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# Graduate Student Research Symposium & Schofield Lecture

Conference Proceedings  
November 16, 2011

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# Introduction

The ninth annual Ontario Veterinary College Graduate Student Research Symposium provides an opportunity for graduate students at the OVC to come together and celebrate their accomplishments. This year, we have the opportunity to showcase a wide variety of cutting-edge research here at the OVC. We hope you enjoy the presentations that OVC graduate students have prepared with the support of their advisors and colleagues.

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# Graduate Student Research Symposium Schedule

<b>Time</b>	<b>Event</b>	<b>Location</b>
10:00 a.m. – 6:30 p.m.	Posters displayed	OVC Lifetime Learning Centre (LLC) 1707 B&C (Cafeteria)
10:30 a.m. - 12:00 p.m.	Posters staffed by graduate students	
12:00 p.m. - 1:00 p.m.	Oral presentations: Session I	OVC LLC 1713 and 1715
1:00 p.m. - 1:15 p.m.	Break	
1:15 p.m. - 2:15 p.m.	Oral presentations: Session II	OVC LLC 1713 and 1715
2:15 p.m - 2:30 p.m.	Break	
2:30 p.m. - 3:45 p.m.	Oral presentations: Session III	OVC LLC 1713 and 1715
4:00 p.m. - 5:00 p.m.	Schofield Lecture	OVC LLC 1714
5:00 p.m. - 6:00 p.m.	Reception and Awards Ceremony	OVC LLC 1707 A (Cafeteria)

## Schofield Lecture

### “The Continuum of Cancer Care in Veterinary Medicine”

Dr. Page received his DVM from Colorado State University and completed specialty training in the field of medical oncology at the Animal Medical Center in New York City. He is board-certified in Internal Medicine and Oncology. He was a faculty member at North Carolina State University for 15 years prior to his appointment at Cornell in 1999. Dr. Page served as the founding director of The Sprecher Institute for Comparative Cancer Research at Cornell University and in 2005 he was appointed Chair of the Department of Clinical Sciences. He returned to Colorado as the Director of the Flint Animal Cancer Center in 2010. He has authored or co-authored about 115 peer-reviewed manuscripts, 30 book chapters and co-edited one textbook.

The primary focus of Dr. Page’s research has been the characterization of large animal models of cancer. Although his research group uses in vitro systems as well as conventional laboratory animal systems, characterization of spontaneous animal tumors in companion animals is their primary model of interest. The scientific value of large animal models relates to the extended sampling and imaging opportunities to address hypotheses not feasible in rodent models. Promising new treatments identified in conventional animal models can be expeditiously refined for human clinical studies in such “intermediate” models.

In the last several years Dr. Page has been involved with the development of a large observational, lifetime study in dogs to define current cancer incidence and characterize the lifestyle, diet and environmental exposures potentially associated with development of cancer and significant health problems in dogs.



**Dr. Rodney Page DVM, MS, DACVIM**  
**Director, Flint Animal Cancer Center**  
**Professor of Medical Oncology**

College of Veterinary Medicine and Biomedical Sciences  
Colorado State University

## Poster Displays – Room 1707 B & C

1	Keating, S	DVSc	Clinical Studies	Effects of Acepromazine or Dexmedetomidine on Cardiopulmonary Performance During Recovery from Fentanyl/Isoflurane Anesthesia in the Dog
2	Park, F	DVSc	Clinical Studies	Assessment of hypercoagulability in canine pituitary-dependent hyperadrenocorticism
3	Snyman, H	DVSc	Pathobiology	Constitutive variation in hepatic expression of innate immune genes in healthy pigs
4	Stasiak, I	DVSc	Pathobiology	The Role of Hepcidin in Regulation of Iron Balance in Bats: A Pilot Study
5	Sunohara-Neilson, J	DVSc	Pathobiology	Intranasal infection of SPF New Zealand White rabbits with leporid herpesvirus-4, a novel alphaherpesvirus of domestic rabbits
6	Brady, E	MPH	Population Medicine	Dog Tales: Digital Storytelling in Two First Nations Communities
7	Gagne, A	MPH	Population Medicine	Review of Canadian Information on Antimicrobial Use and Resistance in Selected Beef Cattle Pathogens
8	Kim, M	MPH	Population Medicine	Developing a Cultural Food Safety Program in Peel Region
9	Koziarski, R	MPH	Population Medicine	Guidelines for Effective Narrative Use in Health Communication Programs
10	Leung, Z	MPH	Population Medicine	EcoHealth and One Health in Ontario: Lessons Learned and Possibilities for Action
11	Azizzeddin, A	MSc	Biomedical Sciences	Sperm swim-up separates X and Y-spermatozoa
12	Barber, A	MSc	Biomedical Sciences	The role of MLH1 and MSH2 expression on tumour growth and drug resistance in human colorectal cancer cells
13	Buechli, M	MSc	Biomedical Sciences	Enhanced Chondrogenic Differentiation of Equine Umbilical Cord Blood-Derived Mesenchymal Stromal Cells in Membrane-Based Cultures
14	Caudle, J	MSc	Biomedical Sciences	Expression of Y chromosome linked genes in bovine pre-implantation embryos
15	Delaney, L	MSc	Biomedical Sciences	Effect of DCA treatment on Bcl-2 family protein expression in colorectal cancer cells
16	Fitzpatrick, C	MSc	Population Medicine	The Objective Assessment of Pain in Dairy Cattle With Clinical Mastitis Through the Use of Pain Pressure Algometers and Rumination Collars
17	Gallienne, J	MSc	Pathobiology	Type I and Type II Cytokine Production in Ontario Dairy Cows
18	Hosein, A	MSc	Clinical Studies	Prevalence of Tritrichomonas foetus in cats in Southern Ontario
19	Kerr, A	MSc	Biomedical Sciences	Chronic, low-grade, systemic inflammation enhances the progression of epithelial ovarian cancer (EOC) in vivo
20	Kim, D	MSc	Biomedical Sciences	Regulation and role of cytochrome P450 2A5, CYP2A5, in protection against bilirubin toxicity
21	Lepage, V	MSc	Pathobiology	'Redtail': bacterial dermatitis in seahorses
22	Martin, C	MSc	Pathobiology	Kinetics of type-I and type-II gene expression, cytokine production and cell proliferation in dairy cattle
23	Medhanie, G	MSc	Population Medicine	The epidemiology of pathogenic and non-pathogenic brachyspira species in Ontario layers
24	Mehdizadeh Gohari, I	MSc	Pathobiology	The possible role of Clostridium perfringens in equine typhlocolitis

25	Oluwole, O	Msc	Biomedical Sciences	Sertoli cell proliferation and thyroid hormone in prepubertal ram lambs
26	Podobed, P	MSc	Biomedical Sciences	Circadian orchestration of normal cardiac proteome is essential for heart structure and function
27	Price, K	MSc	Pathobiology	Modification of commercial replacement layer pullet husbandry to enhance live Eimeria vaccination
28	Reabel, S	MSc	Pathobiology	Evaluation of methods for extraction of DNA from Encephalitozoon cuniculi
29	Richard, A	MSc	Biomedical Sciences	Investigating the dose-dependent signalling of TGF $\beta$ and its effects on angiogenesis
30	Santry, L	MSc	Pathobiology	Detection of maedi visna virus infection in Ontario sheep flocks
31	St. Paul, M	MSc	Population Medicine	Prophylactic treatment with Toll-like receptor ligands enhances host immunity to avian influenza virus in chickens
32	Tsimakouridze, E	MSc	Biomedical Sciences	Circadian genetic and protein biomarkers of cardiovascular disease
33	Turk, R	MSc	Pathobiology	Post-hospital discharge procedure specific surgical site infection (SSI) surveillance in small animal patients
34	Veitch, S	MSc	Biomedical Sciences	Characterization of $\alpha$ -adrenergic receptor function in 3rd vs 1st order mesenteric veins of normal male SD rats
35	Wittrock, J	MSc	Population Medicine	Evaluation of an electronic cow-side glucose meter for diagnosing insulin resistance in Holstein cows
36	Zours, S	MSc	Biomedical Sciences	Characterization of endothelial-mesenchymal-transition (EndoMT) markers in response to transforming growth factor-beta (TGF $\beta$ ) in bovine aortic endothelial cells (BAEC)
37	Adnan, H	PhD	Biomedical Sciences	The effect of menadione on GSTA1-JNK complex dissociation and JNK activation in Caco-2 cells
38	Al-Najeer, A	PhD	Biomedical Sciences	Characterization of gender related differences in vascular reactivity of mesenteric veins to endothelin-1 in a gonadally intact and nurtured rats
39	Anderson, M	PhD	Pathobiology	Observation of patient and surgeon preoperative preparation in companion animal clinics
40	Bauman, C	PhD	Population Medicine	Prevalence of Johne's Disease in the Dairy Goat Industry of Ontario: risk factors and evaluation of test methodologies
41	Carter, T	PhD	Biomedical Sciences	Measuring all the ends: the challenges, solutions, and applications of comprehensive telomere measurement
42	Deng, L	PhD	Pathobiology	Effects of oral inoculation on chickens with wt FAdV-9 and one mutant FAdV-9 $\Delta$ 4
43	Ferris, J	PhD	Biomedical Sciences	Effects of in ovo exposure to cortisol, bisphenol A and the antiestrogen ICI 162,780 on vertebral morphology
44	Gilchrist, G	PhD	Biomedical Sciences	Sex Specific Telomere Analysis In Leukocytes From Newborn Calves
45	Griffin, B	PhD	Pathobiology	Down-regulation of surface class I major histocompatibility complex (MHC-I) by fowl adenoviruses
46	Ho, N	PhD	Biomedical Sciences	Assessment of mitochondrial remodelling following DCA exposure in human colorectal cancer cells
47	Kellenberger, L	PhD	Biomedical Sciences	Secondary active transport of glucose enhances the viability of ovarian cancer cells in high glucose environments
48	Lachowsky, N	PhD	Population Medicine	Comparing younger and older MSM in New Zealand; important differences in HIV-related knowledge, attitudes, and sexual behaviour
49	Meadows, S	PhD	Population Medicine	Determining the seroprevalence and risk factors of Q Fever in sheep, goats and their farm workers in Ontario
50	Ogedengbe, M	PhD	Pathobiology	DNA Barcoding of coccidia (Apicomplexa) and qPCR of Eimeria species infecting chickens

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51	Thompson, J	PhD	Pathobiology	Establishment and characterization of two cell lines derived from canine cutaneous mast cell tumours
52	Wagter-Lesperance, L	PhD	Pathobiology	Feasibility of Transferring High Immune Response (HIR) Technology as a Health Management Tool to the Dairy Marketplace
53	Yu, D	PhD	Pathobiology	Detection of a Hybrid Jaagsiekte Sheep Retrovirus – Murine Leukemia Virus Transcript in JS7 Cells

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## Oral Presentation Schedule - Room 1713

### *Degree Program of Presenters: MSc*

<u>Time</u>	<u>Presenter</u>	<u>Topic</u>
12:00	<b>Kate Bottoms</b>	Towards risk-based surveillance of contagious swine diseases in southern Ontario
12:15	<b>Samantha Payne</b>	An investigation of angiogenesis during multi-tissue reparative regeneration
12:30	<b>Theresa Procter</b>	Prevalence and risk factors for shedding of <i>Campylobacter</i> spp. in dogs that frequent dog parks in southern Ontario, Canada
12:45	<b>Lisa Werden</b>	Factors affecting the abundance of blacklegged ticks ( <i>Ixodes scapularis</i> ) and the prevalence of <i>Borrelia burgdorferi</i> infection in the Thousand Islands region

### **Break**

### *Degree Program of Presenters: PhD/DVSc*

13:15	<b>Belinda Black</b>	Local Anesthesia and MRI of the Equine Foot
13:30	<b>Katharine Woods</b>	Comparison of manual aspiration via polyethylene tubing to suction pump aspiration via suction trap connection for bronchoalveolar lavage in healthy dogs
13:45	<b>Galina Hayes</b>	Investigation of pharmacokinetics of gentamicin following intra-articular implantation of a gentamicin impregnated collagen sponge (GICS) in the inflamed canine stifle joint
14:00	<b>Kayla Perkel</b>	Assessing bovine embryo health by proton nuclear magnetic resonance: a method for non-invasive embryo selection

### **Break**

### *Degree Program of Presenters: PhD*

14:30	<b>Marlene Paibomesai</b>	Parturition Effects on IL-4 and IFN $\gamma$ Concentration in Isolated CD4+ T-cells From High and Low Immune Responder Dairy Cows
14:45	<b>Wendy Pons</b>	Recognizing the Needs of Small Drinking Water Operators in Ontario
15:00	<b>Kathleen Thompson-Crispi</b>	Incidence Rates of Clinical Mastitis in Canadian Holsteins Classified as High, Average and Low Immune Responders
15:15	<b>Chandrika Senthilkumaran</b>	Are Annexin (I & II) diagnostic markers for shipping fever in beef calves?
15:30	<b>Hind Kasab-Bachi</b>	Epidemiological study of <i>Clostridium perfringens</i> and <i>Clostridium difficile</i> in Ontario broiler chickens

**4:00 p.m. in OVC LLC 1714**

### **Schofield Lecture: "The Continuum of Cancer Care in Veterinary Medicine"**

Dr. Rodney Page, PhD, DACVP, Professor and Director, Dept. of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine

**RECEPTION TO FOLLOW (OVC LLC 1707 A)**

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## Oral Presentation Schedule - Room 1715

### *Degree Program of Presenters: PhD*

<u>Time</u>	<u>Presenter</u>	<u>Topic</u>
12:00	<b>Jason Stull</b>	Pet ownership, interactions and animal-associated disease risks in Canadian households
12:15	<b>Olivier Côté</b>	Role of Clara cell secretory protein in lung inflammation
12:30	<b>Valerie Wong</b>	Anti-chemotactic and anti-apoptotic effects of canine activated protein C
12:45	<b>Bimal Chhetri</b>	Comparison of the geographical distribution of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections in the United States (2000-2011)

### **Break**

### *Degree Program of Presenters: PhD*

13:15	<b>Melanie Ammersbach</b>	Proteomic analysis of the feline immunodeficiency virus
13:30	<b>Saad Enouri</b>	Effects of transmural pressure elevation on endothelium-dependent and endothelium-independent vasodilation in isolated rat mesenteric veins
13:45	<b>Steven Roche</b>	Measuring the impact of a participatory approach to accelerating the adoption of practices and procedures to control Johne's Disease (JD) on Ontario dairy farms
14:00	<b>Laura Falzon</b>	The effect of lambing season on the periparturient egg rise of gastro-intestinal nematodes in ontario sheep flocks

### **Break**

### *Degree Program of Presenters: PhD*

14:30	<b>Elizabeth Sinclair</b>	Cardiac myofilament function and proteomic changes in Naturally Occurring Dilated Cardiomyopathy in Doberman Pinschers
14:45	<b>Cynthia Weijs</b>	Facebook and the veterinary profession: exploring attitudes, behaviours and experiences
15:00	<b>Christine Murray</b>	Physiological and behavioural characteristics related to vitality of newborn Holstein dairy calves and the efficiency of immunoglobulin absorption
15:15	<b>Scott Walsh</b>	Experimental infection of newborn lambs with enzootic nasal tumor virus (entv-1)
15:30	<b>Clemence Nash</b>	Measuring cow comfort on commercial dairy farms in Canada

**4:00 p.m. in OVC LLC 1714**

### **Schofield Lecture: "The Continuum of Cancer Care in Veterinary Medicine"**

Dr. Rodney Page, PhD, DACVP, Professor and Director, Dept. of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine

**RECEPTION TO FOLLOW (OVC LLC 1707 A)**

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# Abstracts Oral Presentations

  

## Graduate Student Research Symposium



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## **Towards risk-based surveillance of contagious swine diseases in southern Ontario**

**Kate Bottoms, Zvonimir Poljak, Cate Dewey, Robert Friendship**

### **Department of Population Medicine**

The primary objective of this project is to develop a map of southern Ontario that can be used as a tool in the risk-based surveillance of contagious swine diseases. For this purpose, we are interested in external biosecurity protocols and herd density. Using cluster analysis, we can determine how many biosecurity groups we have in the study region and which factors are most important in defining these groups. The risk map will incorporate spatial information about the distribution of these groups with the density of swine herds in the study region. Additionally, multinomial logistic regression will allow us to determine which demographic variables are significant predictors of biosecurity group membership.

Geographic areas identified as 'high risk' have high densities of swine and/or a low probability of having herds that are classified as having good biosecurity practices. Surveillance of emerging contagious swine diseases would best be focused in these areas. Alternatively, areas identified as 'low risk' have low densities of swine and/or a high probability of having herds that are classified as having good biosecurity practices. Elimination projects would best be focused in these areas. Multinomial regression analysis identified factors that are significant predictors of biosecurity group membership. These include herd size, herd type, site production type and number of swine sites within a three mile radius. This information can be utilized by the swine industry to maximize the benefits of surveillance projects involving contagious swine diseases.

## **An investigation of angiogenesis during multi-tissue reparative regeneration**

**Samantha Payne, Jim Petrik, Matt Vickaryous**

### **Department of Biological Sciences**

Angiogenesis, the sprouting of new blood vessels, has a well-documented role in embryogenesis and tumorigenesis. Angiogenesis also occurs during tissue repair and regeneration, although to date the process has been largely overlooked. We investigated angiogenesis during complex multi-tissue regeneration using the leopard gecko tail as a novel model. As for other lizards, leopard geckos voluntarily shed their tails as an anti-predation strategy, and then spontaneously regenerate a functional replacement. We hypothesize that angiogenesis during regeneration follows a conserved sequence of events regulated by angiogenic factors, and is comparable to blood vessel formation during mammalian development. Using a panel of mammalian angiogenic markers ( $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), vascular endothelial growth factor (VEGF), thrombospondin-1 (TSP-1), and cluster differentiation 36 (CD36)) we demonstrate that expression of these proteins occurs in a conserved sequence during regeneration. After tail loss the wound site is characterized by the development of new capillaries lined by VEGF positive endothelial cells (ECs). Continued maturation of vessels corresponds with the recruitment of  $\alpha$ -SMA positive pericytes, as well as ongoing VEGF expression. TSP-1 and the EC receptor CD36 are not expressed until the later stages of tail regeneration, corresponding with the complete differentiation of tail tissues. To further explore the role of angiogenesis during regeneration we have initiated a study involving the administration of the anti-angiogenic compound ABT-510, a TSP-1 mimetic. Preliminary data indicates that ABT-510 alters the distribution and architecture of newly formed blood vessels. Our results confirm a critical role for blood vessel development during multi-tissue regeneration.

