



**THE OHIO STATE UNIVERSITY**

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COLLEGE OF VETERINARY MEDICINE

**COLLEGE OF  
VETERINARY MEDICINE  
RESEARCH DAY**

**6 APRIL 2017**

**BOOK OF  
ABSTRACTS**

# **PROGRAM**

April 6, 2017

## **POSTER JUDGING**

Graduate Student Posters

8:00 am – 10:30 am

(closed session – only open to  
those being judged)

## **AWARDS PRESENTATION**

Veterinary Medical Center Auditorium

12:00 pm

## **GRADUATE STUDENT and POST DOC PLATFORM PRESENTATIONS**

Dr. Mostafa Ghanem

Dr. Madhav Kodigepalli

## **KEYNOTE SPEAKER**

Veterinary Medical Center Auditorium

immediately following the awards  
and platform presentations

## **DR. X. J. MENG**

University Distinguished Professor

Virginia Polytechnic Institute and State University

***“Animal Reservoirs and Cross-species  
Infection of Hepatitis E Virus”***

## **POSTER SESSION**

1<sup>st</sup> and 2<sup>nd</sup> Floors – Vet Med Academic Building

11:00 am – 5:00 pm

## **CHAired BY**

Dr. Patrick Green

## **ORGANIZED BY**

Michele Morscher

Special thanks to Marc Hardman in the  
College's Technology Services for printing the posters

# POSTER JUDGING SESSIONS

Wednesday, April 5, 2017  
2:00 – 5:00 pm  
Undergraduate and  
Veterinary Student Poster Judging

Thursday, April 6, 2017  
8:00 – 10:30 am  
Graduate Student Poster Judging

Thank you to the following faculty and guests for taking time out of their busy schedules to judge 70 posters.

Jim Belknap

James Blacka

Luciana da Costa

Ian Davis

Jim DeWille

Joelle Fenger

Rebecca Garabed

Julien Guillaumin

Kate Hayes-Ozello

Lauren Holtvoigt

Sanggu Kim

Bill Kisseberth

Krista La Perle

Daniel Marsman

Eric Miller

Mike Oglesbee

Tracey Papenfuss

Bill Saville



# College of Veterinary Medicine

# Research Day

Awards Presentation, Graduate Student and Post Doc  
Platforms, and Keynote Address

Thursday, April 6, 2017

Noon – 2:00 pm

Veterinary Medical Center Auditorium

## “Animal Reservoirs and Cross-species Infection of Hepatitis E Virus”



**X. J. Meng, MD, PhD**

University Distinguished Professor

Virginia Polytechnic Institute  
and State University

Poster Judging:

April 5th, 2 – 5 pm for professional students

April 6th, 8 – 10:30 am for graduate students



**THE OHIO STATE UNIVERSITY**

COLLEGE OF VETERINARY MEDICINE

Office of Research  
and Graduate Studies



Platform Presentation

**CORE GENOME MULTILOCUS SEQUENCE TYPING SCHEME (CGMLST): A GLOBAL STANDARDIZED APPROACH FOR MOLECULAR TYPING OF *MYCOPLASMA GALLISEPTICUM*.** M.Ghanem<sup>1</sup>, L.Wang<sup>2</sup>, Y. Zhang<sup>2</sup>, S. Edwards<sup>3</sup>, D. Harmsen<sup>4</sup>, D. Ley<sup>5</sup> and M. El-Gazzar<sup>1</sup>

<sup>1</sup>Depts. of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA, <sup>2</sup>Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, 8995 East Main Street, Reynoldsburg, OH 43068, USA, <sup>3</sup>Depts. of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, <sup>4</sup>Depts. for Periodontology, University of Münster, Münster, Germany. <sup>5</sup>Depts. of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States of America.

*Mycoplasma gallisepticum* (MG) is the most pathogenic avian mycoplasma species to poultry, causing respiratory disease and reduced production efficiency. Currently, MG strain differentiation is based on the sequence analysis of only 5 loci proved to be insufficient for accurate outbreak investigation. Recently, whole genome sequence (WGS) of many human and animal pathogens has been successfully used for routine microbial diagnosis and outbreak investigation. However, the massive amount of sequence data and the diverse properties of different genes within bacterial genomes resulted in the lack of standard reproducible method for comparison between MG whole genomes. Here, we are proposing the development of cgMLST for typing of MG strains and field isolates. For development of this scheme, a diverse collection of 37 MG genomes was used to identify the cgMLST targets. A total of 425 MG conserved genes (49.85% of MG genome) were selected as core genome targets for this scheme. A total of 81 MG genomes from 5 countries in 4 continents were later typed using this scheme. Analysis of phylogenetic trees generated by cgMLST displayed a high degree of agreement with geographical and temporal information. Moreover, the high discriminatory power of cgMLST allowed differentiation between samples of the same outbreak, resolving the confusion in many historical MG outbreaks. MG-cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation between MG isolates. cgMLST provides stable and expandable nomenclature, allowing for comparing and sharing the typing results between different laboratories worldwide. cgMLST offers an opportunity to harness the tremendous power of next generation sequencing technology in applied avian mycoplasma epidemiology at a local and global level.

Key words: *Mycoplasma gallisepticum*, Molecular typing, Multilocus sequence typing (MLST), Whole genome, Next-generation sequencing.

Platform Presentation

**MECHANISMS OF SAMHD1-MEDIATED ANTI-PROLIFERATION IN CUTANEOUS T-CELL LYMPHOMA AND ACUTE MONOCYTIC LEUKEMIA CELLS.** K. M. Kodigepalli<sup>1</sup> and L. Wu<sup>1,2,3</sup>,

<sup>1</sup>Center of Retrovirus Research, Department of Veterinary Biosciences; <sup>2</sup>Comprehensive Cancer Center; <sup>3</sup>Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA

Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a mammalian dNTP hydrolase that regulates cellular dNTP homeostasis. The *SAMHD1* gene is mutated and its expression is downregulated in cancers including cutaneous T-cell lymphoma (CTCL) and leukemia. We reported that exogenous expression of SAMHD1 in CTCL-derived HuT78 CD4+ T-cells significantly reduces cell proliferation and increases apoptosis. We also reported that SAMHD1 knockout in acute monocytic leukemia (AML)-derived THP-1 cells causes increased cell growth and proliferation, but reduced apoptosis. These results suggest an anti-proliferative function of SAMHD1 in lymphoma and leukemia pathophysiology. The goal of this study is to investigate the mechanisms underlying SAMHD1-mediated anti-proliferation in lymphoma and leukemia cells. We found that exogenous SAMHD1 expression in HuT78 cells significantly reduced mRNA and protein levels of the short-form of cellular FLICE-inhibitory protein (cFLIP<sub>s</sub>), a key anti-apoptotic molecule. Reduced cFLIP<sub>s</sub> levels were partially due to enhanced proteasomal degradation, indicating that SAMHD1-induced effects in CTCL-derived HuT78 cells may be mediated via degradation of cFLIP<sub>s</sub>. In contrast, SAMHD1 knockout in AML-derived THP-1 cells did not alter cFLIP<sub>s</sub> expression, suggesting that SAMHD1-regulated cell proliferation and apoptosis in these cells is independent of cFLIP<sub>s</sub>. Interestingly, SAMHD1 knockout in THP-1 cells significantly increased the activation or expression of key mediators of PI3K and the downstream NF-κB pathway, including serine/threonine kinase Akt, and p100/p52. Inhibition of PI3K activity using specific inhibitor (LY294002) not only reduced p100/p52 levels, but also resulted in greater inhibition cell proliferation in THP-1 cells with SAMHD1 knockout. Together, our results suggest that SAMHD1 inhibits proliferation of CTCL and AML cells via different mechanisms involving cFLIP<sub>s</sub>, or PI3K and NF-κB pathways respectively. A better understanding the mechanisms of SAMHD1-mediated anti-proliferation in CTCL and AML cells will enhance our knowledge on pathogenesis of these cancers and can help to develop novel therapeutic strategies against them.

Keywords: SAMHD1, Lymphoma, Leukemia, PI3K, NF-κB, cFLIP.

# **CLINICAL RESEARCH**

**EFFECTS OF ORAL ADMINISTRATION OF ACETYLSALICYLIC ACID AFTER PARTURITION ON ACTIVITY PATTERNS, PREVALENCE OF DISEASES, MORTALITY AND CULLING RATES IN DAIRY COWS.** A. A. Barragan<sup>1</sup>, L. Bauman<sup>2</sup>, J. Velez<sup>3</sup>, J. D. Rozo Gonzalez<sup>3</sup>, G. M. Schuenemann<sup>1</sup> and S. Bas<sup>1</sup>. <sup>1</sup>Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 43210, <sup>2</sup>Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, <sup>3</sup>Aurora Organic Farms, Boulder, CO 80302.

Dystocia is considered a painful event and has been associated with increased risk of cow morbidity, mortality and culling in dairy farms. Administration of NSAID drugs has been proposed to decrease postpartum discomfort. The objectives of this study were to assess the effects of oral administration of acetylsalicylic acid after calving on: 1) daily activity patterns and 2) prevalence of diseases, mortality and culling rates in lactating dairy cows. Cows from 3 organic dairy herds were enrolled in the present study. Immediately after parturition, cows were blocked by parity and calving ease (eutocia [EUT]; dystocia [DYS]) and were randomly assigned to two treatment groups: 1) ASP (n=278): at ~12 h after parturition cows received 4 consecutive treatments with acetylsalicylic acid (100 mg/kg; 2 boluses) 12 h apart; or 2) placebo (PLC; n=285): at ~12 h after parturition cows received 4 consecutive treatments with gelatin capsules (2 capsules) filled with water 12 h apart. Activity monitors were placed on the rear leg of a subset of cows at enrolment, and were removed 7 d later. Cows in the ASP group tended ( $P=0.05$ ) to have more steps compared to PLC. Furthermore, cows that experienced DYS spent more time lying ( $P<0.05$ ; DYS=590±17 min/d; EUT=511±17 min/d), less time standing ( $P<0.05$ ; DYS=850±17 min/d; EUT=929±17 min/d), and had less steps ( $P<0.05$ ; DYS=3089±126 steps/d; EUT=3587±133 steps/d) than EUT cows. Additionally, ASP cows that experienced EUT tended to spend less time lying and more time standing, and had more steps compared to PLC cows that experienced EUT. No difference was found on the incidence of health events, culling and mortality rates between groups. The results of this study suggest that activity patterns of cows that experience DYS are different from cows that experience EUT, and that administration of ASP after calving may increase activity of dairy cows.

