, histopathologic and immunophenotypic features of feline extra-intestinal lymphoma.

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Lymphoma is the most common hematopoietic neoplasm in cats, however, except for intestinal lymphoma, the histopathologic and immunophenotypic characteristics are incompletely defined. Further, neither the frequency of retroviral infection in cats with lymphoma nor the responses to therapy or survival times are well characterized. In this study, 89 cases of feline lymphoma, affecting primarily

tissues other than the intestine, were retrieved from hospital and pathology databases. For each case, clinical data were compiled, and sections of tumor tissue were evaluated by routine histopathology and after immunohistochemical staining with antibodies to CD3 and CD79a. Ages of affected cats ranged from 0.7 to 18.0 years and included 33 females and 56 males. Serologic testing indicated feline leukemia virus or feline immunodeficiency virus infection in 7.9% and 3.4% of cases, respectively. The most common anatomic location was multicentric (n=33), followed by gastric (n=13), mediastinal (n=11) and renal (n=7). Histopathologically, the tumors were classified as diffuse small lymphocytic (n=20), lymphoblastic (n=19), immunoblastic (n=18), diffuse large (n=11), diffused mixed (n=7), centroblastic (n=5), diffuse small cleaved (n=5) and follicular (n=4). Immunohistochemistry indicated B- and T-lymphocyte origin in 53 and 36 cases, respectively. The mean survival time of cats treated with chemotherapy for lymphomas with B-lymphocyte origin was 15.7 months, and for T-lymphocyte origin was 19.0 months, after diagnosis. Unusual microscopic findings included an immunoblastic histopathology appearance of tumor cells corresponding to T- rather than B-cell origin, and apparent follicular formation of some lymphomas in hepatic parenchyma. Results suggest that in cats B-cell lymphoma is more common than T-cell lymphoma, that B-cell origin prevails at specific sites such as the nose and stomach, and that retroviral infection is not a common feature of contemporary feline lymphoma.

The prevalence and antimicrobial resistance of *Escherichia coli* and *Salmonella* in Canadian spent layer hens

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Non-typhoidal salmonellosis is the second most common bacterial enteric illness in Canada. It is associated with consumption of contaminated food. Eggs are one of the most commonly identified sources. Infection by a *Salmonella* strain with antimicrobial resistance (AMR) can result in treatment failure, a longer course of illness and a greater risk of adverse outcome. Since 2002, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has monitored the prevalence and AMR of *Salmonella* and generic *Escherichia coli* in broiler chickens at slaughter, but not in retail table eggs. Monitoring of retail table eggs can be time-consuming and resource intensive since the per-egg prevalence of *Salmonella* is very low. A previous study at the University of Guelph looked at pre-pasteurization liquid whole eggs from egg breaking stations. One problem posed by this study was that many egg breaking stations accept eggs not destined for the table market, as well as eggs from the United States. Another alternative to sampling table eggs for assessing the

occurrence and resistance of *Salmonella* and generic *E. coli* in the laying industry is to sample laying hens at slaughter. This is done by collecting cecal samples from Canadian spent layer hens, testing them for *E. coli* and *Salmonella*, then serotyping and creating AMR profiles for the positive isolates. The prevalence of *Salmonella* and *E. coli* in Canadian spent layer hens is 24% and 97% respectively. AMR results show that multi drug resistance occurs in 49.6% of *E. coli* isolates, with 3.5% being resistant to 5 or more antimicrobials. It also shows 60% of *Salmonella* isolates are resistant to 1 or more antimicrobials. These results are similar to those found in the aforementioned liquid whole egg study. Given the similarity to the findings from the liquid whole egg study, Canadian spent layer hen sampling can be viewed as an acceptable alternative to testing the consumer risk for retail table eggs.

Actinobacillus suis pathogenomics

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Actinobacillus pleuropneumoniae and *Actinobacillus suis* are similar bacterial pathogens of swine. They cause acute hemorrhagic pleuropneumonia, produce many of the same virulence factors, and have very similar colonial morphology and biochemical properties. *A. suis*, however, has a broader host range than *A. pleuropneumoniae* and can cause septicaemia, enteritis, meningitis, arthritis, skin lesions, and abortion as well as pneumonia. By sequencing the *A. suis* genome, and comparing it with existing *A. pleuropneumoniae* genome sequences, we hope to identify genetic differences and begin to explain the contrast in their tissue and host specificity. The genome was sequenced using a 454 pyrosequencing method and forty-two contigs totaling 2.4 Mb were assembled; this was consistent with initial estimates of 2.3 Mb obtained by pulsed field gel electrophoresis. The contigs were first ordered by paired end sequencing then aligned with an *Af/II* optical map except for some smaller fragments, which are difficult to place precisely because they have very few restriction sites. Using these two approaches twenty contigs could be ordered and thirteen gaps were closed by long range PCR. The positions

of the ribosomal operons were predicted to be between sections of assembled sequence. Preliminary *in silico* analysis was done using Mauve and BLAST searches. The sequence of *A. suis* is very similar to that of *A. pleuropneumoniae*, but large segments of the genome are rearranged. The *A. suis* genome also contains unique regions compared to *A. pleuropneumoniae* that require further investigation, and further analysis is required to study genes of interest.

Effect of oxytetracycline treatment on papillomatous digital dermatitis in dairy cows

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Papillomatous digital dermatitis (PDD) is a contagious and painful disease of dairy cattle. The objective of this study was to determine if application of oxytetracycline in a paste would be as therapeutically effective in the treatment of PDD as the powdered form held in place by a bandage. Cows diagnosed with PDD during routine trimming were randomly assigned to one of three treatment groups, a paste group, a wrap group and a negative control group. Paste was composed of oxytetracycline 1000 with glycol and vinegar to allow for adhesion, while wraps held powdered oxytetracycline 1000 against the lesion and were removed 3 days post treatment. Twenty six cows were included in the trial and data were analyzed in a pairwise fashion using the Fisher's exact test. Evaluation on days 3-7 showed no significant difference between the three treatment groups ($p=0.49$). However, evaluations done on days 8-12 demonstrated a significant difference ($p<0.001$) in the proportion of active lesions, with paste and wrap groups having fewer active lesions than the control group. Oxytetracycline is effective for the treatment of PDD and the use of it in a paste form rather than a powder alone could eliminate the need for bandage application and subsequent removal. From an industry perspective this is useful as it eliminates the need of the producer to remove a bandage 3-5 days post treatment, and could make treating PDD less labour intensive.