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**Prevalence and risk factors for shedding of *Campylobacter* spp. in dogs that frequent dog parks in southern Ontario, Canada**

**Theresa Procter, David Pearl, Rita Finley, Erin Leonard, Nicol Janecko, Richard Reid-Smith, Scott Weese, Andrew Peregrine, Jan Sargeant**

**Department of Population Medicine**

This project examined the prevalence of, and risk factors for, shedding of *Campylobacter* spp. in the feces of dogs that visit dog parks in southern Ontario. From May to August 2009, canine fecal samples were collected at ten dog parks in Guelph and Kitchener-Waterloo, and owners were asked to complete a questionnaire related to pet management factors; questions were asked on diet, exposure to other animals and livestock, treatment with antibiotics, and human illness in the home. A total of 251 fecal samples were collected and 189 were surveys completed. *Campylobacter* spp. were found in 43% (95% CI: 36.8%- 49.2%) of fecal samples. Of the *Campylobacter* spp. detected, 86.1% were *C. upsaliensis* while 13.9% were *C. jejuni*. Multivariable logistic regression was used to identify factors associated with shedding *Campylobacter* spp. and *C. upsaliensis*. Risk factors associated with the shedding of *Campylobacter* spp. included dogs having access to water for swimming or drinking (e.g., lakes, ditches, toilets), and being on their current diet for less than one year. Sparing factors included increasing age, feeding of a commercial dry diet, and exposure to compost. Risk factors for shedding *C. upsaliensis* included water access, human household members experiencing a gastro-intestinal upset in the previous 30 days, and the dog being less than one year of age. Understanding the pet-related risk factors for *Campylobacter* spp. shedding in dogs can help in the development of interventions for potentially reducing the risk of transmission between humans and dogs.

**Factors affecting the abundance of blacklegged ticks (*Ixodes scapularis*) and the prevalence of *Borrelia burgdorferi* infection in the Thousand Islands region**

**Lisa Werden, Amy Sharp, Ian Barker, Jeff Bowman, Emily Gonzales, Patrick Leighton, Robbin Lindsay, Claire Jardine**

**Department of Pathobiology**

Lyme disease, caused by the bacterium *Borrelia burgdorferi* (Bb), is the most common vector-borne disease in the temperate world. Numerous biotic and abiotic factors affect the distribution of the blacklegged tick vector (*Ixodes scapularis*) and the prevalence of Bb infection in eastern North America. The objective of this study was to assess the distribution of ticks and Bb in the Thousand Islands, a new endemic region, and to determine the relative roles of various factors in predicting the presence of ticks and Bb. Data were collected via drag sampling, small mammal trapping, deer population estimates, and microclimate loggers at 12 sites in 2009 and 2010. Infection status of ticks was determined using PCR. Model selection and multimodel inference were used to evaluate variable effects. Our results show dramatic inter-island variation in the abundance of ticks (<1-57 per hour of dragging) and the prevalence of Bb in ticks (0-63%). Interestingly, species richness and the proportion of mice in the small mammal population interacted to affect both the number of ticks and the prevalence of Bb. As predicted, deer and temperature had positive effects on tick numbers, and both the number of ticks and infection prevalence declined with increasing distance from the United States, the presumed source of this new endemic population. Our results will help in devising management strategies aimed at controlling blacklegged ticks and reducing the risk of Lyme disease in the Thousand Islands and will help predict future risk as this disease system becomes further established in Canada.

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## Local Anesthesia and MRI of the Equine Foot

**Belinda Black, Nicola Cribb, Donald Trout,  
Stephanie Nykamp, Jeffrey Thomason**

### Department of Clinical Studies

**Introduction:** The effect of diagnostic anesthesia on the interpretation of MRI is unknown. Our objective was to determine if mepivacaine injection in the foot would cause iatrogenic variation detectable with MRI. We hypothesize that MRI will detect an increase in joint fluid at 24h, but not 72h post-injection and that abnormalities at needle site entries will be detectable.

**Materials and Methods:** Fifteen sound adult horses had baseline MRI performed up to 6 days prior to injection of the podotrochlear bursa (PB), digital flexor tendon sheath (DFTS), distal interphalangeal joint (DIPJ) and palmar digital nerve block (PDNB). MRI was repeated at 24h and 72h post-injection then quantitative and qualitative measurements were made. **Results:** MRI measurements made >24 hours after mepivacaine injection of the DIPJ, PB and PDNB did not alter significantly from baseline values. Compared to baseline values, a significant increase in synovial fluid volume was detected in MRI at 24h and 72h after injection of the DFTS.

**Discussion:** Mepivacaine injected into the DIPJ, PB and a PDNB does not interfere with MRI interpretation >24 hours after, however, DFTS injection caused increased synovial fluid, detectable on MRI for at least 72 hours. In conclusion, injection of the DFTS with mepivacaine can cause an increase in synovial fluid detectable on MRI for at least 72 hours, therefore we recommend waiting greater than three days between injection and evaluation of the DFTS with MRI.

## Comparison of manual aspiration via polyethylene tubing to suction pump aspiration via suction trap connection for bronchoalveolar lavage in healthy dogs

**Katherine Woods, Alice Defarges, Anthony Abrams-Ogg, Howard Dobson, Laurent Viel, Dorothee Bienzle**

### Department of Clinical Studies

The purpose of this study was to compare the diagnostic quality of bronchoalveolar lavage fluid (BALF) acquired by manual aspiration via polyethylene tubing (MAPT) and suction pump aspiration (SPA) via suction trap connection techniques in healthy dogs.

Bronchoalveolar lavage was performed under general anesthesia using bronchoscopic guidance in twelve beagles. MAPT was performed with a 35mL syringe attached to sterile polyethylene tubing wedged in the bronchus via the bronchoscope's biopsy channel. SPA was performed using 5kPa negative pressure applied directly to the bronchoscope's suction valve via a disposable aspiration tube. MAPT and SPA techniques were performed in randomized order in opposite caudal lung lobes of each dog. Two lavages were performed at each site using a weight-adjusted aliquot volume. The BALF samples were centrifuged and analyzed using a subjective scale for quality, percent of retrieved fluid, total nucleated cell count and differential cell count. Results were compared by paired Wilcoxon sign rank test.

The proportion of BALF retrieved was significantly higher (mean difference 19.02%,  $p = 0.001$ ) for SPA than MAPT. A higher percentage of epithelial cells was present in MAPT BALF samples compared to SPA (mean difference = 0.81,  $p = 0.054$ ).

The results indicate that SPA provided BALF samples of higher diagnostic quality than MAPT in healthy dogs. The SPA technique may improve the rate of diagnostic success of BAL in dogs.

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**Investigation of pharmacokinetics of gentamicin following intra-articular implantation of a gentamicin impregnated collagen sponge (GICS) in the inflamed canine stifle joint.**

**Galina Hayes, Tom Gibson, Noel Moens, Ron Johnson**

**Department of Clinical Studies**

**Objective:** To investigate local and systemic pharmacokinetics of gentamicin following intra-articular implantation of a gentamicin impregnated collagen sponge (GICS) in the inflamed canine joint.

**Study Design:** Descriptive repeated measures experimental study. Animals: 9 dogs

**Methods:** Stifle joint inflammation was generated by injection of urate. Twenty-four hours later a GICS was arthroscopically implanted at gentamicin dose=6mg/kg. Synovial fluid and plasma gentamicin concentrations were measured for 14 days post implantation, and pharmacokinetic parameters modeled using a splined mixed level random-intercept random-slope approach to obtain population-averaged estimates.

**Results:** Intra-articular gentamicin concentrations fell to sub-MIC for staphylococci (4ug/ml) by 25.3hrs (95% CI=16.8-33.8hrs). C<sub>max</sub> synovial was 2383ug/ml (95% CI=974 – 3793 ug/ml) at 0.5hr. Comparison with previously reported data suggested that intra-articular gentamicin after GICS declines more rapidly in the presence of inflammation. There was substantial systemic uptake of gentamicin with C<sub>max</sub> plasma =7.4ug/ml (95% CI=5.0-9.7 ug/ml) at 1.5hrs following GICS placement.

**Conclusions:** Intra-articular gentamicin following GICS placement at an IV-equivalent dose reached very high levels but decayed rapidly. The maximum plasma levels attained were approximately 1/3rd of the recommended sub-toxic target for human patients following parenteral gentamicin administration.

**Clinical relevance:** Intra-articular concentrations are theoretically sufficient to kill even multi-drug resistant staphylococci. It is unclear whether the collagen sponge formulation offers any advantage over intra-articular injection of the parenteral formulation, given the rapid decay profile. GICS should be used with caution or avoided in renally insufficient patients. Concurrent parenteral aminoglycoside doses should be avoided or adjusted.

**Assessing bovine embryo health by proton nuclear magnetic resonance: a method for non-invasive embryo selection**

**Kayla Perkel, Payneesh Madan**

**Department of Biomedical Sciences**

**Introduction:** The reproductive success of in vitro produced mammals, including cattle, is largely dependant on early embryonic survival in vitro. Currently, morphological assessment is the preferred method used to determine embryo viability, however this is a poor predictor of embryo viability. Development of refined biological techniques like metabolomics has enabled the health of a cell to be explored based on its secreted metabolite constituents. We hypothesize that embryos developing at different rates differ in their metabolomic signatures. The objective of study was to determine the metabolomic signatures of slow (SG) and fast (FG) growing embryos at timed stages of development.

**Methodology:** Standard IVM and IVF protocols were used on oocytes aspirated from abattoir obtained ovaries. Presumptive zygotes were placed individually in 40 µl culture drops. Media from FG embryos was collected at 2-cell (30 hours post fertilization or hpf), 4-cell (42 hpf), 8-cell (49 hpf), 16-cell (90 hpf), morula (144 hpf) and blastocyst (168 hpf) and from SG embryos 12 hr later to reach equivalent embryo stage. Nuclear magnetic resonance was performed on a 600MHz spectrometer.

**Results:** Preliminary data indicates distinct differences between metabolomic profiles at all embryonic stages for SG and FG groups. However, FG growing embryos consumed more metabolites than their SG counterpart.

**Conclusion:** Lower utilization of metabolites by slow growing embryos may indicate inefficient use or decreased viability. The results provide evidence towards the understanding of metabolism in SG and FG embryos, the use of metabolomics for the development of a non-invasive tool for assessing embryo health.

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## **Parturition Effects on IL-4 and IFN $\gamma$ Concentration in Isolated CD4+ T-cells From High and Low Immune Responder Dairy Cows**

**Marlene Paibomesai, Bonnie Mallard**

### **Department of Pathobiology**

The peripartum period is a period of high stress, transition and management changes for dairy cows. It is associated with higher incidents of both metabolic and pathogenic disease. Both antibody (AMIR; respectively high IL-4) and cell mediated (CMIR; respectively high IFN $\gamma$ ) immune responses play a key role in the maintenance of health in mammals protecting against both intracellular and extracellular pathogens. The aim of this study was to determine the effects of parturition on cytokine production in CD4+ T-cells isolated from highAMIR/lowCMIR (hiAMIR) and highCMIR/lowAMIR (hiCMIR) cows. Previously immune response phenotyped cows were selected based on hiAMIR (n=12) and hiCMIR (n=11) responses to test antigens. Isolated CD4+ T-cells collected at 28 days prepartum, 4 days postpartum, and 21 days postpartum from these groups were stimulated with Concanavalin A (ConA) and incubated for 24hrs (37°C, 5% CO<sub>2</sub>). Cell culture supernatant was collected and IL-4 and IFN $\gamma$  concentrations were quantified. Overall, it was observed that both groups showed immunosuppression for both IFN $\gamma$  and IL-4 at the time of parturition. At all time points IFN $\gamma$  was expressed at higher concentration than IL-4 for both groups. HiAMIR expressed less IL-4 and IFN $\gamma$  than hiCMIR, but showed less decrease in cytokine production around parturition. HiCMIR expressed more IFN $\gamma$  and IL-4 than hiAMIR but showed greater changes than hiAMIR in response to parturition. Different expression kinetics were observed for IFN $\gamma$  and IL-4 from CD4+ cells of hiAMIR and hiCMIR cows during the peripartum period.

## **Recognizing the Needs of Small Drinking Water Operators in Ontario**

**Wendy Pons, Andrew Papadopoulos, Scott McEwen, Katerina Pintar**

### **Department of Population Medicine**

A telephone survey was conducted of small drinking water operators in Ontario to determine water operators knowledge level of drinking water risks, hazards and control measures, in order to identify gaps in knowledge, perceived challenges and future training needs.

A sampling frame was obtained from the Ontario Ministry of Health. Operators were randomly selected and asked to complete a 22 question survey.

The survey found great variety in the experience and knowledge of the water operators. Providing insight into their practices, abilities and challenges they face in protecting the water supply.

The results from this study will be especially valuable in identifying key water safety issues at the operator level and will play an important role in informing and shaping future training requirements.

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## **Incidence Rates of Clinical Mastitis in Canadian Holsteins Classified as High, Average and Low Immune Responders**

**Kathleen Thompson-Crispi, Asheber Sewalem, Filippo Miglior, Bonnie Mallard**

### **Department of Pathobiology**

Mastitis, the inflammation of the mammary gland, is a complex disease associated with significant economic loss in the dairy industry. The immune system controls response to pathogenic challenge. The adaptive immune system can be categorized into two branches: type-1 or more generally cell-mediated immune response (CMIR) and type-2 or antibody-mediated (AMIR). Both AMIR and CMIR are essential for host protection to the variety mastitis-causing organisms. The objectives of this study were to determine the incidence rate of clinical mastitis (IRCM) for Canadian Holsteins from numerous herds on a national scale and to determine differences in IRCM between cows classified as High, Average or Low for AMIR and CMIR based on estimated breeding values. Previously, 690 Holsteins were immunized to measure serum antibody by ELISA (AMIR), and delayed-type hypersensitivity (CMIR). Subsequently, heritability and breeding values of these traits were estimated in order to classify cows. In collaboration with the Canadian Bovine Mastitis Research Network, data on cases of mastitis and causative pathogens were available for cows in this study. The overall IRCM for all cows was 26.3. Cows classified as High for AMIR had significantly lower IRCM (17.1) compared to Average (27.9) and Low (30.7) AMIR cows. Low AMIR cows had significantly higher severe mastitis. No differences were found when cows were classified based on CMIR. Results indicate breeding Holsteins for enhanced AMIR may reduce the incidence and severity of mastitis on Canadian farms. Genotyping with the Bovine Illumina SNP50 is underway to determine genetic associations with enhanced IR.

## **Are Annexin (I & II) diagnostic markers for shipping fever in beef calves?**

**Chandrika Senthilkumaran, Khaled Abdelaziz, Brandon Lillie, Ken Bateman, Joanne Hewson, Shayan Sharif, Jeff Caswell**

### **Department of Pathobiology**

Shipping fever is one of the most economically important diseases for the North American beef industry and it often occurs a few days after weaning and transportation. Stress and concurrent viral infections are important factors for the development of the disease. Antimicrobial proteins present in the epithelial lining fluid (ELF) may play an active role in disease resistance. Discovery of differently expressed proteins in ELF after transportation could be a diagnostic marker for shipping fever. ELF of healthy weaned beef calves was sampled one to two days after transportation. The ELF protein expression of calves that later developed bacterial pneumonia (n=7) was compared to those that remained healthy (n=7) by difference in gel electrophoresis (DIGE). Spots that were significantly different were identified by mass spectrometry. Anti-inflammatory proteins that were significantly lower in calves that later developed pneumonia were: macrophage capping protein, annexin I, annexin II, dihydrodiol dehydragenase 3, peroxiredoxin I, calyphosin, and superoxide dismutase. These findings suggest lower levels of annexins I and II as potential biomarkers of increased susceptibility in recently arrived, at-risk feedlot cattle, perhaps reflecting differences in the stress response that predisposes to development of pneumonia. In addition, these findings have implications in pathogenesis. The influx of inflammatory cells and fibrin deposition is thought to damage the host, and annexins I and II prevent neutrophil influx and fibrin deposition, respectively. Having higher levels of annexin I and II may minimize the damage caused by infection with *M. haemolytica*.

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## Epidemiological study of *Clostridium perfringens* and *Clostridium difficile* in Ontario broiler chickens

Hind Kasab-Bachi, Michele Guerin, Scott McEwen, David Pearl, Andreas Boecker, Durda Slavic

### Department of Population Medicine

Currently, there are no observational studies on the prevalence, characterization, risk factors, and antimicrobial susceptibility patterns of *C. perfringens* and *C. difficile* in Ontario broiler flocks.

The objectives of this study are to: determine the prevalence of *C. perfringens* and *C. difficile* in Ontario broiler chicken flocks; identify the genotypes of *C. perfringens* using polymerase chain reaction; identify bio-security and management practices associated with the presence of *C. perfringens*; identify antimicrobial use practices associated with antimicrobial resistance patterns for *C. perfringens*; determine the antimicrobial susceptibility patterns using minimum inhibitory concentration testing for *C. perfringens* and *C. difficile*; and identify the genotypes of *C. difficile* isolates using enzyme-linked immunosorbent assay and ribotype patterns using PCR ribotyping.