Keywords: Acetylsalicylic acid, activity, calving ease



**USE OF HYPERTONIC MEDIUM TO CRYOPRESERVE SAUGER (*SANDER CANADENSIS*) SPERMATOZOA.** B Blawut <sup>a</sup>, B Wolfe <sup>a</sup>, CR Darr <sup>b</sup>, S Hale <sup>c</sup>, R Zweifel <sup>c</sup>, D Sweet <sup>c</sup>, SA Ludsin <sup>d</sup>, MA Coutinho da Silva <sup>b</sup>

<sup>a</sup> Department of Veterinary Preventive Medicine; <sup>b</sup> Department of Veterinary Clinical Sciences; <sup>c</sup> Ohio Department of Natural Resources, Division of Wildlife; <sup>d</sup> Aquatic Ecology Laboratory, The Ohio State University

Freshwater fish species typically exhibit poor sperm quality following cryopreservation. In anadromous species, cryopreservation extenders hypertonic to the seminal plasma have been used successfully to improve post-thaw sperm quality and fertilization rates. The objective of this study was to determine the effect of extender osmolality on post-thaw sperm quality in an economically valuable freshwater fish, the sauger (*Sander canadensis*). We hypothesized that extenders hypertonic to the seminal plasma would enhance dehydration during cryopreservation resulting in increased post-thaw sperm quality. Fresh milt from 10 male sauger was diluted using extenders with osmolalities of 350, 500, or 750 mOsm/kg (E350, E500 and E750, respectively) containing 10% DMSO, frozen in LN<sub>2</sub> vapor, and stored for 3 months before being thawed and analyzed. Sperm parameters (total motility, progressive motility, velocity, and viability) were objectively assessed at different steps of the cryopreservation process (extended, equilibrated, and post-thaw). Cryoprotectant (CPA) addition decreased sperm velocity in all extenders ( $p < 0.001$ ), but increased progressive motility in E350 and E500 ( $p < 0.001$ ). Total motility was unaffected by CPA addition in E350 and E500 but decreased in E750 ( $p < 0.001$ ). All parameters measured, except progressive motility, were significantly reduced by cryopreservation. E500 yielded the highest post-thaw progressive motility ( $32.20 \pm 1.20\%$ ) and velocity ( $84.97 \pm 5.32 \mu\text{m/s}$ ) whereas both E350 and E500 displayed the highest total motility ( $65.30 \pm 1.40$  and  $68.70 \pm 2.00\%$ ) and viability ( $80.60 \pm 1.50$  and  $78.80 \pm 1.20\%$ ), respectively. By contrast, E750 yielded the lowest post-thaw velocity, viability, total, and progressive motility. In conclusion, the use of a hypertonic extender with osmolality of 500 mOsm/kg resulted in higher sperm velocity and progressive motility post-thaw compared to an isotonic extender. The improvements in sauger sperm cryosurvival obtained in our study lay the foundation for future experiments evaluating the fertilizing capacity of freshwater fish sperm cryopreserved in hypertonic extenders.

Keywords: Spermatozoa, Percidae, Osmolality, Hypertonic, Cryopreservation, Hatchery

**ECHOCARDIOGRAPHIC ESTIMATES OF RIGHT VENTRICULAR SYSTOLIC FUNCTION IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE.** Chapel EH, Scansen BA, Schober KE, Bonagura JD

Right ventricular (RV) dysfunction strongly and independently predicts outcome in people with myxomatous mitral valve degeneration (MMVD). The purpose of this prospective pilot study was to assess RV systolic function in dogs with MMVD.

Client-owned dogs with MMVD not previously receiving cardiovascular medications and without moderate to severe pulmonary hypertension were eligible for enrollment. Dogs presenting in acute congestive heart failure received a single dose of furosemide prior to echocardiographic imaging. Seven echocardiographic indices of RV function were measured: tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), lateral tricuspid annular longitudinal peak systolic velocity ( $S'$ ), global strain ( $St_G$ ) and strain rate ( $StR_G$ ), and longitudinal strain ( $St_L$ ) and strain rate ( $StR_L$ ) of the RV free wall. Groups were compared using one-way ANOVA and Tukey's posthoc test. Frequencies of cases falling outside established reference intervals were compared using Fisher's exact test.

Thirty-six dogs with MMVD at 3 stages of disease were recruited based on statistical power analysis: B1 (n=12), B2 (n=12), and C (n=12). Compared to stage B1, dogs with stage B2 disease had significantly higher values for TAPSE,  $St_L$  and  $StR_L$  (all  $p \leq 0.05$ ). TAPSE exceeded reference limits in 67% of B2 dogs compared with 9% in stage C ( $p = 0.009$ ).

Compensated MMVD with remodeling was associated with hyperdynamic RV systolic function indices; there was a trend toward normalization of these indices in acute congestive heart failure. In conclusion, measures of RV systolic function differed between classes of MMVD. Further studies are necessary to determine the prognostic significance of these findings.

Keywords: right ventricle, mitral valve disease, echocardiography

**MARBLE BURYING FOR ASSESSING POSTOPERATIVE PAIN IN RATS TREATED WITH MELOXICAM OR SUSTAINED-RELEASE MELOXICAM.** K. Galang and R. Onaga., J. Ayers, and L. Kendall. Laboratory Animal Resources, Department of Microbiology, Immunology, and Pathology; College of Veterinary Medicine and Biomedical Sciences, Colorado State University

Rats are a common model for research studies involving the use of pain-inducing events that must be ameliorated with analgesics. Rats bury novel objects as a function of their natural behavior to new objects and is a common method to evaluate anxiety in rats. This study investigates marble burying as a unique means to assess pain in laboratory rats. We hypothesize that rats in pain will bury less marbles. Male Sprague-Dawley rats were castrated or received anesthesia only and divided into 3 respective treatment groups of 6 rats each: meloxicam (2 mg/kg SC once daily for 3 days), sustained-release meloxicam (SRM; 4 mg/kg SC once), or saline. Assessments were done at baseline and 1, 6, 12, 24, and 48 h post-surgery. Rats were observed for 5 minutes to evaluate behavioral indicators of pain including orbital tightening, wound licking, grooming, and rearing. Then, rats were placed in individual marble-containing cages and given 30 m to bury marbles. Castrated rats given SRM had the highest rearing activity post-surgery, while castrated rats given meloxicam had the highest grooming and the least wound licking activities post-surgery. Castrated rats in the saline treated group buried fewer marbles and had the lowest magnitude increase in marble burying from 1 h to 48 h post-surgery as compared to castrated rats that received analgesics and the anesthesia-only groups. This suggests the marble burying test may be used as an adjunct to other pain assessment indicators to identify pain in rats.

Keywords: laboratory animal medicine, rat behavior, animal welfare, analgesia, pain management, meloxicam, pain score

**PREVALENCE AND QUANTIFIED DOSE OF VENOUS AIR EMBOLI IDENTIFIED ON THORACIC COMPUTED TOMOGRAPHIC EXAMINATIONS IN DOGS.** Y.H. Hsieh<sup>1</sup>, G.G. Habing<sup>2</sup>, W.T. Drost<sup>1</sup>, E.M. Green<sup>1</sup>. <sup>1</sup>Department of Veterinary Clinical Sciences and <sup>2</sup>Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 43210.

### **Introduction/Purpose**

Venous air emboli are commonly identified on computed tomographic (CT) images of dogs after intravenous (IV) positive contrast medium injection. The objectives of this study were to 1) Quantify the volume and dose of venous air emboli on CT images and compare to the lethal IV dose; 2) Determine if an IV injection protocol would decrease the incidence and dose of venous air emboli after intravenous positive contrast medium injection.

### **Methods**

Thoracic CT examinations were evaluated in 81 dogs (64 retrospective and 17 prospective). An intravenous positive contrast medium injection protocol was established and used on the prospective examinations. The locations, volumes and doses of venous air emboli on the CT images were identified and calculated. The medical records of dogs with venous air emboli were evaluated for any post-intravenous positive contrast medium injection cardiopulmonary complications.

### **Results**

Multiple locations of venous air emboli were identified, including the right atrium and right ventricle. The doses of venous air emboli with and without the protocol were significantly lower than the reported lethal dose ( $P < 0.001$ ). The dose of venous air emboli with the protocol was significantly lower than that without the protocol ( $P < 0.05$ ). No cardiopulmonary complications were recorded in any animals with venous air emboli.

### **Discussion/Conclusion**

The doses of venous air emboli in this study were significantly lower than the reported lethal dose and the dose of venous air emboli after intravenous positive contrast medium injection decreased with the use of the protocol. Some locations of venous air emboli could increase the clinical concern for life-threatening complications. Great care should be taken for intravenous positive contrast medium injection during CT examinations.

Keywords: CT, dogs, venous air emboli

**EFFECTS OF PERIOPERATIVE BUPIVACAINE AND BUPRENORPHINE IN A RAT THORACIC SPINAL CONTUSION MODEL. DM LeMoine<sup>1</sup> and D McTigue<sup>2</sup>**

<sup>1</sup>Department of Veterinary Preventive Medicine, College of Veterinary Medicine and

<sup>2</sup>Department of Neuroscience, Wexner Medical Center, The Ohio State University, Columbus, OH 43210

Spinal cord injury (SCI) affects the lives of millions of people worldwide, and research into SCI and repair is ongoing. Rat spinal contusion, the most common SCI animal model, requires surgery which may cause postoperative pain. Many investigators are approved to withhold analgesics based on reported lack of sensation, anesthetics used, and potential confounding effects. However, there are no reports evaluating the impacts of perioperative analgesics on pain behaviors and functional and molecular outcomes in this model. Female Sprague Dawley rats (n=24) underwent thoracic spinal contusion, either with analgesia (SC 0.25% bupivacaine 4 mL/kg + 3 doses SC 0.05 mg/kg buprenorphine q8h) or with matching doses of sterile water. Semi-quantitative cageside pain assessment scores were elevated postoperatively in both groups ( $p < 0.05$ ) but were unaffected by treatment. Rat Grimace Scale (RGS) scores in a small subset of animals during the 12h postoperative period indicate increased scores relative to baseline at 4h in both control ( $p < 0.01$ ) and treatment ( $p < 0.05$ ) groups, but no effect of treatment overall. Interestingly, treated animals also showed increased RGS scores relative to baseline 12h after surgery ( $p < 0.05$ ). Treated animals lost less weight during the immediate postsurgical period (significant at 12h and 36h,  $p < 0.05$ ) but the body weight nadir occurred concurrently and to the same magnitude. On the day of surgery, treated animals displayed greater movement time ( $p < 0.05$ ) and more movement episodes ( $p < 0.05$ ) than controls. Treatment did not significantly impact locomotor recovery over time ( $p = 0.21$ ). Expression in the spinal cord of numerous genes involved in pain signaling and Toll-Like Receptor pathways were downregulated in treatment animals relative to controls. These data indicate that rats exhibit pain following thoracic spinal contusion procedures, but that the analgesic regimen used may have been ineffective. While functional recovery was unaffected, the research implications of altered gene expression must be considered.