This study has a target sample size of 240 flocks. After producer recruitment, whole intestines are collected at processing plants. Cecal and cecal/colon swabs are submitted to the Animal Health Laboratory for testing. After sample collection, producers are interviewed to gather information on the farm management practices for the specific flock sampled.

To date, samples have been collected from 180 flocks. Partial preliminary laboratory results are available. Six percent and 79% of 165 flocks tested were positive for *C. difficile* and *C. perfringens*, respectively. All positive *C. perfringens* isolates were type A except for one type E isolate. The beta2 gene was present in 88% of the positive *C. perfringens* isolates.

This baseline surveillance study will be a vital step in prioritizing future research and designing broiler disease prevention programs in Ontario.

## Pet ownership, interactions and animal-associated disease risks in Canadian households

Jason Stull, Andrew Peregrine, Jan Sargeant, Scott Weese

### Department of Pathobiology

**Objectives:** Describe household knowledge, perceptions, and practices of pet ownership and animal interaction for a sample of the general population in Ontario.

**Methods:** A questionnaire was developed and administered at two multi-doctor general practice physician offices in the Waterloo Region, Ontario, Canada.

**Results:** 641 adults from separate households completed the survey (75% response). Pet contact was common, with 64% of participants having one or more pets and 37% of non-pet owning households having at least weekly physical contact with animals. Individuals at higher risk to infections (i.e., immunocompromised) were more likely to reside in non-pet households (65%, 45%), while dog or cat-induced bites/scratches were more likely in pet owning households (36%, 10%). Only 31% of respondents had ever received zoonotic disease information from any source. Respondents from pet-owning households were less concerned, more comfortable with disease knowledge, and had a higher demonstrable knowledge of zoonotic diseases than those from non-owning households. High-risk behaviours and management practices occurred in many pet-owning households with the feeding of raw food products and poor hand/face hygiene being the most common. Many reptile-owning households reported high risk practices.

**Conclusions:** Although animal contact, resulting human injury, and higher risk settings were frequently reported, a minority of respondents received zoonotic disease information. Despite greater comfort with and knowledge of zoonotic diseases, considerable knowledge deficits were evident in pet-owning households. There is a need for consistent, dependable zoonotic disease information for both pet and non-owning households.

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## **Role of Clara cell secretory protein in lung inflammation**

**Olivier Côté, Dorothee Bienzle**

### **Department of Pathobiology**

Recurrent airway obstruction (RAO) is a chronic inflammatory lung disease affecting horses following repeated exposure to environmental substances. Early during inhalation of noxious substances, bronchial cells produce Clara cell secretory protein (CCSP) to prevent tissue damage caused by inflammatory cells. Horses are unique by having 3 copies of the CCSP gene distributed over a region of ~520 kilobases. CCSP copies differ from each other at 8 to 10 nucleotides, and are expected to produce different proteins. Therefore, we hypothesize that CCSP variants are differentially expressed and have distinct functions.

Long-Range PCR was used to validate the presence of 3 different CCSP genomic sequences in every horse analyzed ( $n > 10$ ). Polymorphisms detected in specific copies from different horses were mostly conservative, indicating preservation of each copy over time. End-Point Limiting Dilution assays revealed that 2 of 3 CCSP genes are expressed in adult lung and uterine tissues. CCSP tissue-specific expression was further evaluated in 27 additional tissues. A markedly different expression ratio of the 2 expressed copies was found in lung from RAO affected animals ( $n = 5$ ) compared to controls ( $n = 10$ ), suggesting distinct roles for different CCSP genes. Bioinformatic analysis revealed a stop mutation in the non-expressed gene copy. The precise causes underlying most airway diseases are poorly understood, and most treatments are aimed at reducing the signs of disease rather than at achieving a cure. Findings from these studies may increase our understanding of inflammatory mechanisms in asthma-related disease and identify better targets for therapy.

## **Anti-chemotactic and anti-apoptotic effects of canine activated protein C**

**Valerie Wong, Dorothee Bienzle, Darren Wood**

### **Department of Pathobiology**

Activated protein C (APC) is a natural anticoagulant with anti-inflammatory and cytoprotective effects. Currently, recombinant human APC is the only FDA-approved drug for treating severe sepsis in adults. The biological properties of canine APC (CnAPC) are not well characterized, rendering the therapeutic potential of CnAPC for canine sepsis uncertain. The goals of our study were (1) to determine if CnAPC inhibited chemotaxis of neutrophils *in vitro* and (2) to determine if CnAPC protected canine aortic endothelial cells (CnAoECs) from apoptosis *in vitro*. Protein C zymogen was purified from canine plasma by immunoaffinity chromatography and activated *ex vivo* by Protac. To study the effects of CnAPC on neutrophil chemotaxis, neutrophils were isolated from canine whole blood, labeled with calcein AM, treated with different concentrations of CnAPC, and then allowed to migrate towards canine recombinant IL-8 through a porous membrane. The number of neutrophils that migrated through the membrane was quantified by fluorescence. To study the anti-apoptotic effects of CnAPC, cultured CnAoECs were treated with different concentrations of CnAPC, followed by induction of apoptosis with staurosporine. Apoptosis was measured as expression of caspase 3/7 using a fluorescence-based ELISA. Results were analyzed using a standard linear regression approach after logarithmic or logit transformation of the data to meet assumptions of the regression model. Our results showed that CnAPC inhibited neutrophil chemotaxis and protected cells against apoptosis in a dose-dependent manner within the ranges 0-376 ng/mL ( $p < 0.0001$ ) and 1.5-6 ng/uL ( $p < 0.0001$ ), respectively. We conclude that CnAPC inhibited neutrophil chemotaxis and protected endothelial cells from apoptosis *in vitro*. The biological implications of these results warrant further investigation.

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## **Comparison of the geographical distribution of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections in the United States (2000-2011)**

**Bimal Chhetri, Olaf Berke, David Pearl,  
Dorothee Bienzle**

### **Department of Population Medicine**

It has been suggested that FIV and FeLV infections share similar risk factors and control measures. As both infections are endemic in North America, it is assumed as a working hypothesis that their geographic distributions are similar. This goal of this exploratory analysis was to investigate the geographical distribution of both viral infections.

Counts of FIV (n=17,108) and FeLV (n=30,017) positive serology results (FIV antibody and FeLV ELISA) were obtained for 48 contiguous states of the U.S from the IDEXX Laboratories website. The proportional morbidity ratio of FIV to FeLV infection was estimated by state. Its geographic distribution pattern is visualized by a choropleth disease map. Statistical evidence of an excess in the proportional morbidity ratio was assessed using the spatial scan test statistic under the Bernoulli assumption.

As a result, this study showed distinct spatial distribution patterns in the FIV to FeLV morbidity ratio. The disease map indicates higher number of FIV infections in Southern and Eastern U.S. compared to FeLV. On the contrary, FeLV infections were observed to be higher in Western U.S. compared to FIV. The respective excess in the proportional morbidity ratio was significant with respect to the spatial scan test ( $\alpha=0.05$ ).

In conclusion, the observed variability in the geographical distribution of the proportional morbidity ratio of FIV to FeLV may be related to presence of an additional but yet unknown spatial risk factor. Putative factors are variations in specific virus strains, length of survival of FIV infected cats and prevalence of vaccine use for FeLV.

## **Proteomic analysis of the feline immunodeficiency virus**

**Melanie Ammersbach, Dorothee Bienzle**

### **Department of Pathobiology**

Different strains of the feline immunodeficiency virus (FIV) appear to induce differently severe disease in cats. FIV Petaluma is considered to be minimally pathogenic viral strain while FIV Gammar has been described to induce rapid immunodeficiency.

The objective of this study was to describe the proteome of FIV and to assess differences between the Petaluma and Gammar strains. A large number of lymphocytes from a cat were infected with either FIV Petaluma or FIV Gammar. The virus-containing supernatant was collected on days 7 and 10 post infection, and virus was purified over a sucrose gradient. The viral proteins were separated by electrophoresis in an acrylamide gel, and each lane was cut into 15 bands. Proteins within each molecular weight fractions were analyzed by tandem mass spectrometry and peptide spectra were compared to viral and mammalian protein databases of the National Center for Biotechnology Information (NCBI). FIV Gag, Pol and Env proteins were detected, along with numerous host-derived proteins.

We conclude that mass spectrometry is a useful tool to identify the diversity of viral and host proteins in FIV. Replicate analyses with samples derived from different hosts are required to identify consistent differences among viral strains.

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## **Effects of transmural pressure elevation on endothelium-dependent and endothelium-independent vasodilation in isolated rat mesenteric veins**

**Saad Enouri, Ron Johnson, Gabrielle Monteith**

### **Department of Biomedical Sciences**

The vascular endothelium produces vasodilator substances that play a role in the overall cardiovascular regulation. This vascular regulatory function of the endothelium can be impaired by increases in transmural distending pressures. In this study we investigated the effects of changing transmural distending pressure on endothelial dilator function of rat mesenteric small veins (MSV). In MSV precontracted with PGF2 $\alpha$  (1  $\mu$ M), Bradykinin (BK), SNP, and SNAP concentration response curves (CRCs) were generated at intermediate (6 mmHg) and high (12 mmHg) transmural pressures. BK-induced vasodilation was also examined in the absence and presence of a NOS inhibitor (L-NNA, 100  $\mu$ M), a COX inhibitor (indomethacin, 1  $\mu$ M), a large (BKCa) conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels blocker (paxilline, 500 nM), and a small (SKCa) conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels blocker (apamin, 300 nM). BK, SNP, and SNAP CRCs were not altered by transmural pressure increases. BK-induced vasodilation was significantly reduced by indomethacin and L-NNA at 6 mmHg and L-NNA at 12 mmHg. Apamin and paxilline significantly reduced BK-induced vasodilation. Apamin and/ or paxilline applied with L-NNA resulted in further significant reduction of vasodilation evoked by BK compared with responses obtained with either blocker. Endothelium removal completely abolished BK-induced vasodilation. These findings suggest that 1) venous endothelial dilator function is not affected by an elevation of transmural pressure, 2) BK-induced vasodilation is completely dependent on the presence of functional endothelial cells and mediated in part by NO, BKCa, and SKCa channels while the participation of prostacyclin in mediating vasodilation induced by BK may be important at 6 mmHg.

## **Measuring the impact of a participatory approach to accelerating the adoption of practices and procedures to control Johne's Disease (JD) on Ontario dairy farms**

**Steven Roche, Dave Kelton, Andria Jones, Mike Von Massow**

### **Department of Population Medicine**

The purpose of this study is to design, implement and evaluate a novel knowledge extension program for Ontario dairy producers, focusing on educating producers about innovative and effective management practices for the prevention and control of Johne's Disease (JD). Dairy producers across Ontario volunteered to participate in this process, which includes four separate full-day sessions, where groups of producers are prompted to discuss and view JD from a variety of lenses (e.g. producer, veterinarian, consumer), using a variety of extension techniques (e.g. poster/oral presentations, expert speakers, farm tours). The evaluation of this process involves a pre/post survey, with a 'knowledge assessment' component, that is used to evaluate the changes in producer knowledge of JD. Also, pre/post risk assessments are completed by veterinarians to assess changes in herd risk as a result of adopting new management practices. Several focus groups will also be used to qualitatively assess this process. This information will be compared to data from a group of producers that have not participated in this process to understand its overall impact. While this project is still ongoing, preliminary results suggest that this process is an effective method for disseminating information to dairy producers, increasing dairy producer knowledge about JD, as well as facilitating the implementation of management practices for the prevention and control of JD. Overall, the process to this point has been successful in addressing many 'knowledge gaps' within the dairy community and we anticipate that this process will become a preferred method for extension in Ontario.

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## **The effect of lambing season on the periparturient egg rise of gastro-intestinal nematodes in Ontario sheep flocks**

**Laura Christina Falzon, Krishna Shakya, Paula Menzies, Andria Jones, John VanLeeuwen, Jocelyn Jansen, Jacob Avula, Andrew Peregrine**

### **Department of Population Medicine**

Out-of-season lambing is practiced by approximately 30% of sheep producers in Ontario. The periparturient egg rise (PPER), a major source of pasture contamination with gastro-intestinal nematodes, has only been documented in ewes lambing in the spring. The objective of the study was to determine whether ewes lambing out-of-season show an increase in fecal egg shedding during lambing. Six farms on year-round lambing were selected. The farms were visited five times following a schedule determined by the predicted date when 50% of ewes would have lambed in that season. All farms were visited for three consecutive lambing seasons (winter, spring, fall) using the same schedule. On the first visit for each lambing season, twenty pregnant ewes and twenty non-pregnant/early gestation ewes were randomly selected. On each visit, faecal samples were collected from each animal and processed individually to measure parasite faecal egg counts (FEC). The ewes were re-classified into different productivity stages (maintenance, early gestation, late gestation, early lactation, late lactation) based on information collected during farm visits and lambing dates. During the winter and spring lambing seasons, the FEC were not significantly different from each other, and were lower than the fall lambing season. In both winter and spring lambing seasons, the PPER peaked between early to late lactation; during the fall, the PPER peaked at late gestation and then decreased. The PPER occurred during all 3 lambing seasons, though its magnitude and distribution varied with the lambing season, suggesting that the PPER depends on both animal and seasonal effects.

## **Cardiac myofilament function and proteomic changes in Naturally Occurring Dilated Cardiomyopathy in Doberman Pinschers**

**Elizabeth Sinclair, Allison MacKay, Lynne O'Sullivan, Glen W. Pyle**

### **Department of Biomedical Sciences**

**Objectives:** Dilated cardiomyopathy (DCM) in Doberman Pinschers is an autosomal inherited disease, with a breed prevalence of up to 60%. Overt DCM is rapidly progressive: median survival time for treated dogs is ~11 weeks, while untreated dogs survive ~8 days. The underlying mechanism of this progressive disease is not understood, and the clinical and pathophysiological manifestations between dogs and humans are similar. Our goal is to better understand the underlying mechanisms of disease in end stage DCM and to identify therapeutic targets and markers of disease.

**Methods:** In order to investigate myofilament functioning, cardiac protein and phosphorylation changes in DCM, we used 2 dimensional differential gel electrophoresis (DIGE), myofibrillar Mg<sup>+</sup> ATPase assay, and phosphorylation gels to investigate differences between normal canine cardiac myofilaments and those with DCM.

**Results:** We have found 11 novel protein changes in the cardiac myofilament protein profile, in addition to phosphorylation alterations in myosin binding protein, troponin T and tropomyosin. We have also identified a decreased sensitivity to calcium in DCM myofilaments, and a hyper-response to the calcium sensitizer drug, bepridil.

**Conclusions:** Novel protein changes, alterations in protein phosphorylation and calcium handling provide new information on the pathogenesis of DCM and the cardiac remodeling process in Doberman Pinschers with DCM. Alterations in calcium sensitivity and myofilament response to a sensitizing drug indicates that myofilament function and regulation is altered in DCM. Finally, novel protein changes may provide putative biomarkers of disease.

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## **Facebook and the veterinary profession: exploring attitudes, behaviours and experiences**

**Cynthia Weijs, Jason B. Coe, Serge Desmarais**

### **Department of Population Medicine**

The objective of this study was to explore the behaviours, attitudes, and experiences of veterinarians on the social media site, Facebook. Facebook is the most popular social networking site worldwide, with benefits including sharing information online, and for businesses (including veterinary clinics), connecting with clients and advocating for animals. However, Facebook is still largely unregulated and presents some risks to users such as reputation damage, job loss, and threats to personal security (e.g. theft, bullying). For veterinarians, consequences are potentially compounded by risks to the individual's professional reputation, the reputation of his/her employer and the reputation of the profession.

1587 members (17%) of the American Animal Hospital Association completed an online survey. Seventy-two percent of respondents had a personal Facebook profile, and 75% felt their image on Facebook accurately presented who they are. Participants reported they were likely to post: their birthday (71%), their university (62%), their current profession (65%), pictures of animals they work with (25%), pictures of themselves at work (35%) and in their veterinary role (40%). Just over half of participants reported that their practice had a Facebook page, and 15% of all participants reported a negative experience at their practice related to Facebook.

Early findings of this research suggest a proactive educational approach is warranted. Information from this survey will be used in the development of communication guidelines and educational tools that will support the veterinary profession in using social media in a manner that maximizes its benefits and minimizes risks to individuals and the profession.

## **Physiological and behavioural characteristics related to vitality of newborn Holstein dairy calves and the efficiency of immunoglobulin absorption**

**Christine Murray, Doug Veira, Audrey Nadalin, Ken Leslie**

### **Department of Population Medicine**

This research examined physiological and behavioural characteristics of newborn calves with the aim of developing a newborn calf vitality scoring system strongly associated with absorption of colostrum immunoglobulins, health and performance. A total of 48 calving events ( $n=51$  calves) were continuously monitored from the first sight of foetal membranes. All calves were assessed for measures of vitality at the time of first sternal recumbency (SR), 2 and 24hrs, 7 and 14 days of age. Measurements included time to SR and standing, blood gases, oxygen saturation, lactate, glucose, respiration and heart rates, suckling response, IgG absorption and growth. Calves born from a hard pull (HP) ( $n=10$ ) were more acidotic (mean  $\text{pH} \pm \text{SD}$ :  $7.20 \pm 0.12$  vs  $7.28 \pm 0.05$ ) ( $P=0.014$ ) and took twice as long to attain SR as those born unassisted ( $n=18$ ) ( $\text{SR} \pm \text{SD}$ :  $5.2 \pm 3.0$  versus  $10.3 \pm 4.4$  min) ( $P<0.001$ ). All calves achieved a normal pH ( $7.4 \pm 0.04$ ) within 24hrs of birth. Calves born from a HP had weaker suckling response at SR compared to unassisted calves ( $P=0.01$ ). Significant predictors of weight gain included monocyte concentration at 2hrs ( $P=0.013$ ) and pH at SR ( $P=0.004$ ). Duration of calving is a significant predictor of pH at SR ( $P=0.014$ ) and may be used as an inexpensive, easy to measure variable to predict health of newborn Holstein calves. Overall, calves born from a HP are weaker and less responsive in the first few hours. This may lead to failure of passive transfer and reduced performance. Yet, objective and easily measurable physiological or behavioral outcomes highly correlated to calf vitality and success of passive transfer remains unclear.