Keywords: Spinal cord injury, rat, analgesia, pain management, Rat Grimace Scale, locomotor recovery, RNA



**USING BEHAVIOR TO TIME INITIATION OF OXYTOCIN ADMINISTRATION TO PROLONG LUTEAL FUNCTION IN MARES.** H. S. Manning, E. E. Runcan, M. A. Coutinho da Silva, Department of Veterinary Clinical Sciences

Poor performance and undesirable behavior during estrus are common complaints made by horse owners and trainers. Administration of exogenous oxytocin starting on Day 7 of diestrus has been shown to be effective in extending luteal function and preventing estrus behavior in mares. The current protocol requires serial veterinary examinations to determine the exact date of ovulation. The objective of this study is to use estrus behavior alone to determine the appropriate time for starting the oxytocin protocol. Twenty-two light breed mares were teased by introducing them to a stallion and observing for signs of sexual receptivity. On the day that estrus behavior was observed (Day 0), mares were randomly divided into two groups: Oxytocin (n=11): oxytocin (60 IU, IM) was administered once daily from Day 8 to 17; Control (n=11): did not receive treatment. Blood samples were collected from all mares throughout the experiment to determine serum progesterone concentration (levels >1 ng/ml indicated a functioning corpus luteum). The average interestrus interval between groups was compared by independent samples t-test. Data is presented as mean  $\pm$  SEM. Significance was set at  $P < 0.05$ . The average interestrus interval was higher for oxytocin treated mares compared to controls (21.5 $\pm$ 1.6 vs. 32.4 $\pm$ 4.2 days). In the oxytocin group, the interestrus interval was longer than 31 days in 6/11 (54.5%) mares and up to 45 days in 5/11 mares (45.45%). We conclude that luteal maintenance was attained by once daily oxytocin administrations beginning 8 days following behavioral signs of estrus.

**Keywords:** Oxytocin, Mare, Estrus, Behavior, Corpus luteum

**EFFECT OF BEHAVIORAL ACTIVITY ON LAMENESS IN TRANSITION DAIRY COWS.**

Piñeiro, J.M.<sup>‡</sup>, B.T. Menichetti<sup>‡</sup>, A.A. Barragan<sup>‡</sup>, W.P. Weiss<sup>†</sup>, S. Bas<sup>‡</sup>, and G.M. Schuenemann<sup>†</sup>

<sup>‡</sup>*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210*

<sup>†</sup>*Department of Animal Sciences, The Ohio State University, Wooster, OH 44691*

The objective of this study was to assess the effect of behavioral activity on lameness in transition dairy cows. A behavioral activity index (BAI) was computed for every animal taking into account the number of steps (no./d), standing time (min/d), lying time (min/d), and lying bouts (LB, no./d). A total of 387 Holstein dairy cows (110 primiparous and 277 multiparous) in 3 commercial dairy herds were enrolled at 7 d prior to calving until 14 d post-calving. Weekly, a cohort of 10 to 15 pre-partum heifers and cows were enrolled at each farm, and electronic data loggers (IceQube, Edinburgh, UK) were fitted to the hind leg of individual animals to assess their behavioral activity. Pre-partum heifers and cows were moved into pre-partum pens 21 d before the expected calving date. All heifers and cows were housed in similar pre-partum free-stall barns and moved into a contiguous individual maternity pen for parturition. Animals were screened for lameness using a 3-point scoring system (LS; 1=normal walk, 2=moderately lame, and 3=severely lame) at 7 d prior to calving and at 14 DIM. The BAI was computed for the first 7 DIM to assess differences among LS in primiparous and multiparous cows. Data were analyzed using MIXED procedure of SAS. Primiparous cows ( $P<0.05$ ) had greater BAI compared to multiparous cows, and as cows mature they become less active around the transition period. Regardless of parity, post-partum cows with LS of 3 spent more time lying (742 min/d) than cows with LS of 1 (654 min/d;  $P<0.05$ ). Post-partum cows with LS of 1 had a greater BAI (380) compared to cows with LS of 3 (278). These results suggest that monitoring a combination of behavioral metrics, such as lying time and BAI, could be used to consistently identify lame cows during the transition period.

Keywords: Lameness, Behavior, Dairy Cattle

**OPTIMAL DECELLULARIZATION PROCESS PRODUCES EXTRACELLULAR MATRIX SCAFFOLD FOR *IN VIVO* CARTILAGE REGENERATION.** E. Pinnell, L. Scheuermann, N. Reisbig, H. Hussein, and A. Bertone. Dept. of Veterinary Clinical Sciences.

Decellularized extracellular matrices [ECM] can serve as biologic structural scaffolds for tissue regeneration. Prior work in our laboratory [Reisbig, Pinnell, Hussein, Bertone, In Press, AJVR, 2016\*] produced a novel synovial origin ECM (synECM) meeting specifications for transplantation of low DNA and cellularity while maintaining tissue integrity. The objective of our current study was to optimize cell migration and engraftment in various scaffold sizes designed for application in animal knee models. Synovial tissue was collected and decellularization was performed using 0.1% peracetic acid (PAA) as published\* with either magnetic agitation or shake agitator for 6 hours, repeated twice. DNA content and fragment size, cell viability, and histology were evaluated on the synECMs. Subsequently, 6mm and 2mm synECM scaffolds were positioned in 6mm inserts of co-culture wells (0.4µm pore size) in a medium with a 30% fetal bovine serum gradient. Viable synoviocytes were seeded onto the 6mm and 2mm synECMs in quantities of 0.5, 1, or 2 x 10<sup>6</sup> cells. After 5 days of culture histology, flow cytometry, and trypan blue staining were performed to assess distribution, cell number and viability of engrafted synovial cells on the synECM. Objective outcomes will be compared between methods by Students-t test with a significant level of P<0.05. Both techniques produced synECMs with low DNA content and no DNA fragments and the histology for tissue integrity is still pending. Outcome assessments for the cell seeding experiment is ongoing. The impact of our work is anticipated to produce consistent large numbers of living synECM grafts that could be engineered and implanted *in vivo* adjacent to cartilage defects to promote regeneration.

Keywords: scaffolds, cartilage regeneration, decellularization, synovial tissue

**A POPULATION STUDY OF NORMAL IONIZED MAGNESIUM AND CALCIUM LEVELS AND THE RATIO OF IONIZED CALCIUM TO MAGNESIUM IN THE HORSE**

S Schumacher DVM, A Bertone DVM, PhD, J Yardley, DVM and M Gabour DVM. Department of Veterinary Clinical Sciences. The Ohio State University, Columbus, Ohio 43210

Magnesium is a highly abused substance in equine competition when used as a calming agent. In racing and show horses, magnesium sulfate ( $MgSO_4$ ) has become a substitute for training. Magnesium sulfate is used for the treatment of human medical disorders including preeclampsia, stroke and severe hypertension associated with pheochromocytoma crisis. In equine medicine, there are legitimate uses for the administration of magnesium sulfate but they are limited. The regulation of a substance that is both highly abused and a threat to the welfare of the horse much more difficult when the substance is a common, essential endogenous element. As for all endogenous substances, the challenge is to develop a threshold value for which an excessive amount detected can be definitive for an exogenous administration. The goal of this population survey was to describe the normal circulating plasma levels of ionized calcium and magnesium, as well as, the normal ionized calcium to magnesium ratio in non-racing horses. Blood samples were obtained from 57 horses. Age of the horses ranged from 1 to 31 and included mares and geldings. Samples were analyzed using a Nova, Stat Profile pHox Ultra In vitro blood gas analyzer and values were obtained including ionized calcium and magnesium. Samples were analyzed at two separate laboratories; The Ohio State University Veterinary Medical Center (OSUVMC) and the Equine Drug Testing and Research Laboratory (EDTRL). Values were adjusted based upon sample pH as this can alter the amount of free calcium and magnesium. For samples analyzed at OSUVMC, the mean values  $\pm$ SD for iCa, and iMg were  $6.14 \pm 0.2$  and  $1.51 \pm 0.11$  mg/dl, respectively. For samples analyzed at EDTRL, the mean values  $\pm$ SD for iCa, and iMg were  $1.54 \pm 0.14$  and  $0.64 \pm 0.06$  mmol/L, respectively. The ratios of pH corrected iCa/iMg were  $2.48 \pm 0.2$  and  $2.41 \pm 0.15$ , respectively for OSUVMC and EDTRL. This work will provide a suitable threshold for the regulation of magnesium administration.