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## **Experimental infection of newborn lambs with enzootic nasal tumor virus (ENTV-1)**

**Scott Walsh, Darrick Yu, Andrés Diaz-Mendez, Robert Foster, Paula Menzies, Sarah Wooton**

### **Department of Pathobiology**

Enzootic nasal tumor virus (ENTV-1) is a betaretrovirus of sheep associated with enzootic nasal adenocarcinoma (ENA), a neoplastic transformation of the ethmoid turbinate. Confirmation of the role of ENTV in the pathogenesis of ENA has yet to be resolved due to our inability to propagate the virus in cell culture and the lack of an infectious molecular clone. A molecular clone of ENTV-1 was constructed that could generate viral transcripts in transfected HEK 293 cells. To address the role of ENTV-1 infection in the development of ENA, groups of lambs were infected via nebulization with recombinant ENTV-1 virus derived from the molecular clone or clarified nasal tumor homogenate from ENA affected sheep. 3 months post infection, clinical symptoms of ENA were observed in a lamb infected with tumor homogenate and the lamb was euthanized. A multi-focal nasal tumor was identified on a computed tomography scan performed post-mortem and the presence of gross lesions was confirmed upon necropsy. Immunohistochemistry showed the presence of ovine betaretroviral antigens in nasal tumor cells but absence of these antigens in all other tissues tested. Hemi-nested PCR screening of genomic DNA for the provirus was positive for all tissues, excluding the trachea and the lymph nodes. This is the first report of experimental induction of ENA in sheep and the first use of an ENTV-1 infectious molecular clone. Our molecular clone is the first of its kind and represents a vital step towards the confirmation of ENTV as the etiologic agent of ENA.

## **Measuring cow comfort on commercial dairy farms in Canada**

**Clemence Nash, Jessica Zaffino, Derek Haley**

### **Department of Population Medicine**

This cross-sectional study is part of a national-level cluster project. It involves visiting tie-stall, free-stall and automated dairy farms in Ontario, Alberta and Quebec (N=240) to assess a variety of cow welfare indicators. Only farms milking 40 or more Holstein cows are eligible for the study. To date 60 farms have been visited in Ontario. The main objective of this research is to gather information about a variety of housing and management factors known to affect cow welfare and to examine their relationship with relevant animal-based measures. Measures are being taken over the course of two visits to each farm. Housing and management factors being measured include housing type, stall design, bedding quantity, bedding quality, etc. The animal-based measures are being taken on 40 cows per herd. These include the prevalence of surface injuries, body condition scores, lameness, cow cleanliness and lying behaviour. Inter- and intra-observer reliability for recording these measures is being maintained at a minimum of 75% exact agreement. Preliminary results indicate that cows housed in tie-stall systems lie 1.5 hours more than cows housed in free-stall systems. Lying time was observed to be 1.75 hours higher while injury prevalence was 35% lower in cows housed in deep sand stalls when compared to all other stall bases. This may demonstrate that deep sand stalls are the ideal stall base for housing dairy cows in Ontario. Data collection is to be completed in all three provinces by February 2012 at which time a more complete analysis will be undertaken.

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# Abstracts Poster Presentations

  

## Graduate Student Research Symposium



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## POSTER #1

### Effects of acepromazine or dexmedetomidine on cardiopulmonary performance during recovery from fentanyl/isoflurane anesthesia in the dog

Stephanie Keating, Carolyn Kerr, Alex Valverde, Wayne McDonell, Ron Johnson

#### Department of Clinical Studies

**Objective** – To compare the cardiopulmonary effects of IV dexmedetomidine, acepromazine or saline in dogs recovering from isoflurane anesthesia and receiving a constant rate infusion (CRI) of fentanyl.

**Methods** – In a cross-over study, seven purpose-bred dogs were randomly assigned to receive 1) acepromazine (0.05 mg/kg); 2) dexmedetomidine (2.5 µg/kg); or 3) saline IV. Anesthesia was induced with propofol and maintained with isoflurane delivered via an endotracheal tube. Following instrumentation to measure cardiopulmonary variables a loading dose of fentanyl (5 µg/kg) and CRI (5 µg/kg/hr) (T=0) were administered. Isoflurane was discontinued after 2 hours and the assigned treatment administered at extubation. The fentanyl CRI was continued for an additional hour and variables recorded for 90 minutes post-administration. Data were analyzed using ANOVA for repeated measures followed by Dunnett's or Tukey's analysis (P<0.05).

**Results** – Dexmedetomidine resulted in significantly lower cardiac index (CI), HR, PaO<sub>2</sub> and PvO<sub>2</sub>, and higher MAP and PaCO<sub>2</sub> than acepromazine and saline for up to 90 minutes. Acepromazine and saline produced similar results, except for short-lived increases in CI, PvO<sub>2</sub> and prolonged decreases in MAP with acepromazine compared to saline treatment. Many differences were most pronounced within 10 minutes of treatment administration.

**Conclusions** – On recovery from isoflurane anesthesia, the concurrent administration of a fentanyl CRI and dexmedetomidine caused significant cardiovascular and respiratory compromise, while acepromazine transiently improved hemodynamics compared to saline.

## POSTER #2

### Assessment of hypercoagulability in canine pituitary-dependent hyperadrenocorticism

Fiona Park, Shauna Blois, Tony Abrams-Ogg, Darren Wood, Dana Allen

#### Department of Clinical Studies

Dogs with pituitary-dependent hyperadrenocorticism (PDH) are at increased risk of thromboembolic disease, which may be due to a hypercoagulable state. Routine coagulation tests are insensitive for the diagnosis of hypercoagulability. Thromboelastography (TEG) provides a global assessment of coagulation, and is more sensitive than conventional coagulation tests for detecting hypercoagulability. TEG with platelet mapping (TEG-PM) allows assessment of platelet function in response to the agonists arachidonic acid and ADP.

The objective of this study was to evaluate dogs with PDH for evidence of hypercoagulability at the time of diagnosis and following treatment with trilostane. TEG-PM was performed in 12 dogs with PDH pre-treatment and repeated in 8 of these dogs post-treatment. TEG-PM was also performed on 40 healthy control dogs. Complete blood counts and coagulation profiles (PT, aPTT and fibrinogen) were also performed on all dogs.

Preliminary results for standard TEG show that MA and alpha-angle were significantly higher in PDH dogs than controls (p<0.0001 and p<0.05 respectively). After 3 and 6 months of treatment, MA was significantly lower (p<0.05 and p<0.01 respectively) in dogs with PDH compared to baseline. For TEG-PM, MA for the arachidonic acid assay was significantly higher in dogs with PDH (p<0.05) and did not change significantly following treatment. MA for the ADP assay was not significantly different between PDH dogs and controls. Dogs with PDH have evidence of hypercoagulability based on standard TEG, which significantly improves following trilostane treatment. TEG-PM results also suggest increased platelet reactivity to arachidonic acid, which does not change significantly with treatment.

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## POSTER #3

### Constitutive variation in hepatic expression of innate immune genes in healthy pigs

Snyman HN, Mavin S, Hammermueller JD, Hayes MA, Lillie BN

#### Department of Pathobiology

Infectious diseases are an important factor limiting the production, growth performance, economics and welfare of the global swine industry. Both environmental and genetic factors contribute to this multifactorial problem and the innate immune system represents a critical component in the defence against infectious disease, being particularly important in young animals. Identification of genetic defects in innate immune proteins would allow for selection for swine with improved disease resistance and growth performance. A number of single nucleotide polymorphisms (SNPs) have been identified within some innate immune genes of swine, some of which have been shown to be more prevalent in pigs with various common, economically important infectious diseases (enteritis, serositis, pneumonia) and/or pathogens. Using genome wide microarray technology we are studying constitutive hepatic gene expression to identify innate immune genes with variable constitutive hepatic expression. Agilent microarrays were performed on liver mRNA from 96 healthy market weight pigs. Constitutive variation was identified by determining gene expression ratios of the mean expression values of the top 50% of pigs to the mean expression values of the bottom 5% of pigs after excluding high expressing outliers. The promoter regions of innate immune genes with the highest expression ratios will be sequenced to identify promoter variants, with results confirmed by RT-qPCR. The discovery of these SNPs ultimately will lead to the development of a more complete genetic selection panel leading to increased disease resistance; which will increase production, promote animal health and welfare, and decrease use of antimicrobials.

## POSTER #4

### The role of hepcidin in regulation of iron balance in bats: A pilot study

Iga Stasiak

#### Department of Pathobiology

Hemochromatosis, or iron storage disease, has been associated with liver pathology and mortality in captive *Rousettus aegyptiacus*. Although evolutionary adaptation to low levels of iron in their diet has been implied, the physiologic basis for susceptibility has not been established. In humans, the regulatory hormone hepcidin influences iron absorption in the intestine, recycling by macrophages, and mobilization from hepatic stores. A deficiency or resistance to hepcidin has been implicated in human hereditary hemochromatosis and may play a role in *R. aegyptiacus*. A preliminary investigation into the role of hepcidin in iron metabolism in bats was performed. The coding gene sequence of the hepcidin gene was determined for two megachiropterans (*R. aegyptiacus*, *Eidolon helvum*) and one microchiropteran (*Desmodus rotundus*). The latter two species do not commonly develop hemochromatosis in captivity. Baseline blood and liver iron parameters were compared to those obtained 14 and 30 days after IM administration of iron dextran (25 mg/kg or 100 mg/kg) in two representatives of each of *E. helvum* and *R. aegyptiacus*. Hematologic parameters assessed included plasma ferritin, transferrin saturation, plasma iron, and a complete blood cell count (CBC). Levels of hepcidin gene expression were evaluated and liver biopsy samples were obtained at each of the three time points from all three species. Liver morphology and iron content were assessed using histopathology and atomic absorption spectrophotometry, respectively. Iron injection resulted in increased plasma ferritin and hepatic iron content in all species. Comparisons of hepcidin gene expression in a larger subset of the captive population are underway.

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## POSTER #5

### **Intranasal infection of SPF New Zealand white rabbits with *leporid herpesvirus-4*, a novel alphaherpesvirus of domestic rabbits**

**Janet Sunohara-Neilson, Eva Nagy, Susy Carman, Patricia Turner**

#### **Department of Pathobiology**

*Leporid herpesvirus-4* (LHV-4) is a novel alphaherpesvirus, recently identified as the causative agent of severe systemic disease with high morbidity and mortality in commercial and pet rabbits. The clinical course of infection is anecdotally reported to be rapidly progressive but the disease is not well described. To better characterize disease characteristics, progression, and pathogenesis, an infection trial was conducted using virus isolated from a pet rabbit. Sixteen young adult female New Zealand white rabbits were heavily sedated and intranasally inoculated with  $4.2 \times 10^4$  CCID<sub>50</sub> in culture media, 2 rabbits were sham inoculated with culture media, and 2 rabbits acted as untreated controls. Rabbits were observed for up to 22 days post-infection (dpi) to determine progression of clinical signs, viral shedding, and development of serum antibodies. Groups of 3 rabbits were euthanatized at 3, 5, 7, 14 and 22 dpi to evaluate gross and histologic lesions. The most significant clinical signs, including decreased food consumption, body weight losses, mild pyrexia, lethargy, serosanguinous nasal discharge, and laboured breathing occurred from 5-7 dpi, and affected 12/13 rabbits. Splenic necrosis and rhinitis with virus-induced syncytia and herpetic intranuclear inclusions could be observed at 5 dpi. Virus was isolated from nasal secretions from 2-10 dpi. At 11 dpi, rabbits were seropositive for LHV-4. This study has shown close correlation between the time course for clinical and laboratory infection of rabbits with LHV-4. We have also demonstrated the benefit of evaluating nasal secretions and serum antibody levels for ante mortem diagnosis.

## POSTER #6

### **Dog tales: Digital storytelling in two First Nations communities**

**Emily Brady, Paul Sockett**

#### **Department of Population Medicine**

The goal of this study was to determine if the PhotoVoice approach was effective and should be further developed as a tool to raise awareness of risks and prevention strategies for parasitic zoonotic diseases, dog aggression or other health issues in First Nations communities. The objectives of the study were to: assess the level of involvement of the community participants; assess the cultural sensitivity of the project process; assess the perceived suitability of the PhotoVoice approach; and assess the future use of PhotoVoice in the communities. Two workshops occurred and four PhotoVoice videos were created. These videos incorporated audio recordings and photographs, which included messages on the participants' experiences, emotions, concerns, and suggested solutions. Results show that the participants were involved, comfortable, and were given enough time and opportunity to participate. The majority of the participants felt that the two workshops were culturally sensitive; however, more emphasis on traditional methods and values for caring for dogs was requested and having a small group of representatives speak on behalf of the community was not congruent with First Nation beliefs. The participants learned more from viewing the videos than they did from the educational presentation or the group discussion. The PhotoVoice approach was effective in the two First Nations communities. Possible future uses include holding a full-scale digital storytelling workshop, showing the videos in the band office or at a community gathering, hiring a community member to show the videos door-to-door, and creating videos on other topics.

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**POSTER #7****Review of Canadian information on antimicrobial use and resistance in selected beef cattle pathogens****Ashley Gagne, Carolee Carson, Agnes Agunos, Scott McEwen****Department of Population Medicine**

Antimicrobial resistance (AMR) is a growing animal and public health concern. Antimicrobial use (AMU) is considered a major driver for AMR and AMU in livestock production is coming under increasing scrutiny. The objective of this study was to review Canadian information on AMR and AMU for treatment/prevention of common bacterial pathogens associated with bovine respiratory disease, neonatal enteritis and liver abscesses in order to identify potential implications to clinical efficacy and public health. A scoping study was conducted to identify relevant literature. Public health significance was assessed through categorizing antimicrobials according to Health Canada's classification system, drugs are ranked according to their importance in human medicine from Category I (very high) to Category IV (low). Calculating the mean prevalences of resistance across studies, little or no resistance ( $\mu = 0.25\%$ ; range = 0% to 1 out of 3 isolates) in *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* was identified to Category I antimicrobials. Resistance was documented for antimicrobials used to treat neonatal enteritis caused by *Escherichia coli*, increasingly from Category I (2.3%; 0-3.9%) to Category III (45.35%; 11.7-87%). No Canadian AMR data was available for neonatal enteritis caused by *Salmonella* and for *Fusobacterium necrophorum* and *Actinomyces pyogenes*, the causative agents of liver abscesses. In terms of public health importance, resistance levels to Category I antimicrobials was generally reported to be low (<4%). In terms of animal health, there were licensed or recommended antimicrobials available for all disease conditions studied, in which resistance in the associated bacterial pathogens was low (<4%).

**POSTER #8****Developing a cultural food safety program in Peel Region****Melissa Kim, Tony Camara****Department of Population Medicine**

The increased emergence of cultural foods, or foods from different ethnic backgrounds, has made it challenging for public health inspectors to recognize and make accurate risk assessments in food establishments. Research in secondary literature and informal interviews from key informants from Peel Public Health has revealed the need and associated benefits for a cultural food safety program. A logic model was created that delineated the key components necessary in a new cultural food safety program. The first component consists of an inspection procedure for inspectors to follow when encountering an unfamiliar cultural food. The second component consists of a health promotion strategy that entails a central database that will house fact sheets and other pertinent information about various techniques and preparation methods of cultural foods. Finally, the last component consists of educational in-house workshops for inspectors and food operators through partnerships with community agencies. This program aims to ensure public health inspectors have the necessary support to properly assess various cultural foods and improve communication strategies with food operators and owners. The long-term objective of this program is to reduce the incidence of foodborne illnesses by ensuring all cultural foods are safely prepared, stored and served for the general public.

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**POSTER #9****Guidelines for effective narrative use in health communication programs****Robert Koziarski****Department of Population Medicine**

Complex health information is difficult to communicate effectively to both the general public and policy makers. Narratives have been suggested as a way to make health information understandable for the public, while being more persuasive than statistical information alone. This purpose of this project was to develop guidelines for effective narrative use by practitioners, and to evaluate the use of such guidelines in health communication programs. A scan of recent literature was conducted, and guidelines for effective narrative use extracted. The internet was searched for programs utilizing narratives to communicate complex health information. Each program was evaluated for the use of guidelines previously extracted from the literature.

In total, 6 guidelines were taken from the literature were evaluated for their use in 7 health communication programs. All programs used multiple narratives, and demonstrated both internal consistency and external realism. Lacking in all but one program was the use of a deeply embedded scientific theme. Both plain-language and first-person narratives were seen in 4 programs.

Guidelines for effective narrative use are currently being used inconsistently in practice, with most programs lacking a clear scientific theme. The use of a specific guideline may be related to the objective of a given program. Future research should focus on the matching of guidelines to program objectives, while practitioners should ensure they are making use of current literature available.

**POSTER #10****Ecohealth and One Health in Ontario: Lessons learned and possibilities for action****Zee Leung****Department of Population Medicine**

There is a growing recognition that many public health issues are complex and can be best understood by examining the relationship between human health and the health of the larger ecosystem. EcoHealth and One Health have emerged as two approaches towards a more holistic understanding of health.

The goal of this study was to better understand how public health actors in Ontario are influenced by the holistic principles underlying EcoHealth and One Health, and what lessons could be learned from their experiences.

Ten participants from the broad public health sphere in Ontario were interviewed. Participants encompassed diverse perspectives including infectious disease, food systems, urban agriculture, and environmental health. Semi-structured interviews were administered, recorded, and transcribed.