**FEASIBILITY OF VIABLE SYNOVIAL ENGINEERED TRANSPLANTS FOR PARACRINE INFLUENCE ON CARTILAGE REGENERATION.** Scheuermann L, Pinnell E, Reisbig N, Hussein H, Berton AL. Department of Veterinary Clinical Sciences

Synovium, a tissue lining the inside of joints, synthesizes growth factors that are anabolic to articular cartilage and improve shock-absorbing matrix. Our objective was to recapitulate intrinsic signaling with viable engineered synovial grafts designed to overexpress the growth factor BMP-2 and transplant the grafts adjacent to injured cartilage. Frozen 293A cells were thawed, expanded and used to propagate adenoviral (Ad) BMP-2 vector. Cryopreserved, viable equine synoviocytes were thawed, cultured and high numbers were transduced with AdBMP-2 to seed on prepared decellularized synovial scaffolds. ELISA confirmed the living grafts for BMP-2 overproduction. Viable cells ( $1 \times 10^6$ ), measured by flow cytometry, were driven by serum gradient to engraft in the scaffolds. Living grafts were secured to synovium adjacent to cartilage in cadaver rat knees. We successfully produced a >90% viable synovial graft with a mean of  $1 \times 10^6$  cells that overproduced and released soluble BMP-2 (mean  $1.04 \times 10^{-5}$  ng/cell). Grafts were transplanted subpatellar using a standard surgical approach that can be transposed to an arthroscopic approach and lie directly appositional to cartilage for potential paracrine influence on cartilage regeneration. Our work is the first to report a method for novel synovial engineered tissue grafts to potentially promote cartilage regeneration, as opposed to extensive literature on the, as of yet, poorly successful cartilage grafts for cartilage replacement. We documented the feasibility of this strategy for knee application that may benefit humans and animals with knee cartilage damage and osteoarthritis. Our future goal is to move to a clinical model of knee injury in dogs and horses to validate cartilage influence.

Keywords: Cartilage, Regeneration, Synovium, Transplants, Mesenchymal Stem Cells, Bone Morphological Protein 2



**IN VITRO BACTERICIDAL ACTIVITY OF BLUE LIGHT (465-NM) PHOTOTHERAPY ON METHICILLIN-SUSCEPTIBLE AND METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS.** A. Schnedeker, L. Cole, G. Lorch, S. Diaz, J. Bonagura, J. Daniels. Department of Veterinary Clinical Sciences

*Staphylococcus pseudintermedius* is the most common cause of bacterial skin infections in dogs. Methicillin-resistant infections have become more common and are challenging to treat. Blue light phototherapy may be an option for treating these infections. The objective of this study was to measure the *in vitro* bactericidal activity of 465-nm blue light on methicillin-susceptible *Staphylococcus pseudintermedius* (MSSP) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). We hypothesized that irradiation with blue light would kill MSSP and MRSP in a dose-dependent fashion *in vitro* as previously reported for methicillin-resistant *Staphylococcus aureus* (MRSA). In six replicate experiments, each strain (MSSP: n=1), (MRSP ST-71 [KM1381]: n=1) and (MRSA [BAA-1680]: n=1) were cultivated on semisolid media, irradiated using a 465-nm blue light phototherapeutic device at the following cumulative doses: 56.25, 112.5, and 225 J/cm<sup>2</sup> and incubated overnight at 35°C. Controls were not irradiated. Colony counts (CC) were manually performed. Descriptive statistics were performed and treatment effects assessed using the Mann-Whitney-Wilcoxon rank-sum test. Bonferroni-corrected rank sum tests were performed for post-hoc analysis when significant differences were identified. There was a significant decrease in CC with blue light irradiation at all doses for MRSA ( $P=0.0006$ ) but not for MSSP ( $P=0.131$ ) or MRSP ( $P=0.589$ ). Blue light phototherapy significantly reduced CC of MRSA, but not of MSSP or MRSP. The mechanism for the relative photosensitivity of the MRSA isolate is unknown, but is hypothesized to be due to an increased concentration of porphyrin in *S. aureus* relative to *S. pseudintermedius*, which would modulate blue light absorption.

Keywords: *Staphylococcus pseudintermedius*, phototherapy, blue light

**TWO-DIMENSIONAL LONG-AXIS ECHOCARDIOGRAPHIC RATIOS FOR ASSESSMENT OF LEFT ATRIAL AND VENTRICULAR SIZE IN HEALTHY DOGS AND DOGS WITH MITRAL REGURGITATION.** L.E. Strohm<sup>1</sup>, L.C. Visser<sup>1,2</sup>, W.T. Drost<sup>1</sup>, and J.D. Bonagura<sup>1</sup>. <sup>1</sup>Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210. <sup>2</sup> Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, CA 95616.

Mitral valve endocardiosis is the most common heart disease of dogs and leads to mitral regurgitation (MR) with progressive left atrial (LA) and left ventricular (LV) dilatation. The severity of enlargement relates to staging, prognosis, and indications for intervention. The aims of this study were to establish canine reference values for long-axis aortic ratios in healthy dogs and test the utility of this method in dogs with different stages of chronic MR.

Eighty healthy dogs >8 months of age of both sexes and of various bodyweights were recruited. Right parasternal long-axis image planes were used for all measurements. The LV and LA internal dimensions were measured at end-diastole and end-systole, respectively. Aortic dimensions (Ao) were measured in early systole between the maximally-opened valve leaflets. Using these three measurements, the following ratios were calculated: (1) LV/Ao; (2) LA/Ao; and (3) LA/LV. Reference intervals were calculated using the CLSI robust method. Comparisons of ratios from normal dogs to dogs with MR were conducted using a t-test or Mann-Whitney test. The ability of LV/Ao to detect cardiomegaly was compared to values published by Cornell, et. al. (2004) using McNemar's test. Significance was  $p < 0.05$ .

Reference values were: LV/Ao - 1.8 to 2.4; LA/Ao - 1.8 to 2.4; LA/LV - 0.9 to 1.1. The ratios of LV/Ao, LA/Ao, and LA/LV were greater in dogs with MR ( $p < 0.001$ ). Of the 25 dogs with MR, 68% of the dogs demonstrated LV dilation using the LV/Ao ratio method, whereas only 36% exceeded previously-published values for LV diastolic dimension ( $p = 0.002$ ).

This study establishes normal two-dimensional, long-axis LV and LA to aorta ratios based on a relatively large sample of healthy dogs of different body weights. This method provides a simple and sensitive approach to detecting LV and LA enlargement in dogs and has clinical applicability across a range of body weights.

Keywords: canine, cardiac, echocardiography, ultrasound, ratios

**RETROSPECTIVE ANALYSIS OF PULMONARY HYPERTENSION IN CATS WITH LEFT-SIDED CONGESTIVE HEART FAILURE.** T Vezzosi, KE Schober, Department of Veterinary Clinical Sciences

Pulmonary hypertension (PH) caused by left-sided CHF (L-CHF) is common in dogs and contributes to clinical signs and outcome. Anecdotal evidence suggests that PH in cats secondary to L-CHF is much less frequent. However, data on the prevalence of PH in cats with L-CHF is not available. This study addresses the general hypothesis that PH secondary to L-CHF is rare, and that echocardiographic findings suggestive PH are different between dogs and cats.

Retrospective observational study including 56 healthy cats and 131 cats with L-CHF imaged between 2004 and 2016. Diagnostic variables included tricuspid regurgitation (TR) velocity, right atrial (RA) and right ventricular (RV) size and function, RV wall thickness, pulmonary artery size, systolic time intervals of PA flow (STIs), presence of septal flattening, and variables characterizing LA size and LV size and function. PH was identified if TR peak velocity was  $>2.7$  m/s.

Tricuspid regurgitation was present in 57/131 (44%) of cats with L-CHF, and PH was present in 22/131 of those cats (17%). In 15/22 cases PH was associated with cardiomyopathy, in 5/22 with congenital heart disease, and in 2/22 with other causes. All cats with PH (22/22, 100%) had subjectively-assessed right-sided enlargement, with larger RA and RV diameters ( $P<0.0001$ ), thicker RV wall thickness ( $P<0.05$ ) and higher prevalence of septal flattening (6/22, 27%,  $P<0.0001$ ) compared to cats without PH. Maximum RA diameter ( $>21.5$  mm; Sensitivity [Se] 0.82, Specificity [Sp] 0.42) and RV diameter ( $>7.8$  mm; Se 0.91, Sp 0.64) were identified as the most accurate diagnostic cutoffs to predict PH compared to other variables.

Compared to dog, PH is not a common finding in cats with L-CHF. Right-sided enlargement is the main finding in cats with PH, and right-heart dimensions should be used to suspect PH if TR is absent or difficult to measure.

Keywords: Feline, echocardiography, heart failure, pulmonary hypertension

**ASSOCIATION OF PROGESTOGENS WITH INFLAMMATION AND IMMUNITY IN CRITICALLY ILL FOALS.** S.M. Vijan, K.A. Dembek, S. Reed, R.E. Toribio. Department of Veterinary Clinical Sciences

Septicemia is the leading cause of mortality in newborn foals. Progesterone is mainly known for its role in pregnancy; however, it is also a precursor to adrenocortical steroids and likely plays important functions in disorders of the equine neonate. Human studies have demonstrated that progesterone modulates immunity and predisposes to inflammatory conditions. The relevance of high progesterone concentrations in the systemic inflammatory response to sepsis in sick foals remains unclear. The goal of our study was to measure blood concentrations of inflammatory cytokines and serum amyloid A (SAA) in healthy and hospitalized foals, and to determine their association with progestogens, disease severity, and mortality in foals. We hypothesized septic foals will have higher progesterone, 17 $\alpha$ -hydroxyprogesterone and cortisol concentrations that will be associated with the inflammatory response (cytokines and SAA), disease severity and mortality. A total of 52 hospitalized foals (septic and sick non-septic [SNS]) and 10 healthy were included. Hospitalized foals were also divided into survivors and nonsurvivors. Blood samples were collected on admission. Hormones and cytokines were measured by immunoassays. Interleukin1 $\beta$ , SAA, progesterone, 17 $\alpha$ -hydroxyprogesterone, and cortisol concentrations were significantly higher, while leukocyte count and IgG concentrations were lower in septic and SNS compared to healthy foals. Interleukin 4 was significantly higher in non-survivors. IgG and interleukin 6 concentrations were positively correlated. Progesterone was significantly lower in non-survivors and negatively correlated with SAA. These results suggest that progesterone, in addition to cortisol, is involved in the adrenocortical response to stress from critical illness in foals. This is the first study reporting an association between progestogens with markers of inflammation, severity of disease and mortality in hospitalized foals.