Coding and thematic analysis were performed on transcripts totalling over 48,000 words. Analysis revealed a number of themes including discussions around health, the environment, barriers and support systems. Participants were found to be heavily influenced by holistic concepts couched in EcoHealth and One Health literature, despite being unfamiliar with these fields. Participants also identified governance and models of collaboration as key factors that could either inhibit or foster holistic and integrative practices.

This study provides some insight into the role of holistic and integrative philosophies in the practice of public health. A number of challenges towards operationalizing these ideas were identified. Further exploration is warranted into how various tools such as governance frameworks may allow EcoHealth and One Health to better inform the practice of public health in Ontario.

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**POSTER #11****Sperm swim-up separates X and Y-spermatozoa****Arshia Azizeddin, Fazl Ashkar, Tamas Revay, Allen King****Department of Biomedical Sciences**

The ability to select the sex of an offspring at the onset of conception remains among the most sought-after reproductive technologies to date. Separating X- and Y-chromosome bearing spermatozoa is one way to achieve this goal. Knowing that the X-chromosome is about 3.9% larger than the Y and also contains 2.9% more genetic material, we hypothesized that the genetic make-up and weight of the spermatocytes directly affects its speed and motility during swim-up in sperm TALP medium. To distinguish between and separate X and Y-bearing spermatozoa, 13 replicate samples of bovine sperm from 3 unrelated bulls were injected into capillary tubes divided into 8 segments and allowed to swim up for different time intervals during incubation at 38.5°C. Our observations showed that the ideal incubation time for obtaining the most motile spermatozoa in the top segment was about 45 minutes. Concentrated spermatozoa obtained from different zones of the swim-up column were fixed on slides and subjected to fluorescence in situ hybridization using X- and Y-chromosome specific probes. Chi-square test was used for statistical analysis. Control (unswum-up) samples did not differ from the expected 50/50 percent ratio of X- and Y-bearing sperm while the top 7th and 8th fractions showed significant enrichment of Y-sperm (60/40) in both bulls, indicating that Y-bearing sperm have a faster swim-up speed and travel further in the capillary. Therefore, after a short term swim-up in a capillary tube, there is an increased probability of selecting a Y-bearing enriched sperm fraction to fertilize oocytes with.

**POSTER #12****The role of MLH1 and MSH2 expression on tumour growth and drug resistance in human colorectal cancer cells****Amanda Barber, Kristen Lacombe, Brenda Coomber****Department of Biomedical Sciences**

Loss of genomic stability is associated with a variety of diseases, including cancer. One way in which a cell maintains its genome is through DNA mismatch repair, a family of enzymes which recognize and repair single base mismatches or insertions/deletions. Two important enzymes in this family are MLH1 and MSH2. To investigate the impact that MLH1 and MSH2 have on tumour growth, derivatives of the human colorectal cancer cell line CaCo2 were developed. siRNA was used to stably knock down expression of these proteins, as confirmed with western blot. Knockdown cells were xenografted into SHO hairless SCID mice and tumour growth was quantified. Both MLH1 and MSH2 knockdown cells showed increased tumour take, decreased tumour latency, and increased tumour growth rate compared to controls. Tumour pieces were used to develop cell lines, which maintained the MMR protein knockdown. Cells from the tumours tested negative for over 260 activating point mutations in oncogenes using a commercially available assay (Sequenom OncoCarta, sensitivity for detection of 10%). Cells from the tumours were then tested for resistance to the chemotherapeutic agent 5-fluorouracil. Preliminary results suggest that MLH1 and MSH2 knockdown cells may be resistant to apoptosis caused by exposure to 5-fluorouracil. These results suggest that levels of MLH1 and MSH2 influence the growth of cancer cells *in vivo*. However, despite evidence of progression in these knockdown cells, residual amounts of MLH1 and MSH2 may have been sufficient to prevent detectable point mutations in known mutation targets.

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## POSTER #13

### Enhanced chondrogenic differentiation of equine umbilical cord blood-derived mesenchymal stromal cells in membrane-based cultures

Midori Buechli, Jonathon Lamarre, Thomas Koch

#### Department of Biomedical Sciences

Mesenchymal stromal cells (MSCs) are multipotent cells that are capable of being differentiated towards chondrogenic, adipogenic and osteogenic lineages *in vitro*. MSCs derived from equine cord blood (eCB-MSCs) are a potential cell source for the repair of articular cartilage defects. Chondrogenesis of MSCs has traditionally been induced by micromass pellet culture system, whereby MSCs are centrifuged into pellets in polypropylene tubes, resulting in the formation of cell aggregates, to which differentiation media containing TGF- $\beta$ 3 is applied. However, surface tension and the formation of diffusion gradients within the pellet often results in cell death in its center as well as heterogeneous staining for collagens I, II and X.

Our lab has developed a protocol to differentiate eCB-MSCs towards a chondrogenic fate on fibronectin-coated polytetrafluoroethylene membrane inserts. Currently the micromass pellet system ( $2.5 \times 10^5$  cells/pellet) and the membrane-based system ( $2.0 \times 10^6$  cells/membrane) are being compared. Preliminary results have revealed that the cells show a chondrogenic response in both culture systems, as evidenced by positive proteoglycan staining. However, we hypothesize that the membrane-based cultures will show more homogeneous distributions of collagen II. Additionally, following collagenase digestion of the differentiated tissues, a step that would precede seeding onto a construct, the ratio of live cells/seeded cells is expected to be significantly higher in the membrane-based culture system. Thus, we anticipate a new method of enhancing *in vitro* chondrogenesis that can easily be integrated into cartilage engineering strategies.

## POSTER #14

### Expression of Y chromosome linked genes in bovine pre-implantation embryos

Jeffery Caudle

#### Department of Biomedical Sciences

Different growth rates for male and female embryos are suggesting that Y chromosome linked genes may be active and play a role in the development of the early embryo. mRNA for SRY which is a Y-linked gene involved in testis development at the 6-7 weeks stage of development has been identified in the pre-implantation bovine embryo indicating that sexual dimorphic gene expression occurs early in development. Therefore, the aim of this study was to further investigate the transcription of Y-linked genes in the pre-implantation bovine embryo. mRNA was extracted from 30 pools of 5 *in vitro* produced bovine blastocysts of unknown sex and reverse transcribed to cDNA by standard procedures. Primers were designed for four Y chromosomal genes (USP9Y, ZFY, EIF1AY, and DDX3Y). Each pool of cDNA was screened for the presence of mRNA from the four genes by polymerase chain reaction and visualized using gel electrophoresis. cDNA from female kidneys and testis were used as controls. Evidence of transcription for all four genes was found in the pools of cDNA. It was concluded that mRNA is actively transcribed from the Y chromosome at or prior to the blastocyst stage. These genes act as transcription or translation factors that may be crucial for male development as early as the blastocyst stage. While the full effect of these transcripts is unknown their presence introduces the possibility of a second method of embryo sexing using RNA as well as using these genes as developmental markers in male embryos.

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**POSTER #15****Effect of DCA treatment on Bcl-2 family protein expression in colorectal cancer cells****Leanne Delaney, Brenda Coomber****Department of Biomedical Sciences**

Dichloroacetate (DCA) is a metabolic reprogramming agent that has been suggested as a potential cancer therapy. However, our laboratory has shown that some colorectal cell lines may not react to treatment as predicted, but the mechanism by which DCA alters these cells' behavior is currently unknown. To understand the reaction of these cells to DCA treatment, one area of interest is the Bcl-2 family proteins, which are involved in cell survival and death pathways. These proteins have in common certain domains, the combination of which determines whether the protein acts to enhance or prevent apoptosis. Bcl-2 proteins can act either directly on the mitochondrial outer membrane to initiate apoptosis, or indirectly through interactions with each other. It is therefore of interest to study the effect of DCA on their expression levels relative to one another, as certain ratios may act as a predictor of whether the cell will die or survive. We used cell culture and western immunoblotting to examine changes in key members of the Bcl-2 family in several human colorectal cancer cell lines, particularly in HCT116 cells. Mcl-1 is a Bcl-2 family protein that may act to either inhibit or enhance apoptosis, depending on the isoform present. Preliminary results suggest that the presence and levels of Mcl-1 isoforms in HCT116 vary and that these effects are exaggerated when cells are treated with DCA. Further research may provide insight into the reason why some human cancer cell lines are not susceptible to DCA-induced cell death.

**POSTER #16****The objective assessment of pain in dairy cattle with clinical mastitis through the use of pain pressure algometers and rumination collars****Colleen Fitzpatrick, Nria Chapinal, David Kelton, Todd Duffield, Trevor DeVries, Christins Petersson-Wolfe, Ken Leslie****Department of Population Medicine**

As Clinical mastitis has negative effects on profitability and cow welfare, with significant discomfort and pain. The objective of this study was to evaluate the use of various technologies to objectively assess pain and discomfort in dairy cattle experiencing experimentally-induced clinical mastitis. This study involved 42 (19 primiparous and 23 multiparous) lactating Holstein cows enrolled in a lipopolysaccharide (LPS) endotoxin challenge study. Each animal was challenged in one rear mammary quarter by intramammary infusion with 25 µg or 100 µg of *E. coli* LPS. Subsequently, all animals received a placebo injection, as they were all control subjects for a NSAID drug trial. The animals were monitored for two days prior to, and two days following, the intramammary challenge. Pain sensitivity was monitored using a handheld pain pressure algometer, and rumination activity was monitored using rumination collars. There was an effect of time on pain sensitivity, as well as parity, as  $2.9 \pm 0.5$  l bf ( $p = 0.0002$ ) more pressure could be placed on the quarters of multiparous cows as compared to primiparous cows. For rumination, there was also an effect of time ( $p < 0.0001$ ), the amount of endotoxin used ( $p < 0.0001$ ) and parity ( $p = 0.001$ ), when observed on an hourly basis. These results indicate the potential for using continuous measurement of rumination and pain pressure sensitivity for objective assessment of pain due to illness in cases of clinical mastitis.

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## POSTER #17

### Type I and Type II cytokine production in Ontario dairy cows

Jacqueline Gallienne, Kathleen Thompson-Crispi, Claire Martin, Marlene Paibomesai, Bonnie Mallard

#### Department of Pathobiology

Breeding dairy cattle for enhanced immune response (IR) has been demonstrated to decrease disease occurrence. Cows with high or enhanced type-2 antibody-mediated (AMIR) and type-1 cell-mediated IR (CMIR) have been shown to have lower occurrence of disease. The adaptive IR traits AMIR and CMIR have been found to be negatively genetically correlated. The objective of this study was to identify mechanisms that may contribute to these diverse IR phenotypes. The hypothesis was cows with High AMIR will produce more type 2 cytokine, IL-4, compared to High CMIR. Further, High CMIR cows will produce more type 1 cytokine, IFN $\gamma$ , upon stimulation. Cows previously identified as High AMIR Low CMIR (H-AMIR) and High CMIR Low AMIR (H-CMIR) were used in this study. Peripheral blood mononuclear cells (PBMC) were isolated and stimulated with the mitogens Con-A and PHA. Production of IL-4 and IFN $\gamma$  were measured at 24, 48, 72 and 96 hours post stimulation by ELISA. Results of the study show H-AMIR cows produced more IL-4 at all time points to both mitogens compared to H-CMIR cows, significant at 48hrs for Con-A and 72hrs for PHA. H-CMIR cows produced more IFN $\gamma$  at all time points than H-AMIR, significant at 72hrs for Con-A. Overall, stimulation with Con-A produced significantly more IL-4 and IFN $\gamma$  compared to PHA. This suggests H-AMIR cows may have an inherent bias towards type-2 response which could provide enhanced protection to extracellular pathogens and H-CMIR towards intracellular pathogens. Con-A may be a more suitable mitogen for stimulating bovine PBMC.

## POSTER #18

### Prevalence of *Tritrichomonas foetus* in cats in Southern Ontario

Ansarah Hosein, Stephen Kruth, David Pearl, Danielle Richardson, Jocelyn Maggs, Andrew Peregrine

#### Department of Pathobiology

*Tritrichomonas foetus* is a protozoan parasite that has recently been associated with chronic diarrhea in cats. The objectives of the study were to: (1) Determine the prevalence of *T. foetus* carriage in cats in Southern Ontario. (2) To determine associations between the presence of *T. foetus* and potential pet management, health, and demographic risk factors.

**Methods:** A cross sectional study including 140 cats from a cat clinic in Guelph (CCG), 26 cats from a humane society in Guelph (HSG) and 30 cats from a cat show in London, ON was performed. Cats that were over the age of 12 weeks were eligible for inclusion. Demographic and risk factor information were assessed through a client questionnaire. Rectal swabs or freshly voided feces were collected from all cats and the In Pouch™ TF (feline) culture method was used to determine the presence of *T. foetus*.

**Results:** The prevalence of *T. foetus* infection and 95% CI's in cats from the three sample populations was estimated at 0.71% (95% CI: 0.02 – 3.92 %; n=140) from the CCG, 3.85% (95% CI: 0.10-19.64%; n= 26) from the HSG and 30 % (95% CI: 14.73 – 49.4%; n= 30) from the cat show.

**Conclusion:** Based on three sampling sources, the prevalence is quite variable among cats in Southern Ontario. *T. foetus* infection appears to be more common in cats from cat shows which may be due to breed or group housing. Further sampling from cat shows is on-going to provide adequate statistical power to address this issue.

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**POSTER #19****Chronic, low-grade, systemic inflammation enhances the progression of epithelial ovarian cancer (EOC) *in vivo*****Amanda Kerr, Lisa Kellenberger, James Greenaway, Jim Petrik****Department of Biomedical Sciences**

An inflammatory environment can enhance tumorigenesis, although specific mechanisms are not well understood. Epidemiological data have described a link between chronic inflammatory diseases, such as diabetes or obesity, and ovarian cancer suggesting that systemic inflammation can increase the risk of EOC. The systemic inflammation associated with these chronic diseases may potentially synergize with the local ovarian inflammation that occurs during every ovulation.

The purpose of this study was to identify the impact of prolonged exposure to chronic low-grade inflammation on EOC tumor progression *in vivo*. We hypothesized that exposure to chronic inflammation would enhance the growth of epithelial ovarian tumors by increasing cell survival, angiogenesis and metastatic capability.

Chronic inflammation was induced in C57Bl6 mice via daily intraperitoneal injections of bacterial endotoxin lipopolysaccharide (LPS). Ovarian tumors were induced with an orthotopic injection of tumorigenic murine ovarian surface epithelial (ID8) cells. At 60 days post tumor-initiation (PTI) ovarian tumors from mice treated with LPS were significantly larger than vehicle controls. This trend continued 80 days PTI and preliminary data suggest that LPS-induced systemic inflammation causes accelerated disease progression and decreased time to death.

Evaluation of the relationships between chronic, systemic inflammation and the progression of EOC may lead to the development of anti-inflammatory treatment approaches and could provide insight into the role of inflammation in the progression of other human cancers. Additionally, as the rate of metabolic disorders increases in the Western world the results from this work may facilitate the advancement of complimentary therapeutic interventions for all related cancer types.

**POSTER #20****Regulation and role of cytochrome P450 2A5, CYP2A5, in protection against bilirubin toxicity****Sangsoo (Daniel) Kim, Monica Antenos, Gordon Kirby****Department of Biomedical Sciences**

Bilirubin (BR) is the end product of heme catabolism with cytoprotective potential at physiological levels and cytotoxic potential at supraphysiological levels. The objective of this study was to examine the regulation and role of cytochrome P450 2A5 (CYP2A5) in protection against BR toxicity. Primary mouse hepatocytes were exposed to increasing concentrations of BR (1-100  $\mu$ M). BR exposure significantly increased CYP2A5 mRNA and protein levels, 2- and 3- fold, respectively at 100  $\mu$ M, in a dose-dependent manner. Previous studies show that BR also induces apoptosis in mouse hepatocytes. BR induced caspase-3 activation in primary mouse hepatocytes. To investigate the role of CYP2A5 in BR toxicity, a CYP2A5-V5 vector was overexpressed in mouse hepatoma Hepa 1-6 cells, which lack endogenous CYP2A5. Caspase-3 activation was also determined in BR-treated Hepa 1-6. Exposure to 100  $\mu$ M BR induced caspase-3 activation, but the induction was partially blocked by 30% in CYP2A5 overexpressing Hepa 1-6. Finally, to investigate if the regulatory mechanism of CYP2A5 by BR is transcriptional, a CYP2A5 promoter reporter construct was transiently transfected into both primary mouse hepatocytes and Hepa 1-6 for luciferase activity assays. BR had no effect on luciferase activity in either cell types. BR induces CYP2A5 mRNA and protein expression in primary mouse hepatocytes, however our data suggests that the mechanism may not be transcriptional. Also overexpression of CYP2A5 in Hepa 1-6 partially blocked caspase-3 activation. The results suggest that BR regulates CYP2A5 and this enzyme may play a cytoprotective role in BR-induced apoptosis.

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## POSTER #21

### **'Redtail': bacterial dermatitis in seahorses**

**Véronique LePage, Elena Contador, John Lumsden**

#### **Department of Pathobiology**

Seahorses are part of the family of fish, syngnathidae, which also includes pipefish and seadragons. Many seahorses and their relatives are currently listed as threatened or endangered under appendix II of the Convention on International Trade of Endangered Species (CITES). Some syngnathid species provide a serious challenge in captivity for aquarists while overexploitation of syngnathids continues in the wild. Research related to syngnathid disease is slowly emerging; however, publications are relatively few when compared to other captive bred fish. It is therefore critical that we understand disease related to husbandry, as captive rearing of syngnathids may be an invaluable tool in relieving pressures on wild populations. A twelve-year retrospective study of syngnathid diseases observed at the Toronto Zoo (1998-2010) was undertaken, comprising of over 170 cases. The major reoccurring problems were; erosive to ulcerative bacterial dermatitis, bilaterally symmetrical myopathy, mycobacteriosis, gas bubble disease, cryptosporidiosis and several neoplasms. Bacterial dermatitis was the most common diagnosis, occurring in over thirty percent of cases. This dermatitis appears to be associated with the clinical condition 'redtail'. Further investigation of the bacterial dermatitis, which is a significant clinical concern, was therefore undertaken. We hypothesized that the filamentous bacterium seen in the erosive/ulcerative dermatitis lesions by light microscopy are associated with the development of 'redtail'. Bacterial isolation and biochemical characterization, immuno-histochemistry and polymerase chain reaction using 16S rDNA primers were used to identify lesion-associated bacteria. To date, lesions have been found to be most commonly associated with bacteria of the family Flavobacteriaceae, Vibrionaceae, and Bacillaceae.