Keywords: progesterone; sepsis; cytokines; equine neonates

**ACCESSING POTENTIAL CAUSES OF REPEAT BREEDER DIARY COWS THROUGH CYTOLOGY, BACTERIOLOGY, AND HISTOPATHOLOGY.** M. Werner, A. Szczotka, C. Premanandan, and S. Bas. Department of Veterinary Preventative Medicine and Department of Veterinary Biosciences

Reproductive failure leads to increased culling rates, decreased milk yield, and decreased genetic progress; thus, negatively affecting the economic return of dairy operations. A repeat breeder (RB) is defined as a cow without clinically detectable reproductive disorders that regularly cycles every 17 to 25 days and that fails to become pregnant after three or more inseminations. The reported prevalence of RB dairy cattle ranges between 5% and 36%. The objective was to evaluate the gross pathology, bacteriology, cytology, and histopathology of the reproductive tracts of RB dairy cows to determine potential causes of decreased fertility. We hypothesized that RB cows would have increased gross and microscopic pathological conditions and increased bacterial growth when compared to non--RB cows. Immediately after slaughter, the reproductive tracts of 6 RB and 6 non--RB cows were assessed for gross anatomical findings. In addition, cytology, biopsy and swab samples were obtained from the cervix, uterine body, uterine horns, and oviducts. Macroscopic evaluation of the reproductive tracts revealed that 50% of RB and 16.6% of non--RB cows presented anatomical alterations. Based on cytology 26% (6/24) of the samples from RB and 0% of non--RB cows had subclinical endometritis. Bacteriology preliminary results show increased bacterial growth (*E. coli*) in RB (16.7%) when compared to non--RB cows (5%). In addition, focal sites of infection based on culture and cytology were observed. Our preliminary findings illustrate gross and microscopic pathological differences between RB and non--RB dairy cattle. Furthermore, our results illustrate variances of infection and inflammation to focal areas of the reproductive tract.

Keywords: Repeat Breeder Cows. Subclinical endometritis. Histopathology.



**EVALUATING THE CONSUMPTION OF ORAL NSAID PRODUCTS AS PART OF MULTIMODAL PAIN MANAGEMENT IN A MYOCARDIAL INFARCTION MODEL IN C57BL/6 MICE.** S Young<sup>1</sup>, V Shettigar<sup>2</sup>, C Freed<sup>1</sup>

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SBS-Physiology and Cell Biology, The College of Medicine, The Ohio State University, Columbus, OH <sup>2</sup>

At our institution, Ibuprofen added to the drinking water is commonly used to provide non-steroidal anti-inflammatory drug (NSAID) for rodent surgical models and clinical cases. More selective NSAIDS are available in various oral formulations but products are not always preferentially consumed. The purpose of this study was to quantify consumption of Carprofen diet gel (C) and Meloxicam tablets (M) relative to Ibuprofen water (I) in a mouse thoracotomy model. Ten-week-old C57BL/6 male mice were housed 3/cage. Surgical group (Sx): Under Isoflurane anesthesia mice were intubated, ventilated, and a myocardial infarction created. Twice daily subcutaneous buprenorphine (0.1 mg/kg) was provided for 72 hours and cages (n=9) were assigned to 3 NSAID treatment groups; C (17.9 mg/kg), M (20 mg/kg), or I (40 mg/kg). NSAIDS were provided for four days starting 24 hours before surgery. A non-surgical group (NS) of mice were anesthetized and intubated. Mice received buprenorphine, and were assigned to the same three groups (n=9). To determine the impact of opioids on consumption, a second NS group was completed (n=9) without buprenorphine, although no differences were observed. Individual body weight was tracked. Behavioral assessments, food and NSAID consumption were tracked by cage. Body weight decreased for all mice following anesthesia regardless of surgery. Post-operative mortality in the Sx group limited consumption data analysis. Consumption of I water in the NS group was significantly greater on days 1 and 2(vs. C and M), and day 3(vs. M). On average, two products were consumed by NS groups in sufficient amounts (5g/mouse) for therapeutic efficacy, based on product labels; C prior to anesthesia while I consumption was sufficient on day 1, 2, and 3 post-anesthesia. In conclusion, additional information is needed to correlate consumption and clinical efficacy if using these products in practice and close oversight is needed to ensure appropriate analgesia.

Keywords: Analgesia, Oral NSAIDS, Ibuprofen, Carprofen, Meloxicam, Thoracotomy, Myocardial Infarction

**EPIDEMILOGY  
AND  
APPLIED RESEARCH**

**COMPANION ANIMAL, EQUINE, AND LIVESTOCK ENVIRONMENTAL SURFACES CONTAMINATED WITH EXTENDED-SPECTRUM CEPHALOSPORIN, CARBAPENEM, AND FLUOROQUINOLONE RESISTANT *ENTEROBACTERIACEAE***

Adams RJ<sup>1</sup>, Mathys D<sup>1</sup>, Mollenkopf D<sup>1</sup>, Kim S<sup>1</sup>, Daniels J<sup>1</sup>, Wittum T<sup>1</sup>

<sup>1</sup>The Ohio State University, Department of Veterinary Preventive Medicine

**ABSTRACT:**

Antimicrobial resistant bacteria represent an important concern impacting both veterinary medicine and public health. The rising prevalence of extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase, carbapenemase (CRE), and fluoroquinolone-resistant *Enterobacteriaceae* continually decreases the efficiency of vital antibiotics. Moreover, antibiotic resistant enteric bacteria can be transmitted between animals and people, thus serving as a dangerous zoonotic health risk. Our objective was to evaluate the prevalence of antibiotic resistant bacteria on human contact surfaces in various animal environments. Environmental surfaces from companion animal shelters, private equine facilities, dairies, livestock auction markets, and livestock areas of county fairs were collected using electrostatic-cloths. Samples were screened for AmpC, ESBL, CRE, and fluoroquinolone phenotypic resistance using selective media and cephalosporin resistance phenotypes were confirmed using standard PCR techniques. Livestock auction markets and county fairs revealed higher levels of both cephalosporin and fluoroquinolone resistance than equine, dairy, and companion animal environments. Equine facilities harbored more cephalosporin resistance than companion animal shelters, but less fluoroquinolone resistance. The regular use of cephalosporins (ceftiofur) in livestock species could account for the heightened levels of resistance in livestock species environments compared to companion animal and equine facilities. Human surfaces as well as human and animal surfaces were contaminated with various resistant bacteria regardless of species environment. Detecting these bacteria on common human contact surfaces suggests that the environment can serve as a reservoir for antimicrobial resistance genes. Identifying interventions to lower the prevalence of antibiotic resistant bacteria in animal environments will protect both animal and public health.

Keywords: antimicrobial resistance, environmental contamination, livestock, companion animals

**COMPARISON OF THE MICROBIOLOGICAL QUALITY OF FRESH PRODUCE FROM SEASONAL FARMER'S MARKETS AND RETAIL GROCERY STORES IN OHIO.** A. Albers, S. Feicht, D. Mathys, D. Mollenkopf, T. Wittum

The frequent use of antimicrobial drugs in veterinary medicine can result in the emergence and dissemination of antimicrobial resistance in a variety of animal populations.  $\beta$ -lactamases confer bacterial resistance to critically important antimicrobials used in both human and veterinary medicine. Livestock are an important emergence reservoir for zoonotic food-borne transmission of resistant enteric bacteria including *Salmonella* spp. Fresh produce can serve as a vehicle for the zoonotic food-borne transmission of resistant enteric bacteria as a result of livestock fecal contamination. The objectives of this study were to measure the frequency of  $\beta$ -lactamase microbial contamination, and to determine both *Salmonella* and coliform contamination on fresh produce. Samples of leafy greens, tomatoes, and cucumbers were purchased each week from various local farmer's markets and grocery stores throughout May to August 2015 and 2016. The produce samples were placed in buffered peptone water (BPW) and inoculated onto spread plates for detection and quantification of coliform bacteria. An aliquot of the BPW was cultured for the presence of *Salmonella*. To test for the presence of  $\beta$ -lactamase-producing bacteria, samples were enriched in a nutrient broth with cefotaxime, then inoculated onto three MacConkey agar containing ceftiofur, cefepime, or meropenem. We sampled 36 farmer's markets and 33 grocery stores to obtain a total of 364 fresh produce samples. There were 24 (6.59%) samples which produced isolates resistant to ceftiofur and cefotaxime antimicrobials, with 11 (3.02%) confirmed by PCR to contain *bla*<sub>CMY</sub>. There were 5 (1.37%) samples which produced isolates resistant to cefepime and cefotaxime antimicrobials, with one (0.27%) confirmed by PCR to contain *bla*<sub>CTX</sub>. No carbapenem resistant isolates or *Salmonella* spp. were recovered from fresh produce samples. Our results indicate that there is little difference in microbiological quality between farmer's market and grocery store produce measured by the presence of antimicrobial resistant enteric bacteria or coliform contamination.

Keywords: farmer's markets, zoonotic, antimicrobial contamination

## EAR - 3

### **THE EFFECT OF DISEASE ON EARLY MATERNAL BEHAVIORS IN DAIRY CATTLE.**

E. Bratton, N. Perier, K. Proudfoot. Dept. of Veterinary Preventive Medicine and Vetagro Sup CVM, Lyon, France

Dairy cows are at high risk of becoming ill after giving birth. Cows with infectious disease behave differently compared to those that remain healthy, but no research has studied the effect of illness on maternal behavior directed toward the calf. The objective was to determine the effect of disease on maternal behavior after calving. Twenty multiparous Danish Holstein dairy cows were housed in individual pens with their calves for 4 d after calving. Of these cows, 10 were diagnosed with an infectious disease (mastitis, retained placenta, pneumonia, and metritis) by the veterinarian within 3 d of calving. Ill cows were matched for parity with 10 healthy cows. Calves were provided 4 L of colostrum by bottle within 6 h of birth. The duration of time that the dam spent grooming the calf, the duration of time spent lying, and the amount of time the calf spent drinking colostrum (from the dam or bottle) were measured continuously by video for 2 d after calving. Data were analyzed using mixed models including match, health status, day and a health by day interaction. There was no effect of health status on the amount of time the dam spent grooming the calf ( $P = 0.42$ ), but cows spent less time grooming on day 2 compared to day 1 ( $P < 0.0001$ ). Ill cows spent more time lying compared to healthy cows on d 1 and 2 ( $P = 0.03$ ). Calves from healthy and ill cows spent the same amount of time drinking milk ( $P = 0.77$ ) overall, however calves spent less time drinking milk on d 2 compared to d 1 ( $P = 0.08$ ). Awareness of changes in maternal behavior after calving may significantly impact the way that producers manage calves from sick dams, and could improve overall herd health and productivity.