## POSTER #22

### **Kinetics of type-I and type-II gene expression, cytokine production and cell proliferation in dairy cattle**

**Claire Martin, Marlene Paibomesai, Seyedmehdi, Jackie Gallienne, Kathleen Thompson-Crispi, Bonnie Mallard**

#### **Department of Pathobiology**

Dairy cattle can be classified as High (H), Average (A) or Low (L) immune responders based on both antibody-mediated immune response (AMIR), dominated by type II cytokines, such as IL-4, and the cell-mediated immune response (CMIR), dominated by type I cytokines, such as IFN- $\gamma$ . The immunological and genetic mechanisms that lead to these unique bovine immune response phenotypes remain unclear. The objective of this study was to determine how biased immune response phenotypes, H-AMIR/L-CMIR and H-CMIR/L-AMIR, influence peripheral blood mononuclear cell (PBMC) cytokine gene expression, cytokine production, and cell proliferation upon stimulation with the T-cell mitogen, concanavalin A (conA). Relative expression was determined by RT-qPCR at 4, 16, 24, 48 hours for both AMIR (*Tbx21*, *IFNG*) and CMIR (*GATA3*, *IL4*) transcription factor and cytokine genes. Cytokine concentrations (IFN- $\gamma$  and IL-4) and PBMC proliferation were determined by ELISA and water soluble tetrazolium salt (WST-1) proliferation assay at 24, 48, 72, 96 hours. Results show distinct kinetics of H-AMIR/L-CMIR and H-CMIR/L-AMIR responses from 4-96 hours post-stimulation. *Tbx21* and *GATA3* expression showed no change over the time course in each group. Cytokine mRNA expression peaks at 4 hours, followed by the downstream peak in cytokine production at 48 hours for IL-4 and 72 hours for IFN- $\gamma$ . PBMC proliferation increased up to 72 hours for both phenotype groups, but no differences between groups was seen. Differential gene expression and cytokine production of IL-4 and IFN- $\gamma$  in H-AMIR/L-CMIR and H-CMIR/L-AMIR groups may indicate a mechanism for the difference in H-CMIR and H-AMIR immune response phenotypes.

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## POSTER #23

### The epidemiology of pathogenic and non-pathogenic *Brachyspira* species in Ontario layers

Genet Medhanie, Michele Guerin, Scott McEwen, Durda Slavic, Lloyd Weber, Leanne Cooley, Scott Houghton, Babak Sanei

#### Department of Population Medicine

Faecal staining of eggshells due to chronic diarrhoea caused by the bacterium, *Brachyspira*, has been reported. Down-grading of eggs due to faecal-stained shells has occurred in Ontario for two decades; however, the layer industry has recently been under pressure from the Canadian Food Inspection Agency to reduce the number of dirty eggs.

Our objectives were to investigate the presence of *Brachyspira* in Ontario layer flocks; compare the prevalence of *Brachyspira* in flocks with a dirty egg problem (dirty egg cohort) to flocks without a dirty egg problem (clean egg cohort); and identify factors associated with the presence of *Brachyspira*. Fresh pooled fecal samples were collected from flocks and submitted to the Animal Health Laboratory. A face-to-face interview was administered to participating producers. Logistic regression with a random effect for farm was used to identify risk factors.

We collected 534 samples from 89 flocks. Forty-two flocks (47%) were positive for *Brachyspira*; the percentage of positive flocks in the dirty egg cohort (79%) was significantly higher than in the clean egg cohort (21%). The pathogenic species *B. pilosicoli* was isolated from 26% of *Brachyspira*-positive flocks, although there was no significant difference in the percentage of *B. pilosicoli*-positive flocks between cohorts. Flocks from multi-age farms were positively associated with the presence of *Brachyspira* species. The presence of *Brachyspira* was also significantly higher in older flocks than in younger ones. This study provides valuable information on the association of *Brachyspira* species with factors that contribute to down-grading of eggs.

## POSTER #24

### The possible role of *Clostridium perfringens* in equine typhlocolitis

Iman Mehdizadeh Gohari, John Prescott

#### Department of Pathobiology

Typhlocolitis is a serious and sometimes fatal disease in horses; up to 60% of fatal cases have no known cause. *Clostridium perfringens* has been associated with disease but its importance is still unclear. To investigate the possible role of *C. perfringens*, fecal samples from horses typhlocolitis were examined quantitatively for *C. perfringens*, as well as for *C. perfringens* alpha, beta2 toxin, and enterotoxin by ELISA; isolates were genotyped for known toxin genes. Fifty five fecal samples obtained from horses with acute typhlocolitis were examined. Feces that tested positive for *Salmonella* or *C. difficile* or its toxins were excluded from study. Three to five *C. perfringens* isolates per sample were analyzed via multiplex real-time PCR for the toxin genes: *cpa*, *cpb*, *cpb2*, *cpe*, *etx*, *itx*, *netB* and *tpel*. No *Salmonella* were detected but *C. difficile* toxins were present in four samples. *Clostridium perfringens* was isolated in moderate numbers in 22 cases. The atypical *cpb2* gene was present in 11 cases and the *cpb* toxin gene in one case; no *cpe* (enterotoxin) genes were identified. Other clostridia including *C. bifermentans* (5 cases), *C. butyricum* (3 cases), *C. tertium* (3 cases), and *C. innocuum* (1 case) were also recovered and identified using MALDI-TOF MS. Further work is examining the toxicity of the *C. perfringens* and other clostridia for an equine cell line *in vitro*. This study has contributed to understanding the role of *C. perfringens* in severe colitis of horses.

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**POSTER #25****Sertoli cell proliferation and thyroid hormone in prepubertal ram lambs****Olutobi Oluwole, Ann Hahnel, Pawel Bartlewski****Department of Biomedical Sciences**

Studies in rodents have suggested that serum Thyroid Hormone (TH) contributes to stopping Sertoli cell proliferation during the first wave of spermatogenesis, and thus affects maximum sperm output of testes. It is not clear that TH plays a similar role in all species under normal conditions. The purpose of this research was to compare serum TH and Follicle Stimulating Hormone (FSH) levels to Sertoli cell proliferation and examine expression of TH receptor (THR) in ram testes during first wave of spermatogenesis in normal (euthyroid) ram lambs. The experimental design was novel in that weekly biopsies and blood were collected to compare hormone levels (radioimmunoassay) to Sertoli cell divisions (anti-PCNA antibody) and differentiation (seminiferous tubule lumen formation and spermatogenesis) in the same animals during the first wave of spermatogenesis. We found that: the number of dividing Sertoli cells was already decreasing at the beginning of the first wave of spermatogenesis and continued until spermatocytes were seen in 90% of tubules; serum T3 and T4 decreased after the decrease in Sertoli cell divisions; decrease in T3 and T4 occurred after lumens had begun to form in the seminiferous tubules. 2 isoforms of THR were expressed at the early stage while only 1 isoform was expressed by the end of the first wave of spermatogenesis; there was no significant change in serum FSH during first wave of spermatogenesis. Although higher levels of serum TH are present as Sertoli cells stop dividing and differentiate, other mechanisms might be involved in stopping Sertoli cell proliferation.

**POSTER #26****Circadian orchestration of normal cardiac proteome is essential for heart structure and function****Peter Podobed, Michael Bennardo, Jeremy Simpson, Tami Martino****Department of Biomedical Sciences**

**Objective:** Heart structure and function depends on temporal protein expression, therefore we conducted a systematic analysis of the cardiac circadian proteome. We hypothesize that normal hearts have a circadian rhythm in protein expression and that rhythmicity is essential for vital cardiac processes.

**Methods:** Murine (C57Bl/6) hearts were collected every 4h starting at 1h before lights on, for 24h across the light/dark cycle, n=3/timepoint. Soluble cytoplasmic proteins were isolated with Urea/CHAPS lysis buffer. The proteome was detected using two-dimensional difference in gel electrophoresis (2D-DIGE), Typhoon 9410 scanner and DeCyder software. Proteins were sequenced with tandem mass spectrometry and MASCOT database followed by validation with Western blots. Normal circadian rhythms were disrupted by alteration of the light/dark cycle and protein expression was measured with Western blots. Left ventricular developed pressure (LVDP) was measured with the Langendorff apparatus. Energy consumption of isolated cardiomyocytes was determined by actomyosin MgATPase assay.

**Results:** We were the first to demonstrate the normal cardiac circadian proteome. Cycling proteins identified to-date were important for metabolism, stress response and growth (e.g. ALDH4A1, STIP1, IGF2). Disruption of normal circadian rhythms altered expression of these proteins. Actomyosin MgATPase assay revealed reduction in ATP consumption in sleep (light) vs. wake (dark). Langendorff analysis revealed increased LVDP in wake compared to sleep. This difference was lost after circadian rhythm disruption.

**Conclusion:** There is a circadian rhythm in expression of essential cardiac proteins. In the future we will further characterize cardiovascular circadian proteome and use cardiomyocyte-specific clock mutant mice to mechanistically study regulation of circadian proteome.

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**POSTER #27****Modification of commercial replacement layer pullet husbandry to enhance live *Eimeria* vaccination****Kayla Price, Mike Petrik, Linnea Newman, John Barta****Department of Pathobiology**

Coccidiosis continues to burden the poultry industry. With drug-resistance concern alternate methods of control other than antimicrobials are urgently needed. Practically, live *Eimeria* vaccination stimulates protective immunity resulting from the first small dose of vaccinal oocysts and enhanced through cycling parasites fecal-orally. Without cycling, only limited protective immunity may develop and pullets risk coccidiosis when challenged. Cycling is influenced by poultry husbandry. Pullets are frequently reared in a tiered cage system with mesh floors to allow feces to fall onto a conveyor belt. Some producers have used paper coverings from hatch for 10 days to promote cycling; yet these birds frequently suffered from coccidiosis in the layer barn, possibly due to paper decomposition (inadequate cycling). An alternate method to promote cycling was proposed using a fibre tray covering a percentage of the cage floor and lasting approximately five weeks. Commercial pullets were vaccinated with a functional dose of a live vaccine by oral gavage and reared under simulated commercial conditions with 0, 20 or 40% floor coverage for 6 weeks from hatch. At 42 days, birds were challenged with mixed *Eimeria* species. Lesion scores and oocyst output were subsequently determined. Lesion scores were relatively mild and the 20% and 40% coverage groups that had lower lesion scores also had proportionately lower oocyst shedding after challenge. Overall, as floor coverage percentage increased the total oocyst output and corresponding lesion scores decreased. Increased access to oocysts over five weeks allowed for adequate cycling and improved protective immunity.

**POSTER #28****Evaluation of methods for extraction of DNA from *Encephalitozoon cuniculi*****Stephanie Reabel, Scott Weese, John Barta****Department of Pathobiology**

*Encephalitozoon cuniculi* is a microsporidial pathogen that causes subclinical infections and severe clinical disease in domestic rabbits. Cell culture and serology are commonly used for diagnosis, but these methods are labourious, and are generally lacking in sensitivity and specificity. PCR has been reported to have low sensitivity for detection of *E. cuniculi* spores from rabbit urine; however published studies have not reported assessment and validation of DNA extraction or PCR methods. The objective of this study was to assess the efficacy of different DNA extraction methods. Combinations of 6 DNA extractions kits and 4 spore disruption methods were performed in on a suspension containing 100,000 spores/sample. DNA concentrations ranged from 1.42-92.0 ng/ $\mu$ l, with most methods producing <5 ng/ $\mu$ l. Only one method (enzymatic disruption followed by prepGEM™ kit) resulted in a concentration of >90 ng/ $\mu$ l. This method was analysed using a spore dilution series including 100,000, 10,000, 1,000, 100 and 10 spores/sample to determine the lower limit of detection (LOD). Final DNA concentrations ranged from 2.05-45.96 ng/ $\mu$ l. Real-time PCR results indicate that DNA extraction using prepGEM™ and enzymatic disruption is a sensitive method of detection of *E. cuniculi* spores, with an LOD of 100 spores. Since DNA extraction methods can result in markedly different DNA yields, poor efficiency of extraction could account for reports of poor sensitivity of PCR. Studies using evaluated and optimized DNA extraction and PCR methods are needed to understand the epidemiology of this pathogen and facilitate rapid and accurate diagnostic testing.

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**POSTER #29****Investigating the dose-dependent signaling of TGF $\beta$  and its effects on angiogenesis****Amy Richard, Alicia Vilorio-Petit****Department of Biomedical Sciences**

Transforming growth factor-beta (TGF $\beta$ ) is an important signaling molecule, which regulates several cellular processes including angiogenesis. However, its effects on angiogenesis are complex, with TGF $\beta$  promoting angiogenesis only at low concentrations. Evidence suggests that downstream signaling pathways of TGF $\beta$  may be activated in a dose-dependent fashion. In fact, the Vilorio-Petit lab has shown that the Par6 polarity pathway gets preferentially activated at low concentrations. Considering the different cellular effects of downstream signaling pathways, we propose that TGF $\beta$  may modulate its effects on angiogenesis via differential activation of the canonical Smad and non-canonical Akt, FAK and Par6 polarity signaling pathways.

Based on this premise, Bovine aortic endothelial cells were treated with a range of TGF $\beta$ 1 concentrations, from 0.01 to 5 ng/mL. The activation patterns of canonical and non-canonical signaling pathways were studied via Western blotting; with the use of phospho-specific antibodies against Smad2, Akt and FAK. Preliminary results reveal that lower concentrations of TGF $\beta$ 1 (0.01 to 0.5 ng/mL) cause preferential activation of Akt and FAK, while, high concentrations (5 ng/mL) cause preferential activation of Smad2. These results suggest the effect of TGF $\beta$  on angiogenesis is mediated through its ability to preferentially activate a subset of downstream signaling pathways when present at high versus low concentrations. Although dose-dependent Smad activation has been demonstrated previously in endothelial cells, this is the first study to investigate the potential dose-dependent activation of non-canonical signaling pathways; in hopes of linking their differential activation to the ability of TGF $\beta$  to act as an angiogenic inhibitor versus stimulator.

**POSTER #30****Detection of maedi visna virus infection in Ontario sheep flocks****Lisa Santry, Sarah Wootton, Denise Yates, Susy Carmen, Beverly McEwen, Jocelyn Jansen, Paula I. Menzies****Department of Pathobiology**

Maedi visna virus (MVV) is a lentivirus of small ruminants that causes chronic pneumonia, wasting and poor milk production in adult sheep. Maedi visna (MV) disease manifestation can take 3 to 5 years, making it difficult to control. Since MV is common in Ontario flocks, the Ontario Sheep Marketing Agency offers an accreditation program<sup>1</sup> to assist producers and veterinarians in their effort to eradicate MV from infected flocks. The test used by the program was developed by the Canadian Food Inspection Agency (CFIA), which detects the presence of antibodies to MVV, in order to cull all infected animals. Continued access to this test is in doubt, therefore the objective of this study was to compare the specificity and sensitivity of 6 commercially available ELISA kits to the CFIA ELISA using sera from Ontario sheep flocks. Furthermore, a PCR-based assay that amplifies a well-conserved 1211bp region of the MVV genome was employed as an additional comparative method. 12 Ontario flocks were screened for MVV by the CFIA ELISA. A total of 150 positive and 150 negative randomized blood samples were then collected from these farms and evaluated using the various tests. Of the 250 samples tested, 18 CFIA positive samples were negative by PCR, while 6 CFIA negative samples were positive by PCR. PCR amplification of this region has allowed for sequencing and phylogenetic analysis of Ontario MVV strains. This information can be used to enhance currently available tests and aid in the development of new tests.

<sup>1</sup>[http://www.uoguelph.ca/~pmenzies/mv/OMVFSP\\_Program.htm](http://www.uoguelph.ca/~pmenzies/mv/OMVFSP_Program.htm)

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## POSTER #31

### Prophylactic treatment with Toll-like receptor ligands enhances host immunity to avian influenza virus in chickens

Michael St. Paul, Leah Read, Éva Nagy, Shayan Sharif

#### Department of Pathobiology

Avian influenza viruses (AIV) pose a threat towards the health of both poultry and humans. To interrupt the transmission of the virus, novel prophylactic strategies must be considered which may reduce the shedding of AIV. One potential approach being the use of Toll-like receptor (TLR) ligands to promote cell activation and the production of cytokines. TLR ligands have been employed as prophylactic treatments to enhance host resistance to pathogens both in mammals and chickens. Therefore, the present study was conducted to determine whether TLR ligands may be used prophylactically in chickens to enhance host immunity to AIV. Chickens received intramuscular injections of either low or high doses of the TLR ligands poly I:C, lipopolysaccharide (LPS) and CpG ODN. Twenty-four hours post-treatment, chickens were infected with the low pathogenic avian influenza virus H4N6, and both oropharyngeal and cloacal virus shedding were assessed on days 4 and 7 post-infection. To identify potential correlates of immunity, spleen and lung were collected on days 2, 4 and 7 post-infection for RNA extraction. The results of the virus shedding titration assay suggest that the TLR3 ligand, poly I:C, conferred the greatest AIV immunity compared to control birds, followed by CpG ODN and LPS. Furthermore, transcriptional analysis of gene expression in the spleen and lungs suggest IFN- $\alpha$ , IL-8 and IFN- $\gamma$  as correlates of immunity. In conclusion, TLR ligands, have the ability to enhance host protection against AIV, and future studies may consider exploring the combinatory effects of poly I:C prophylaxis in conjunction with AIV vaccination.

## POSTER #32

### Circadian genetic and protein biomarkers of cardiovascular disease

Elena Tsimakouridze, Peter Podobed, Allison Mackay, Heather Chin, Jeremy Simpson, Patricia Huether, Monica Antenos, Jon LaMarre, Ron Johnson, Marty Straume, Tami Martino

#### Department of Biomedical Sciences

**Introduction:** Cardiovascular physiology is regulated by circadian rhythms in mammals, including humans. Examining changes in murine cardiac gene expression over 24hrs provides novel insights into our understanding of cardiac structure/function and remodelling in cardiovascular disease (CVD).

**Methods:** We used a Transverse Aortic Constriction (TAC) model of pressure-overload induced cardiac hypertrophy. We collected hearts from SHAM vs TAC C57Bl/6 mice starting 1h before lights on, then every 4h for 24hrs. RNA was isolated for microarrays and RT-PCR. We created a de-novo algorithm, DeltaGene, to identify circadian genes from microarray data as potential biomarkers of CVD. Using a second sample set, we purified proteins for Western blots to examine rhythmic protein cycling.

**Results:** We identified 300 genes that exhibited time-of-day difference expression in SHAM vs. TAC hearts by microarray and DeltaGene analysis. We selected 20 of the genes with 1.4-fold change and validated by RT-PCR. Some genes at 8-weeks post-TAC showed changes in regulation across the 24h cycle, while others at specific times-of-day. The latter are newly discovered biomarkers of CVD. We determined that these could be CVD progression markers by comparing 1- vs. 8-week TAC. Because proteins are ideal for diagnostic tools, we examined these novel biomarkers for protein cycling and found significant differences for 4 proteins. Mechanistically, we examined promoter regions of new biomarkers for E-box elements, which could indicate *Clock/Bmal* transcriptional regulation.

**Conclusion:** We identified novel biomarkers of CVD at gene and protein level by applying circadian concepts. These CVD biomarkers could be used for diagnosis, prognosis and treatment.

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## POSTER #33

### Post-hospital discharge procedure specific surgical site infection (SSI) surveillance in small animal patients

Rylen Turk, Ameet Singh, J. Scott Weese

#### Department of Pathobiology/Clinical Studies

Surgical site infections (SSIs) are an inherent risk of any surgical procedure and have been increasing concurrently with the international epidemic of methicillin-resistant (MR) staphylococcal infections. The objectives of this study were 1) Describe the incidence of SSI in small animals undergoing surgical procedures at the Ontario Veterinary College Health Sciences Centre (OVCHSC) over a 1-yr period, 2) Describe and compare procedure-specific SSI rates and 3) Identify risk factors for development of SSI.

SSIs were identified in 20 out of 570 surgeries overall; 12/271 (4.4%) orthopaedic procedures, 8/239 (3.3%) soft tissue procedures, and 0/54 neurological or ophthalmologic procedures ( $P=0.27$ ). SSI occurred in 11/195 (5.6%) of procedures associated with a surgical implant versus 9/375 (2.4%) of procedures not associated with a surgical implant ( $P=0.057$ ). Among orthopedic procedures, the SSI rate for tibial plateau leveling osteotomy (TPLO), a procedure anecdotally associated with a high rate of MRSP infections in many facilities, was 5/55 (9%). Only 8 of the 20 cases of SSI were recorded in the medical record, showing a 60% rate of underreporting. MR staphylococci predominated in cases where cultures were performed, particularly MR *S. pseudintermedius*, which accounted for 6/10 cases.

The variable and sometimes high rates of MR-staphylococcal specifically *Staphylococcus pseudintermedius* SSI in veterinary hospitals internationally, the morbidity and mortality associated with those infections and concerns about antimicrobial use in animals indicate that ongoing active surveillance as well as underreporting of cases should be considered part of an SSI (and MRSP) control program.

## POSTER #34

### Characterization of $\alpha$ -adrenergic receptor function in 3<sup>rd</sup> vs 1<sup>st</sup> order mesenteric veins of normal male SD rats

Shawn Veitch

#### Department of Biomedical Sciences

The venous system contains ~70% of total blood volume, with ~25% located within the mesenteric veins and venules. A major regulator of venous capacitance is the SNS; unfortunately the mechanisms involved in SNS mediated regulation of venous tone are not well established. For this reason, the contribution of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor activity involved in mesenteric vein contractility was investigated, focusing on branching order. This was accomplished by examining the effects of endogenously released NE through electrical field stimulation (EFS), and of topically applied norepinephrine (NE) and phenylephrine (PE), in isolated 3<sup>rd</sup> and 1<sup>st</sup> order mesenteric veins (MV). EFS and NE had similar effects in both 3<sup>rd</sup> and 1<sup>st</sup> order MV. PE however appeared to be a more potent agonist in 3<sup>rd</sup> order MV compared to 1<sup>st</sup>. Further analysis of receptor activity was done through the use of the selective  $\alpha_1$ -antagonist, prazosin, and the non-selective  $\alpha$ -antagonist, phentolamine. In 3<sup>rd</sup> order mesenteric veins, both prazosin and phentolamine acted as competitive antagonists, completely blocking EFS and NE mediated effects. In 1<sup>st</sup> order mesenteric veins, prazosin attenuated EFS and NE mediated contractility, with a greater reduction to NE mediated effects. Phentolamine caused an even greater inhibition to sympathetic neurogenic contractility, while acting as a competitive antagonist to exogenous NE. Taken together the results suggest that 1)  $\alpha$ -adrenergic contractility in 3<sup>rd</sup> mesenteric veins involves the predominant contribution of  $\alpha_1$ -adrenergic receptor activity; and 2)  $\alpha$ -adrenergic contractility in 1<sup>st</sup> order mesenteric veins involves contribution from both  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors.

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**POSTER #35****Evaluation of an electronic cow-side glucose meter for diagnosing insulin resistance in Holstein cows****Julie Wittrock, Stephen LeBlanc, Todd Duffield, Sara Riuzzi****Department of Population Medicine**

The purpose of this study was to evaluate the diagnostic performance of a hand-held electronic glucometer (Precision Xtra; Abbott) for cow-side use in dairy cattle. The objective was to assess the accuracy of whole blood glucose measurements from the glucose meter relative to a reference chemical analyzer. Duplicate blood samples were taken concurrently with the preservative sodium fluoride (the gold standard for glucose analysis) or without any additives. Additionally, the suitability of the glucometer to classify cows as insulin resistant (IR) with a glucose tolerance test (GTT) was evaluated, with the ratio of (glucose concentration at 80 minutes after dextrose infusion/concentration prior to infusion) > 1.05 defined as insulin resistant. In 195 peripartum cows, there was a strong correlation in 463 samples between the Precision Xtra and serum samples preserved with NaF ( $R^2=0.88$ ,  $P<0.0001$ ), and in 523 samples between the glucometer and blood without additives ( $R^2=0.87$ ,  $P<0.001$ ). In 47 GTTs, the Precision Xtra had a sensitivity of 50% and specificity of 86% for classifying IR. Using a ROC curve to identify the optimal test threshold, a cut point of 1.09 for the IR ratio yielded sensitivity of 83% and specificity of 72% relative to the serum preserved with NaF and analyzed in a diagnostic lab. Although more health data are required to identify a meaningful IR ratio cut point for predicting disease, the handheld glucometer appears to be a suitable tool for rapid measurement of glucose, including estimation of insulin resistance, under field conditions in dairy cattle.

**POSTER #36****Characterization of endothelial-mesenchymal-transition (EndoMT) markers in response to transforming growth factor-beta (TGF $\beta$ ) in bovine aortic endothelial cells (BAEC)****Sonja Zours, Patricia Huether, Alicia Vilorio-Petit****Department of Biomedical Sciences**

Angiogenesis, the formation of new blood vessels by sprouting from pre-existing ones, is required for tumor growth and metastasis. Transforming growth factor-beta (TGF $\beta$ ) promotes angiogenesis and is also known to induce endothelial-mesenchymal-transition (EndoMT), a phenomenon of cellular plasticity by which endothelial cells become fibroblastic and motile. We hypothesize that TGF $\beta$ -induced EndoMT enables endothelial cells to detach from the blood vessel lining and migrate to form the sprout that gives origin to a new vessel during angiogenesis. This study characterized EndoMT in response to TGF $\beta$ , and TGF $\beta$  plus vascular endothelial growth factor (VEGF), an angiogenic factor known to cooperate with TGF $\beta$  in promoting angiogenesis. For this purpose, bovine aortic endothelial cells (BAEC) were stimulated with varying concentrations of TGF $\beta$  (0.1– 5 ng/mL) plus VEGF (1- 50 ng/mL) for 48, 96 and 144 hours. Confocal imaging and immunoblotting analyses revealed the strongest EndoMT response at 5 ng/mL of TGF $\beta$  and after 144 hours of exposure, which was visualized by loss of the tight junction marker ZO-1 and the adherens junction marker VE-cadherin, in addition to induction of Snail, an EndoMT-inducing transcription factor. VEGF does not significantly potentiate TGF $\beta$ 's effects. These results suggest that EndoMT induction in BAECs requires high concentrations and prolonged exposure to TGF $\beta$  and is not significantly influenced by VEGF. We are currently investigating the potential relationship between EndoMT and blood vessel sprouting using in vitro models of angiogenesis. Our findings will help define TGF $\beta$ 's role in angiogenesis and its potential use as a target for anti-angiogenic cancer therapy.

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**POSTER #37****The effect of menadione on GSTA1-JNK complex dissociation and JNK activation in Caco-2 cells****Humaira Adnan, Monica Antenos, Gordon Kirby****Department of Biomedical Sciences**

Glutathione-S-transferases (GSTs) are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a variety of electrophilic substances. The human GSTA1 is critical in the cellular defence against the deleterious effects of oxidative stress through their selenium-independent peroxidase activity. GSTs also act as modulators of MAPK signal transduction pathways via a mechanism involving protein-protein interactions. We have demonstrated that GSTA1 forms complexes with JNK. However the factors that regulate the dissociation of GSTA1 from JNK have not been identified. We hypothesized that menadione-mediated oxidative stress activates JNK, causes dissociation of GSTA1-JNK complexes and that the degree of oxidative stress depends on the level of GSTA1 expression. Human colonic adenocarcinoma cells (Caco-2) show a progressive increase in GSTA1 protein expression from preconfluency to postconfluency. We demonstrate that the pro-oxidant menadione causes dissociation of GSTA1-JNK complexes and JNK activation in preconfluent Caco-2 cells only, whereas postconfluent cells are predominantly resistant to menadione-mediated complex dissociation. Our results provide novel evidence that GSTA1 plays a direct role in regulating JNK activation through complex formation. Preconfluent cells are more sensitive than postconfluent cells to menadione-induced cytotoxicity. Additionally, transient exposure of menadione significantly reduces cellular cytotoxicity. Modulated levels of GSTA1 affect GSTA1-JNK complex association and N-acetyl cysteine blocks menadione-induced JNK activation in Caco-2 cells. These results suggest that the mechanism of menadione-mediated JNK activation involves the production of reactive oxygen species and that the level of intracellular GSH plays an important role in preventing menadione-induced GSTA1-JNK complex dissociation.

**POSTER #38****Characterization of gender related differences in vascular reactivity of mesenteric veins to endothelin-1 in gonadally intact and nurtered rats****Afrah Al-Najeer, Ron Johnson****Department of Biomedical Sciences**

The ET-1 system and integrated venous function are under the influence of sex steroids. However, there is limited understanding of the influence of gender and steroid hormones on ET-1 mediated vascular responses in the veins. Vascular reactivity in unpressurized mesenteric vessels of gonadally intact and neutered male and female rats was measured in vitro using computer assisted video microscopy. Concentration response curves were generated to ET-1 and the ETB receptor agonist, sarafotoxin (S6c) in presence and absence of ETA receptor antagonist (BQ-610 10<sup>-7</sup>M) and/or ETB receptor antagonist (BQ-788 10<sup>-7</sup>M). No differences in vascular reactivity to ET-1 were reported between intact groups. However, S6c produced greater maximum responses in the veins of intact male veins compared to females. ETA receptor blockade produced greater inhibition of ET-1 induced contractions in female veins than in males. When ETB receptors were blocked (BQ-788), BQ-610 produced similar inhibition of ET-1 responses in female and male veins. Ovariectomy (OVX) enhanced venoconstrictor responses to S6c and ET-1. OVX and castration caused reduction in ETA receptor blockade of ET-1 induced contraction in veins. These results suggest that intact male veins produce greater ETB receptor mediated contractions, and estrogen can modulate ETB mediated contractions while ETA receptors are under the influence of both sex steroid hormones.

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**POSTER #39****Observation of patient and surgeon preoperative preparation in companion animal clinics****Maureen Anderson, Brittany Foster, J. Scott Weese****Department of Pathobiology**

Prevention of surgical site infections begins with preparation of the patient and surgeon. There has been no objective investigation of compliance with preoperative preparation procedures guidelines in veterinary practices. The objectives of this pilot study were to describe a range of preoperative hand scrub and surgical site preparation practices in veterinary clinics, including any areas that may require improvement.

Observation of preparation practices was performed in each of ten clinics over 9-14 days using 2-3 small wireless surveillance cameras. Data were coded for 148 surgical patients, and 31 surgeons performing a total of 190 hand scrubs. Contact time with soap ranged from 18-369s (mean 82s, median 53s), and with alcohol from 3-220s (mean 41s, median 30.5s). Application of alcohol or antiseptic using a “cleanest to dirtiest” pattern was infrequent (29/66 (44%) and 11/83 (13%), respectively). Contact time during surgeon hand preparation ranged from 7-529s (mean 144s, median 124s) for soap and water and from 4-123s (mean 34s, median 25s) for alcohol-based hand rub. Proximal-to-distal scrubbing was noted in 95/142 (67%) of soap and water scrubs. No significant changes in scrub times were identified over the course of the observation period.

Some preoperative preparation practices were fairly consistent between clinics, while others varied considerably. Contact times with preparatory solutions were often far shorter than recommended, and the techniques with which they were applied were highly variable. The camera system used did not appear to have a significant time-dependent effect on the behavior of participants.

**POSTER #40****Prevalence of Johne’s disease in the Dairy Goat Industry of Ontario: Risk factors and evaluation of test methodologies****Cathy Bauman, Paula Menzies, Jocelyn Jansen, Dave Kelton****Department of Population Medicine**

Johne’s disease, or paratuberculosis, is a chronic wasting disease of primarily ruminant animals caused by gastrointestinal infection with the bacteria *Mycobacterium avium subspecies paratuberculosis*. This disease has been extensively researched in both the beef and dairy cattle industry, however little is known about the prevalence of this disease in the small ruminant industry. The objective of this study was to determine the prevalence of Johne’s disease in the dairy goat industry of Ontario and evaluate the current commercial testing methods in this species. Twenty-nine herds of goats were randomly selected from across Ontario to participate; from each herd 20 animals provided fecal, serum, and milk samples and one bulk tank milk sample was evaluated. A total of seven different tests were performed on each animal’s samples and two tests were performed on the bulk tank samples. The results were compared to the ‘gold standard’ of fecal culture. Preliminary results to date indicate that 93% of farms had at least one animal testing positive.

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## POSTER #41

### Measuring all the ends: the challenges, solutions, and applications of comprehensive telomere measurement

Timothy F. Carter, Daniel J. Gillis, Dean H. Betts, Pavneesh Madan, Poul Hyttel, W. Allen King

#### Department of Biomedical Sciences

Telomeric DNA repeats (TTAGGG)<sub>n</sub> cap vertebrate chromosomes and provide a protective buffer between the genome and the ends of our chromosomes, which are incompletely replicated during cellular division. The number of repeats, or telomere length, is dynamic and changes in repeat number are associated with many different chromosomal, genetic, epigenetic, physiological and environmental factors. These influences combine to give cellular populations distinct patterns in chromosomal arm telomere length, or a telomere profile, which has potential to serve as a comprehensive and powerful biomarker for various cellular characteristics. The overall goal of this study is to investigate potential applications of normalized quantitative fluorescence *in situ* hybridization (Q-FISH) and telomere profiling during induced pluripotent stem cell (iPS cell) generation. At present, complete DAPI karyotypes and telomere measurements (186 variables/metaphase) were made for a trial group of female lymphocyte metaphases (n=6). Initial statistical investigations by ANOVA and Tukeys Honest Significant Difference (HSD) test have revealed metaphase, chromosome, chromosome arm and chromosome pair to be variables providing significant differences that could be leveraged in more advanced telomere models and investigations. Within the current metaphases, 11p, 22p, 8q and Xq were profiled as displaying unique length dynamics. This preliminary result supports the hypothesis that telomere profiles have potential as biomarkers in iPS cell procedures, helps to validate the measurement methodology developed to date, and provides strong justification for the measurement of individual telomeres.

This research is supported by the OVC Doctoral Scholarship, Canada Research Chairs (CRC) and the Canadian Institutes of Health Research (CIHR).