Keywords: Lying time, grooming, nursing

## EAR - 4

### **EFFECT OF LOWER STARCH DIET ON HEALTH AND BEHAVIOR OF SLOTH BEARS (*MELURSUS URSINUS*) AT CLEVELAND METROPARKS ZOO**

K. Cashin, P. Dennis, K. Anderson, L. Bernstein Kurtycz. Dept of Preventative Medicine

Tropical and polar bear populations housed in human care are recognized as having a higher prevalence of biliary cancer than wild populations. These carcinomas are not seen commonly in domestic species. In humans, biliary carcinomas have been linked to liver flukes and identified in populations that consume large proportions of fish in their diets. Chronic inflammation may be a precursor to the formation of neoplasia and will be a main focus of this study. Sloth bears in zoos are fed a diet that is not typical of what would be consumed in the wild. The increased carbohydrates and starch in the captive diet may be leading to increased inflammation and stereotypic behaviors. Serum inflammatory markers, insulin resistance parameters, and behavior of the two sloth bears (*Melursus ursinus*) housed at the Cleveland Metroparks Zoo will be compared before and after a lower starch diet is introduced. Specific markers to be analyzed include insulin, insulin: glucose ratio, and serum amyloid A. Behavior observations are conducted using video recording while the bears are on exhibit. It was hypothesized that providing the sloth bears with a diet that is lower in starch will lead to decreased inflammation and stereotypic behaviors. Comparing the activity budgets of the sloth bears before and after the diet change could support the hypothesis that diet influences behavior.

Keywords: sloth bear, behavior, zoo

## EAR - 5

### **DISSEMINATION OF ANTIMICROBIAL RESISTANT ENTERIC BACTERIA IN A ZOO ENVIRONMENT.** S. M. Feicht, D. A. Mathys, D. F. Mollenkopf, T. E. Wittum, Department of Veterinary Preventive Medicine

Both antimicrobial resistant bacteria and *Salmonella* can contaminate the environment of public animal exhibits such as zoos, which can pose a potential health hazard to both the visitors and the animal population. The objective of this study is to determine the prevalence *Salmonella* contamination as well as extended-spectrum beta-lactam and fluoroquinolone resistant *Enterobacteriaceae* on surfaces of human and animal areas of a large metropolitan zoo. Individual electrostatic cloths were used on flat surfaces of human and animal contact areas, and then enriched in nutrient broth with 2 µg/ml cefotaxime or 16 µg/ml naladixic acid. Incubated cefotaxime broth was inoculated onto MacConkey agar with 8 µg/ml of cefoxitin, 4 µg/ml of cefepime, or 1 µg/ml of meropenem, to identify the *bla*<sub>CMY</sub>, *bla*<sub>CTX-M</sub>, and carbapenemase phenotypes. Naladixic acid broth was inoculated onto MacConkey agar with 2 µg/ml of ciprofloxacin or 16 µg/ml naladixic acid, to identify fluoroquinolone resistant phenotypes. A third cloth was enriched in buffered peptone water and Rappaport-Vassiliadis broth, and subsequently inoculated onto XLT-4 agar for the isolation of *Salmonella*. Phenotypic *bla*<sub>CMY</sub> isolates were found on 34.9% of surfaces, while *bla*<sub>CTX-M</sub> isolates were found on 12.7% of surfaces. Naladixic acid resistant isolates were found on 36.7% of surfaces, but ciprofloxacin resistant isolates were found on only 18.1% of sampled surfaces. Meropenem resistant isolates were recovered from 4.8% of surfaces sampled. Recovery of antimicrobial resistant bacteria varied between human and animal contact surfaces, with no consistent pattern observed. *Salmonella* were recovered from only 0.6% of surfaces. These results suggest that the zoo environment harbors coliform bacteria resistant to clinically important antimicrobials, and provides an opportunity for a diverse population of humans and animals to be exposed to bacteria expressing multiple antimicrobial resistant phenotypes.

Keywords: Pathogen, Antimicrobial Resistance, Zoo, Environment

**CORE GENOME MULTILOCUS SEQUENCE TYPING SCHEME (CGMLST): A GLOBAL STANDARDIZED APPROACH FOR MOLECULAR TYPING OF *MYCOPLASMA GALLISEPTICUM*.** M.Ghanem<sup>1</sup>, L.Wang<sup>2</sup>, Y. Zhang<sup>2</sup>, S. Edwards<sup>3</sup>, D. Harmsen<sup>4</sup>, D. Ley<sup>5</sup> and M. El-Gazzar<sup>1</sup>

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*Mycoplasma gallisepticum* (MG) is the most pathogenic avian mycoplasma species to poultry, causing respiratory disease and reduced production efficiency. Currently, MG strain differentiation is based on the sequence analysis of only 5 loci proved to be insufficient for accurate outbreak investigation. Recently, whole genome sequence (WGS) of many human and animal pathogens has been successfully used for routine microbial diagnosis and outbreak investigation. However, the massive amount of sequence data and the diverse properties of different genes within bacterial genomes resulted in the lack of standard reproducible method for comparison between MG whole genomes. Here, we are proposing the development of cgMLST for typing of MG strains and field isolates. For development of this scheme, a diverse collection of 37 MG genomes was used to identify the cgMLST targets. A total of 425 MG conserved genes (49.85% of MG genome) were selected as core genome targets for this scheme. A total of 81 MG genomes from 5 countries in 4 continents were later typed using this scheme. Analysis of phylogenetic trees generated by cgMLST displayed a high degree of agreement with geographical and temporal information. Moreover, the high discriminatory power of cgMLST allowed differentiation between samples of the same outbreak, resolving the confusion in many historical MG outbreaks. MG-cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation between MG isolates. cgMLST provides stable and expandable nomenclature, allowing for comparing and sharing the typing results between different laboratories worldwide. cgMLST offers an opportunity to harness the tremendous power of next generation sequencing technology in applied avian mycoplasma epidemiology at a local and global level.

Key words: *Mycoplasma gallisepticum*, Molecular typing, Multilocus sequence typing (MLST), Whole genome, Next-generation sequencing.



**ANTIBIOTIC ALTERNATIVES FOR THE REDUCTION OF MORBIDITY AND MORTALITY IN VEAL CALVES.** E Holder\*, J Pempek\*, K Proudfoot\*, M Masterson\*, J Lakritz†, and G Habing\*

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Diarrhea is a leading cause of morbidity and mortality in veal and dairy calves. Improvements in antibiotic stewardship necessitate alternative therapies to improve calf health while reducing the need for antibiotics important to human health. Alternative therapies, such as lactoferrin (iron-binding protein found in milk) and cinnamaldehyde (essential oil of the cinnamon plant) have antimicrobial properties in vitro and have been shown to increase calf performance. This study investigated the effect of lactoferrin and cinnamaldehyde on body weight (**BW**), morbidity, and mortality in special-fed veal calves. On the day of arrival to the farm (3 -5 d of age), calves (n = 80 per treatment) were randomized to 1 of 3 treatments: 1) control (no supplement), 2) lactoferrin (1 g/d in milk replacer (**MR**) for 7 d), or 3) cinnamaldehyde (1 g/d in MR for 21 d). BW was measured on day of arrival, 21, and 42 d of age. Health assessments were performed twice weekly through 21 d, and mortality records were obtained through 6 wk of age. A repeated measures ANOVA (PROC MIXED, SAS) was used to compare BW, and a generalized linear mixed model (PROC GLIMMIX, SAS) was used to test differences in the incidence risk of diarrhea (fecal score  $\geq 2$ ). Average BW was similar ( $P = 0.82$ ) between treatment groups (mean  $\pm$  SE; Control:  $57.5 \pm 0.3$  kg, Lactoferrin:  $57.3 \pm 0.3$  kg, Cinnamaldehyde:  $57.6 \pm 0.3$  kg). Neither supplementation with lactoferrin or cinnamaldehyde had an effect on the incidence of diarrhea. Mortality through 6 wk was low, with 4, 1, and 0 deaths from control, lactoferrin, and cinnamaldehyde treatments, respectively. Thus, supplementing lactoferrin or cinnamaldehyde was not associated with improved growth or decreased incidence of diarrhea in special-fed veal calves.

Keywords: antibiotic alternative, Lactoferrin, Cinnamaldehyde, veal calf, diarrhea, mortality, weight gain

**RATES AND RISK FACTORS FOR SURGICAL SITE INFECTIONS AT THE OHIO STATE UNIVERSITY VETERINARY MEDICAL CENTER.** C. Kramer, Dr. J. Daniels. Departments of Veterinary Biosciences and Veterinary Clinical Sciences

Surgical site infections (SSIs) are among the most devastating complications of surgical procedures, resulting in prolonged pain and recovery time, increased expenses, subsequent procedures, and occasionally death. It is therefore preferable to prevent SSIs than treat them. Furthermore, it is more difficult to treat infections caused by antibiotic-resistant bacteria, which are the cause of many SSIs. Understanding the factors that contribute to SSIs is key to decreasing their prevalence. The prevalence of SSIs in canine Tibial Plateau Leveling Osteotomies (TPLOs), a common orthopedic procedure, has been reported to range from 3% to 13%. This retrospective study seeks to determine the prevalence and risk factors for SSIs in TPLOs performed at the OSU Veterinary Medical Center. Medical records from canine patients (n=865) that underwent TPLOs between 1/1/2012 and 12/31/2014 were analyzed and evaluated. Data collected (36 variables) included patient demographics, clinical history, anesthesia duration, type of procedure (arthroscopy or arthrotomy), postoperative antibiotics prescribed, postoperative followup, and diagnosis and treatment of postoperative infections. Of the 865 cases evaluated, 147 cases were lost to followup and 51 surgical site infections were recorded. The prevalence of SSIs, not including the 147 TPLOs lost to followup, was 7.1%. This study will greatly assist the OSU VMC in monitoring the prevalence of SSIs and implementing new procedures and policies to lower the occurrence of these devastating complications.