## POSTER #42

### Effects of oral inoculation on chickens with wt FAdV-9 and one mutant FAdV-9Δ4

Li Deng, Éva Nagy, Sara Languay

#### Department of Pathobiology

Fowl adenoviruses (FAdVs) have a worldwide distribution and appear to be ubiquitous in poultry farms. Our laboratory is studying the non-pathogenic FAdV-9 virus at the molecular level and investigating its use as a vaccine vector. Previously we showed that one mutant virus (FAdV-9Δ4) lacking six ORFs in the left end of the genome replicated less efficiently in intramuscular infected chickens. The aim of this study is to assess the effects of oral inoculation with wild type FAdV-9 and FAdV-9Δ4 on chickens as that is the natural route for FAdV infection. One hundred and thirty five specific-pathogen-free chickens were divided into three groups and orally inoculated with  $2 \times 10^7$  pfu/chick wt FAdV-9, FAdV-9Δ4, and PBS, respectively. Cloacal swabs, serum, and tissues including spleen, liver, thymus, ceecal tonsil and bursa of Fabricius were collected for assessment of virus shedding, antibody response, viral genome copy number and cytokine expression. The results showed that the titer of wt FAdV-9 group chickens was up to  $3.97 \times 10^3$  pfu/ml in the feces, while chickens from FAdV-9Δ4 group did not shed any viruses. Moreover, we showed that both wt FAdV-9 and FAdV-9Δ4 can induce the expression of IFN- $\alpha$ , IFN- $\gamma$  and IL-12 in spleen and liver compared to the control group, although in liver the induction by wt FAdV-9 was significant while it was not by FAdV-9Δ4. Therefore, it is speculated that the deleted 6 ORFs must play an important role in viral replication and modulating the immune response against viral infection.

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**POSTER #43****Effects of *in ovo* exposure to cortisol, bisphenol A and the antiestrogen ICI 182,780 on vertebral morphology****Jacqueline Ferris****Department of Biomedical Sciences**

Vertebral morphology of rainbow trout embryos was examined in order to evaluate the developmental effects of cortisol, bisphenol A (BPA) and the antiestrogen ICI 182,780 on notochord development. Histological analysis was performed using transverse sections of paraffin-embedded late stage rainbow trout embryos. The resulting sections were dyed with haematoxylin and eosin. The results revealed that co-administration of cortisol and BPA *in ovo* caused marked effects on notochord development indicated by an absence of the chordoblast layer in these embryos. This response was not found in embryos reared from oocytes that had been incubated in BPA without co-incubation with cortisol, or in embryos reared from oocytes that had been incubated in cortisol alone. This suggests an interaction between BPA and cortisol is bringing about the observed morphological changes in the notochord. The absence of chordoblast cells suggests that notochord development can be interrupted by exposure to the combination of the cortisol and BPA prior to fertilization.

**POSTER #44****Sex specific telomere analysis in leukocytes from newborn calves****Graham C. Gilchrist, Christine K. Hamilton, Dean H. Betts, Pavneesh Madan, W. Allen King****Department of Biomedical Sciences**

The ends of mammalian chromosomes are capped with telomeres, which are dynamic protective structures. Telomeres are tandem repeats of nucleic acid and specialized proteins that stabilize the chromosomes from erosion and fusion with self or other chromosomes. Telomeres shorten with each round of cellular division and a build up of critically short telomeres induces replicative senescence and therefore is considered a biomarker for aging and age-related diseases. It has been hypothesized that the rate of telomere attrition differs in sexes and alternately, the differences in age-related pathologies is attributed to this difference in telomere length. The purpose of this study was to identify sex related difference in the telomere length of male and female bovine newborn leukocytes. Blood samples were collected in triplicate, from less than ten days old, male (n=10) and female (n=10) calves. Blood samples were spun and lysed overnight, and then the DNA was extracted using standard phenol-chloroform methods. Relative telomere lengths (RTL) were measured using quantitative real-time PCR. A ratio of telomere amplification to a single-copy reference gene (*ZARI*) was used to determine RTL. Analyzing 3 technical and 3 biological replicates, showed no statistically significant differences between male and female newborn telomeres (p-value = 0.4969). This trend in newborns is consistent with human newborn studies, which found no difference in telomere length between newborn males and females. This confirms the potential use of bovine as a suitable model for humans, as well as it indicates that the involvement of telomeres in sex related pathologies occur post-natally.

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**POSTER #45****Down-regulation of surface class I major histocompatibility complex (MHC-I) by fowl adenoviruses****Bryan Griffin, Éva Nagy****Department of Pathobiology**

Adenoviruses (AdVs) are members of the family *Adenoviridae* and are nonenveloped, icosahedral viruses that infect a wide range of vertebrate hosts. AdVs of birds belong for the most part to the genus *Aviadenovirus* and include the species, Fowl Adenovirus (FAdV) A through E. Some FAdVs are associated with inclusion body hepatitis (IBH), characterized by hepatic necrosis and intranuclear inclusion bodies. Analysis of the complete genomes of FAdV-1, FAdV-4, FAdV-9, and FAdV-8 (species FAdV-A, -C, -D, and -E, respectively) has shown that the FAdVs share a common genome organization. The central genomic region consists of ancestral adenoviral genes, dubbed the genus-common genes that are conserved in all adenovirus genera. The terminal genomic regions consist of novel genus-specific genes, that show no homology to the corresponding regions of the mastadenoviruses and are predicted to function in interactions with the host cell. In the most studied FAdV, FAdV-1, protein functions have been demonstrated for only three of the predicted 19 genus-specific genes, ORF1, ORF22, and *Gallus anti morte-1* (GAM-1). Human adenoviruses (HAdVs) have been shown to down-regulate MHC-I surface expression via the virally encoded E3-19K protein. We hypothesized that one or more of the FAdV genus-specific genes down-regulate surface expression of MHC-I. Flow cytometry analysis with anti-chicken MHC-I revealed that chicken hepatoma cells (CH-SAH) displayed high MHC-I surface expression that was much reduced upon infection with FAdV-9. We have sought to investigate the mechanism(s) of MHC-I surface down-regulation.

**POSTER #46****Assessment of mitochondrial remodelling following DCA exposure in human colorectal cancer cells****Nelson Ho, Siranough Shahrzad, Brenda Coomber****Department of Biomedical Sciences**

While glycolysis is a suboptimal metabolic strategy used by cells under hypoxic conditions, many cancer cells preferentially utilize glycolysis for energy production, even in the presence of oxygen, a phenomenon known as the “Warburg Effect”. Dichloroacetate (DCA) is a small metabolic modulator that shifts cellular metabolism to glucose oxidation by inhibition of PDH kinase (PDK). Furthermore, DCA has been shown to induce apoptosis through a mitochondria-dependent pathway in cancer cells but not normal cells. Previous work in our laboratory has shown that some human colorectal cancer (CRC) cells are protected from hypoxia-induced apoptosis upon exposure to DCA. We hypothesize that CRC cells resistant to hypoxia in the presence of DCA do not undergo mitochondrial remodelling and subsequent metabolic changes as a result of upregulation in PDK expression, thus being protected from cellular damage that would otherwise occur from switching to glucose oxidation. Mitotracker dyes and a lactate production assay were used to examine the effects of DCA on mitochondrial function and cellular metabolism, respectively, in resistant and susceptible cells. Several CRC cell lines appear to have a biphasic response upon exposure to DCA. All CRC cell lines examined showed a reduction in lactate production upon exposure to DCA. CRC cell lines with reduced susceptibility to DCA also showed the least reduction in lactate production. We also determined the expression of PDK isoforms by qRT-PCR and western blotting. Based on this study, we hope to determine the mechanisms by which some CRC cells are protected from DCA-induced apoptosis.

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**POSTER #47****Secondary active transport of glucose enhances the viability of ovarian cancer cells in high glucose environments****Lisa Kellenberger, Alison Holloway, Jim Petrik****Department of Biomedical Sciences**

Cancer cells rely on the energetically inefficient process of glycolysis as their preferred metabolic pathway, meaning they must consume significantly higher amounts of glucose than normal cells to maintain ATP production. Glucose utilization by the cells is dependent upon glucose availability and transport. Because glucose uptake is a rate-limiting step in cellular glucose metabolism, membrane transporters are enticing targets for intervention.

Using the mouse ovarian cancer cell line ID8, we identified expression of three glucose uptake transporters: GLUT-1, GLUT-3, and sodium glucose transporter-2 (SGLT-2). SGLT-2 is poorly described in cancer cells, so we initially evaluated protein expression of SGLT-2 in human ovarian cancer cell lines and found it overexpressed compared to immortalized normal ovarian epithelium. Because SGLT-2 has a much higher saturation point than GLUT-1, we hypothesize that these transporters will play an important role in glucose uptake and contribute to tumorigenicity in hyperglycemic conditions.

To evaluate the functional role of SGLT-2, we used the SGLT-specific competitive inhibitor phlorizin to elucidate its effects on glucose uptake and cell viability. Preliminary results showed that in the presence of high glucose (25mM), but not physiological glucose (6mM), phlorizin inhibited glucose consumption. Interestingly, the GLUT-specific inhibitor cytochalasin B showed the inverse effect. While phlorizin did not affect cell number compared to control, it did inhibit migration in a scratch wound assay in the presence of 25 mM glucose. These findings are especially important given the prevalence metabolic dysfunction in the adult population and may point to a novel therapeutic avenue.

**POSTER #48****Comparing younger and older MSM in New Zealand; important differences in HIV-related knowledge, attitudes, and sexual behaviour****Nathan J. Lachowsky, Peter J.W. Saxton, Nigel P. Dickson, Anthony J. Hughes, Cate E. Dewey****Department of Population Medicine & Biomedical Sciences**

The aim of this study is to compare HIV-related risk outcomes between younger men who have sex with men (YMSM) 16-29 years and older MSM (OMSM). A pooled sample of 3387 YMSM and 5602 OMSM was created from the 2006, 2008, and 2011 rounds of the Gay Auckland Periodic Sex Survey and Gay men's Online Sex Survey. Statistical analyses controlled for survey year.

Compared to OMSM, YMSM were more likely to be ethnically diverse, recruited online, and report a bisexual identity. Generally, YMSM reported less HIV knowledge than OMSM. While attitudes were similar across age, more YMSM expected a man to disclose being HIV-positive before sex. Recent sexual health testing/treatment and STI diagnosis rates did not differ by age, except that YMSM were less likely to have tested HIV-positive. YMSM reported fewer sexual partners in the last 6 months than OMSM. Among participants with a current regular partner, YMSM were more likely to report high condom use with boyfriends than OMSM. Among participants with casual partners, YMSM reported lower condom use than OMSM. Regardless of partner type, YMSM were more likely to have "receptive only" anal intercourse compared to OMSM. Largely, concurrent partnering did not differ by age.

This analysis provides the first comprehensive comparison of MSM by age group in New Zealand. Compared to OMSM, YMSM reported fewer sexual partners, less (but riskier) casual sex, but better condom use with boyfriends. Knowledge and attitudes measures, along with sexual behaviour, highlight the need for strategic evidence-based HIV/sexual health promotion targeting YMSM.

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## POSTER #49

### Determining the seroprevalence and risk factors of Q Fever in sheep, goats and their farm workers in Ontario

Shannon Meadows, Paula Menzies, Andria Jones, Scot McEwen, Jocelyn Jansen

#### Department of Population Medicine

Q Fever is a zoonotic disease caused by *Coxiella burnetii*. An ongoing human outbreak in the Netherlands connected to small ruminants has drawn attention to the surveillance and control of this infectious disease. Therefore the objectives of this study are to better understand the extent of exposure and risk factors associated with Q Fever in Ontario small ruminants and their farm workers. To ascertain the current seroprevalence in Ontario this cross-sectional serological survey examines sheep and goats in both meat and dairy sectors; and the farm workers who have routine contact with these animals on farm. Multi-stage random sampling is used to select farms and animals. Blood samples are drawn from selected animals for serological analysis via the IDEXX Enzyme-linked immunosorbent assay (ELISA). Farm workers have the option of having a blood sample drawn for serological analysis using the Immunofluorescence assay (IFA).

The results to date show that from the first 126 farms 73 had at least one animal test positive on the Q Fever ELISA. Results from the human serum samples indicated that from 36 farms, 62 of 82 people tested positive on the IFA test. These 62 people were from 30 different farms. Therefore 30 of 36 farms had at least one human test positive on the IFA test. After data collection is complete, the seroprevalence of Q Fever in small ruminants and their farm workers will be determined, as well as any significant risk factors associated with the Q Fever status in both small ruminants and humans.

## POSTER #50

### DNA Barcoding of coccidia (Apicomplexa) and qPCR of *Eimeria* species infecting chickens

Mosun Ogedengbe, John Barta

#### Department of Pathobiology

Partial mitochondrial cytochrome *c* oxidase subunit I (mt COI) sequences were tested as a molecular species identification tool (DNA barcode) for coccidian parasites of veterinary and medical importance. Quantitative real-time PCR of a small region of the gene (a “mini-barcode”) was developed for detection of all 7 chicken *Eimeria*; speciation from a mixed reaction appears to be possible because melting peaks for products from each *Eimeria* vary.

First, we developed degenerate PCR primers and generated partial COI sequences from many coccidia through PCR and amplicon sequencing. Over 300 partial sequences were aligned using Geneious (Ver. 5+) and utility of COI for species delimitation was tested. Using >100 sequences from chicken *Eimeria*, sequences were searched for a useful COI region to produce a qPCR diagnostic method for chicken *Eimeria* species.

Six degenerate COI PCR primers from the 5' portion of mt COI were developed. COI sequences from more than 35 different coccidia have been obtained. A pair of qPCR primers (400F/605R) was designed for all chicken *Eimeria* spp.; qPCR melting peaks for 6 *Eimeria* spp varied from 75°C-80°C providing a quick diagnostic method to determine the *Eimeria* species in a sample. Currently, parasite DNA extraction methods from fecal samples are being optimized. Additional species-specific primer design will be followed by qRT-PCR assay development and a validation study.

COI is a good candidate as a DNA barcoding target ushering in new, broadly applicable, molecular identification and phylogenetic method for medically and economically important apicomplexan parasites infecting humans, domestic and wild animals.

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## POSTER #51

### Establishment and characterization of two cell lines derived from canine cutaneous mast cell tumours

Jennifer J. Thompson, Sarah Boston, Jodi Morrison, Robert Foster, Brenda L. Coomber

#### Department of Pathobiology

Canine cutaneous and subcutaneous mast cell tumours (MCT) represent about 21% of skin tumours in this species. Some are aggressive with high rates of reoccurrence and metastasis. The behaviour, prognosis and response to therapy cannot be reliably predicted for many, and no additional clinical studies can help without a molecular breakthrough. Many *in vitro* studies of neoplastic canine mast cells using the limited number of cell lines available have restricted our understanding of the biological diversity of these tumours. Also, many of these cell lines were passaged in mice, potentially causing unforeseen phenotypic alterations which limit their value. We recently established two cell lines derived from cutaneous MCT using a fibroblast co-culture technique. Both cell lines are important breakthroughs because they possess mutations in *c-KIT* and exhibit independent cell growth without the addition of exogenous stem cell factor (SCF). The cells also have phosphorylated KIT and vascular endothelial growth factor (VEGFR2) receptors without the addition of recombinant canine SCF or VEGF. Both receptors demonstrate a dose-dependent increase in phosphorylation with supplementation of their respective ligand. Phosphorylation of KIT and VEGFR2 in both cell lines is downregulated by the tyrosine kinase inhibitor, toceranib. The cells also produce and secrete high levels of VEGF as determined by ELISA analysis of conditioned media, suggesting an autocrine loop. These new cell lines are invaluable for our understanding of pathogenic mechanisms of canine MCT, tyrosine kinase receptor signaling, and have wider implications for human and animal cancer intervention.

## POSTER #52

### Feasibility of transferring high immune response (HIR) technology as a health management tool to the dairy marketplace

Lauraine Wagter-Lesperance

#### Department of Pathobiology

High Immune Response (HIR) is an evaluation technology that has the potential to improve the health and food quality of dairy cattle through the reduction of antibiotics and enhanced resistance to economically important diseases like mastitis. Dairy cattle with a high immune response to test antigens are at a lower risk for developing disease compared to average and low responding animals. Focus groups conducted in two Ontario dairy regions indicated significant interest in HIR (75% of producers) for culling, grouping, treating, and breeding animals. A quantitative market assessment indicated producers would like a way of identifying disease prone dairy cattle and when introduced to the concept of HIR, 20% of dairy producers were highly, and 69% moderately interested in trying HIR. HIR testing was also conducted on a group of AI cull sires (N=15) and indicated variation in immune response with no adverse reactions or cross-reactivity with national health testing. A validation study of previous research to rank cattle based on antibody from milk in lieu of blood (N=21 cows) indicated that antibody response is significantly correlated in blood and milk. Beta-testing of HIR in two or more Ontario dairy herds is being used to further demonstrate the economic value of HIR. Finally, knowledge transfer is being accomplished using various communication media, attending dairy symposia, and inviting producers and veterinarians to participate in workshops about HIR. HIR is being demonstrated as an effective health management tool to help dairy producers identify healthier cows, calves and sires.

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**POSTER #53****Detection of a hybrid jaagsiekte sheep retrovirus – Murine leukemia virus transcript in JS7 cells****Darrick Yu, Sarah Wootton****Department of Pathobiology**

Jaagsiekte sheep retrovirus (JSRV) is an oncogenic sheep retrovirus that causes a form of lung cancer known as ovine pulmonary adenocarcinoma (OPA). A cell line known as JS7, which is derived from a spontaneous OPA case, harbors an integrated JSRV provirus. However, these cells do not express JSRV proteins. The objective of this study was to reactivate the latent provirus in JS7 cells using chemical agents. JS7 cells were treated with prostratin (a drug known to activate the NF-Kb pathway), sodium butyrate and TNF $\alpha$  in an attempt to induce expression from the JSRV promoter. Cellular RNA was extracted and reverse transcriptase PCR was conducted to generate cDNAs corresponding to transcripts originating from the JSRV long terminal repeat sequences. Screening of cDNA sequences using PCR and subsequent sequencing showed induced expression of viral transcripts upon treatment with each drug. Unexpectedly a hybrid transcript containing both JSRV and murine leukemia virus (MLV) sequences was recovered from the JS7 cells after treatment with prostratin. This would suggest that co-infection and subsequent recombination had occurred. These results suggest that JS7 cells, a sheep cell line, are able to be infected by MLV, a mouse virus, and also that recombination may occur between JSRV and MLV sequences.

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