Keywords: Tibial Plateau Leveling Osteotomy (TPLO), Surgical Site Infection (SSI), Surgical Complications, MRSP, Retrospective Study

**CASE REPORT: H1N1 INFLUENZA A VIRUS-ASSOCIATED CLINICAL DISEASE AND MORTALITY IN AN EXHIBITION PIG** J. N. Lorbach<sup>1,2</sup>, J. M. Nolting<sup>1</sup>, S. W. Nelson<sup>1</sup>, C. L. Siepker<sup>3</sup>, R. L. Poulson<sup>4</sup>, D. E. Stallknecht<sup>4</sup>, and A. S. Bowman<sup>1</sup>

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Influenza A virus (IAV) is a respiratory pathogen of great importance with respect to human and animal health. Swine are widely considered to be a mixing vessel allowing for viral reassortment of IAV and host adaptation to humans, a key development in the evolution of pandemic IAV. Over recent years domestic swine have been increasingly associated with zoonosis and reverse-zoonosis of IAV. Exhibition swine at agricultural fairs have been a documented source of human infection with IAV and remain an important population for surveillance efforts. In 2012 zoonotic transmission of IAV resulted in regional outbreak of H3N2 variant (H3N2v) influenza in humans with exposure to swine characterized by limited human-to-human transmission of the virus. Here we describe the case of a six month-old Hampshire gilt that presented for clinical evaluation of respiratory disease. The pig was housed with ten other swine that showed no signs of disease. The animal died while in clinical care, and post-mortem examination and collection of lung tissue samples were performed. RNA was extracted from lung tissue homogenate, and reverse-transcription polymerase chain reaction (RT-PCR) for IAV matrix segment indicated the presence of IAV in the sample (Ct=25.95). PCR subtyping identified the virus subtype as H1N1, and whole genome sequencing is pending. This case demonstrates the importance of clinical workup and diagnostic testing for IAV in swine demonstrating respiratory signs; disease is often subclinical but may progress rapidly and can result in fatal complications as was the apparent case in this animal. Furthermore, owners from different agricultural backgrounds may fail to adequately recognize the potential human disease risk associated with these animals. Appropriate education of owners and increased clinical awareness will be integral to timely diagnosis of IAV in exhibition swine and minimizing the potential human health impact of this virus.

Keywords: Orthomyxovirus, influenza A virus, exhibition swine, zoonosis, pandemic

**THE IMPLEMENTATION OF HAND HYGIENE RECOMMENDATIONS AT AGRICULTURAL FAIRS TO PROTECT PUBLIC HEALTH.** A. Martin, M. Zentkovich, J. Nolting, D. Mathys, D. Mollenkopf, A.S. Bowman. Department of Veterinary Preventive Medicine

Agricultural fairs are a prime location for zoonotic disease transmission. Specifically, the transmission of influenza A virus (IAV) from exhibition swine to humans occurs in this setting via direct and indirect contact. Public health organizations have established hand hygiene guidelines for animal settings to prevent the spread of zoonotic disease. We hypothesized that although these guidelines are well established, adoption of these recommendations by fair officials and/or fairgoers is low. Over 3 years, 107 fairs were analyzed to determine presence and functionality of hand hygiene stations, as well as presence of signage for human health risks and hand wash procedures. Overall, 87.9% of fairs had functional hand hygiene stations. Interestingly, 9 fairs added wash stations the year after exhibition swine tested IAV positive. During 2016, presence of IAV and antimicrobial resistant bacteria on these stations, and their usage by fairgoers, was tested at 20 fairs. Up to 5 functional hand hygiene stations were tested per fair representing animal housing and human only use areas. Stations were wiped with an electrostatic cleaning cloth, moistened with BHIB and taken to the lab for testing. All 93 samples were RRT-PCR negative for IAV, while 73 of those samples tested positive for antimicrobial resistant bacteria. Bacteria found included *Escherichia coli*, *Salmonella* and other undistinguishable gram-negative bacteria. Samples were found to be resistant to cephalosporins and/or fluoroquinolones. At 19 of the fairs, up to 2 of the tested stations were observed for an hour each to determine the number of proportion of fairgoers using stations upon exiting an animal area. Only 7.0% of people used these stations. Overall, fairs have followed hand hygiene guidelines by providing hygiene stations. However, the gap identified between these guidelines and their adoption by fairgoers illustrates a strong need to educate the public on zoonotic disease transmission and prevention.

Keywords: hand hygiene, public health, agricultural fairs, influenza A virus, antimicrobial resistant bacteria

**CARBAPENEM RESISTENT *ENTEROBACTERIACEAE* (CRE) PRESENT IN WASTEWATER TREATMENT PLANT (WWTP) EFFLUENT AND NEARBY SURFACE WATERS IN THE US.** D. A. Mathys, D. F. Mollenkopf, S. M. Feicht, R. J. Adams, A. Albers, D. Stuever, J. B. Daniels, T. E. Wittum. Depts. Of Veterinary Preventive Medicine and Veterinary Clinical Sciences

CRE are a critically important threat to the public health, and have been identified as an urgent threat by the CDC and WHO. CREs are rare but highly resistant organisms most commonly associated with hospitalized patients. Metropolitan WWTPs filter water from large geographic areas which often include hospitals, and can serve as maintenance reservoirs for CRE. However, little is known about the potential impact of these WWTP CRE on the local surface water. If CREs are present in the downstream surface water, they may ultimately disseminate to intensively-managed animal agriculture facilities. We obtained one-liter samples from the effluent and both the upstream and downstream surface water from 50 WWTPs throughout the United States. Samples were vacuum filtered using a series of sterile filters culminating in a 0.45  $\mu\text{m}$  pore size filter. All filters were incubated overnight with 100 ml of MacConkey broth modified with 0.5  $\mu\text{g/ml}$  of meropenem and 70  $\mu\text{g/ml}$  of zinc sulfate, then inoculated onto similarly enriched MacConkey agar to identify carbapenem-resistant phenotypes. Isolates then underwent CarbaNP testing to confirm carbapenemase production and were species identified using MALDI-TOF mass spectrometry. Of the 50 WWTPs, 26 were from large metropolitan areas, while 24 came from small towns with populations less than 10,000. We found that 18%, 36%, and 24% of WWTPs harbored CRE in the upstream, effluent, and downstream water samples, respectively. There was no difference in the frequency of CRE recovery between metropolitan and rural WWTPs, nor between upstream, effluent, and downstream samples. These results indicate that our surface water in the United States is routinely contaminated with clinically important, hospital associated CRE. This a major concern for public health and agriculture, because introduction of CRE into intensively managed agricultural environments could lead to amplification and foodborne dissemination to large populations.

Keywords: Surface water, CRE, Epidemiology

**CARBAPENEMASE-PRODUCING *ENTEROBACTERIACEAE* RECOVERED FROM THE ENVIRONMENT OF A SWINE FARROW-TO-FINISH OPERATION IN THE UNITED STATES.** D. F. Mollenkopf<sup>1</sup>, J. W. Stull<sup>1</sup>, D. A. Mathys<sup>1</sup>, A. S. Bowman<sup>1</sup>, S. M. Feicht<sup>1</sup>, J. B. Daniels<sup>2</sup>, T. E. Wittum<sup>1</sup>. <sup>1</sup>Department of Veterinary Preventive Medicine, The Ohio State University College of Veterinary Medicine, Columbus, OH. <sup>2</sup>Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH.

Carbapenemase-producing *Enterobacteriaceae* (CPE) present an urgent threat to public health. While carbapenem antimicrobials are restricted in food-producing animals, other  $\beta$ -lactams, such as ceftiofur, are used in livestock. This use may provide selection pressure favoring the amplification of carbapenem resistance but this relationship has not been established. Previously unreported from US livestock, plasmid-mediated CPE have been reported from livestock in Europe and Asia.

Environmental and fecal samples were collected from a 1,500 sow, US farrow-to-finish operation during 4 visits over a 5-month period, 2015. Samples were screened using selective media for the presence of CPE, with resulting carbapenemase-producing isolates further characterized.

Of 30 environmental samples collected from a nursery room on our initial visit, 2 (7%) samples yielded 3 isolates: 2 ST 218 *Escherichia coli* and 1 *Proteus mirabilis*, carrying the metallo- $\beta$ -lactamase gene *bla*<sub>IMP-27</sub> on an IncQ1 plasmid. We recovered 15 IMP-27-bearing isolates of multiple *Enterobacteriaceae* species from 11 of 24 (46%) environmental samples from 2 farrowing rooms collected on our third visit. These *bla*<sub>IMP-27</sub> isolates were also carried on IncQ1 plasmids. CPE isolates were recovered later from piglet fecal swabs or sow fecal samples.

To control disease, piglets on this farm receive ceftiofur at birth, with males receiving a second dose at castration ( $\approx$ day 6). This selection pressure may favor the dissemination of *bla*<sub>IMP-27</sub>-bearing *Enterobacteriaceae* in this farrowing barn. The absence of this selection pressure in the nursery and finisher barns likely resulted in the loss of the ecological niche needed for maintenance of this carbapenem resistance gene.

Keywords: Swine, antimicrobial resistance, carbapenemase-producing *Enterobacteriaceae*, *bla*<sub>IMP-27</sub>,

**FECAL MICROBIOME OF PERIPARTURIENT DAIRY CATTLE AND ASSOCIATIONS WITH THE ONSET OF SALMONELLA SHEDDING.** L. Muñoz-Vargas,<sup>1</sup> S. O. Opiyo,<sup>2</sup> R. Digianantonio,<sup>1</sup> M. Williams,<sup>2</sup> A. Wijeratne,<sup>2</sup> and G. Habing<sup>1</sup>. <sup>1</sup>Department of Veterinary Preventive Medicine, College of Veterinary Medicine. <sup>2</sup>Ohio Agricultural Research and Development Center, The Ohio State University.

Non-typhoidal *Salmonella* enterica is a zoonotic pathogen with critical importance in animal and public health. Disruptions in the microbial structure facilitate the intestinal colonization of this bacterium. To overcome the colonization resistance of the health gut microbiome, *Salmonella* uses invasion strategies and the host inflammatory response to survive, proliferate, and establish infections with diverse clinical manifestations. Cattle serve as an important reservoir of *Salmonella*, and periparturient cows have a higher prevalence of *Salmonella* shedding; however, little is known about the association between the gut microbiome and the onset of *Salmonella* shedding during the periparturient period. Therefore, the objective of this study was to assess the association between changes in bacterial communities and the onset of *Salmonella* shedding in cattle approaching parturition. In a prospective cohort study, fecal samples of 98 cows from four dairy farms were collected at four time points relative to calving (-3, -1, +1, +3 wks). *Salmonella* was identified in fecal samples using a culture protocol. Sequencing of the V4 region of the 16s rRNA gene through the Illumina platform was used to evaluate the fecal microbiome. Analysis of community similarities using the Jaccard distance with a permutation strategy, Yue and Clayton dendrograms, and principal component plots were generated for community comparisons. Individual cow fecal microbiomes, predominated by Bacteroidetes and Firmicutes phyla, experienced significant changes associated with parturition. The diversity of the microbiome significantly increased after calving. Microbial communities from different farms were distinguishable based on cluster analysis. Although there were significant differences in some bacterial taxa between *Salmonella* positive and negative samples, our results did not identify associations between the onset of *Salmonella* shedding and fecal microbial diversity or structure. Understanding factors that influence the exacerbation of *Salmonella* shedding is important to develop of strategies to decrease foodborne transmission.

Keywords: microbiome, *Salmonella*, cattle

**CLOSTRIDIUM DIFFICILE IN DOGS: RISK FACTORS FOR COLONIZATION, INFECTION AND OWNER TRANSMISSION.** J. O'Day<sup>1</sup>, R. O'Shaughnessy<sup>1</sup>, A. Alexander<sup>2</sup>, S. Wang<sup>2</sup>, and J. Stull<sup>1</sup>

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*Clostridium difficile*, an intestinal-dwelling bacterium, can lead to serious disease in both humans and companion animals such as dogs. While most human infections are linked to hospital care, recent epidemiologic data indicates that up to 30% of *C. difficile* infections (CDI) do not have the classic risk factors and appear to become initially infected in the community. While the sources of human CDI can be complicated by numerous sources, the role of dogs as a source of CDI has been poorly defined. To investigate this, we hypothesized that identifiable husbandry, owner and dog risk factors are associated with *C. difficile* colonization in dogs, and that dogs living with owners recently diagnosed with CDI will have a higher prevalence of *C. difficile* colonization than dogs living with owners not diagnosed with CDI. Patients recently tested for *C. difficile* at The Ohio State University Wexner Medical Center were screened for eligibility, and enrolled if they owned a dog. A survey was conducted to obtain household, husbandry and patient data, and both human and canine fecal samples were collected, cultured and analyzed for presence of the *C. difficile* triose phosphate isomerase (*tpi*) gene. To date, 114 human patients have been enrolled, and 27 samples were confirmed to have the *tpi* gene (23.7%). Twenty-six percent (30/114) of enrolled patients submitted samples from a total of 41 dogs; 8 were *tpi* positive (19.5%). Positive human and dog samples will be further analyzed via PCR-based assays to characterize the presence of *C. difficile* toxin genes (*tcdA*, *tcdB*, *tcdC*, *cdtA* and *cdtB*), isolates will be ribotyped and compared to identify likely dog-human transmission for each household.

Key Words: *Clostridium difficile*, dogs, zoonotic disease, community-associated



**A GEOSPATIAL DESCRIPTION OF FOOT-AND-MOUTH DISEASE IN THE FAR NORTH REGION OF CAMEROON AND TRANSBOUNDARY TRADE OF CATTLE. J. Pelot<sup>1</sup>, J. Tovuo<sup>2,3</sup>, M. Moritz<sup>2</sup>, E. Root<sup>3</sup> and R. Garabed<sup>1</sup>. Depts. of Veterinary Preventative Medicine<sup>1</sup>, Anthropology<sup>2</sup>, and Geography<sup>3</sup>.**

**J. Pelot<sup>1</sup>, J. Tovuo<sup>2,3</sup>, M. Moritz<sup>2</sup>, E. Root<sup>3</sup> and R. Garabed<sup>1</sup>. Depts. of Veterinary Preventative Medicine<sup>1</sup>, Anthropology<sup>2</sup>, and Geography<sup>3</sup>.**

Foot-and-mouth disease (FMD), while absent in US, poses a great risk in terms of economic loss and animal suffering to endemic areas such as tropical regions of Africa. As transboundary cattle herders from Chad and Sudan drive their cattle to market in Nigeria, they cross the Far North Region of Cameroon from east to west. This influx of cattle into the country make it difficult for Cameroon to control the spread of FMD in their local herds (which include transhumant herds that travel a north-south route).

The purpose of our preliminary research is to provide a geospatial description of the possible overlaps of the paths of the local herds with the paths of transboundary herds in order to develop better surveillance of the risk of introduction of new FMD virus strains in Cameroon. These overlaps take into account the persistence of FMD virus in the environment. Specifically, we will be looking at the years 2012-2013, as we have complete data on cattle entering the country and location of local cattle camps for those years. Follow-up research will include looking at weather, geopolitical, and cultural effects on seasonal timing of peak numbers of transboundary cattle moving across Cameroon. This geospatial model can then be transitioned to other cattle-borne pathogen surveillance, such as the emerging, zoonotic Rift Valley Fever.

Keywords: foot-and-mouth disease; Cameroon; geospatial; surveillance; trade

**DAIRY CALF MANAGEMENT – A COMPARISON OF PRACTICES AND PRODUCER ATTITUDES AMONG CONVENTIONAL AND ORGANIC HERDS.** J. A. Pempek, G. M. Schuenemann, E. Holder, and G. G. Habing. Department of Veterinary Preventive Medicine.

Dairy calves are at high risk for morbidity and mortality early in life. Understanding producer attitudes is important for implementation of best-management practices to improve calf health. The objectives of this study were to evaluate usage frequency and producer attitudes on key calf management practices between conventional and organic dairy operations. A cross-sectional survey was mailed to conventional and organic dairy producers in Ohio and Michigan, USA that included questions on cow-calf separation, colostrum management, and vaccination use. The overall survey response rate was 49% (727/1488); 449 and 172 conventional and organic producer respondents, respectively, were included in the final analysis. Binary, cumulative, and multinomial logistic regression models were used to test differences within and between herd types for management practices and producer attitudes. The majority of conventional (64%, 279/439) producers reported separating the calf from the dam 30 min to 6 h after birth. More organic (34%, 56/166) than conventional (18%, 80/439) producers reported separation 6 to 12 h after birth, and organic producers were more likely to agree that time prior to separation is beneficial. Few conventional (10%, 44/448) and organic (3%, 5/171) producers reported measuring colostrum quality. Most conventional producers (68%, 304/448) hand-fed the first feeding of colostrum, whereas the majority of organic producers (38%, 69/171) allowed calves to nurse colostrum. Lastly, 44% (188/430) of conventional producers reported vaccinating their calves for respiratory disease, compared to 14% (22/162) of organic producers; organic producers were more likely to perceive vaccines as ineffective and harmful to calf health. Thus, the usage frequency and perceived risks and benefits of calf management practices vary considerably between conventional and organic dairy producers. These findings provide helpful information to understand decision making at the herd-level regarding key calf management and health practices, regardless of production systems.

Keywords: calf health, cow-calf separation, colostrum, vaccination

## VEAL CALF HEALTH ON THE DAY OF ARRIVAL TO GROWERS IN OHIO

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Veal calves are at risk for disease and mortality in early life. Stressors, including long transport times and going through auction, may contribute to poor health of calves upon arrival to the grower. Our objectives were to: 1) estimate the prevalence of poor health outcomes in veal calves on arrival to growers in Ohio, 2) determine if auction site was a risk factor for these outcomes on arrival, and 3) determine if health outcomes on arrival predicted early mortality. A physical examination was conducted on approximately 30 calves from 12 cohorts (n = 383). Exams included a blood sample to determine packed cell volume ('PCV', dehydration estimate, cut-off > 46%) and total protein (passive transfer estimate, cut-off < 5.5 g/dl). Diarrhea, respiratory disease, depression, navel inflammation, and a skin tent test (a second indicator of dehydration) were recorded. Mortality within 4 wk of age was collected from farm records. Descriptive statistics were used to determine prevalence of calves with poor health at arrival, and a generalized linear model was used to identify risk factors for poor health on arrival and early mortality. Upon arrival, 6% of calves had failure of passive transfer, 14% had diarrhea, 0.5% had respiratory disease, 14% were depressed, and 27% had inflamed navels. Furthermore, 35.1% of calves were dehydrated using a skin tent test, but only 1.3% were dehydrated using PCV. Auction site was associated with depression ( $P=0.0008$ ), and tended to be associated with skin tent on arrival ( $P=0.08$ ). No health variables predicted early mortality; however, overall mortality was low (4.3%), limiting our interpretation of these results. Results show that veal calves are in poor health upon arrival to the growers, and some health outcomes are dependent on auction site. Therefore, there is opportunity to intervene before the calves arrive to growers to improve their health.

Keywords: Dehydration, failure of passive transfer, auction

**COMPARISON OF FIELD RESEARCH METHODS TO CHARACTERIZE FREE-ROAMING CAT POPULATIONS IN A MIXED-URBAN ENVIRONMENT**

E.C. Vincent, A.J. Yoak, J. O'Quin, and R.B. Garabed, Department of Veterinary Preventive Medicine

Free-roaming cat overpopulation presents a public health, environmental, and animal welfare concern in communities across the United States. However, population control methods are often implemented without preliminary data about free-roaming cat population locations, their specific distributions, resources that may impact the presence of cats, or populations of other wildlife that may affect cat populations. To study population size and distributions of free-roaming cats on and near The Ohio State University campus, two field research methods were compared: line transect and trail cameras. Trail cameras were also used to record locations and activity patterns of other wildlife species. The locations of resources, such as uncovered trash cans, that may contribute to the presence of feral cats on campus and in the surrounding area were also mapped. Six free-roaming cats were sighted in 5/100 sampled zones using the line transect method. Ninety-two free-roaming cat photographs were recorded using the trail camera method in 9/23 sampled zones. Cats were most often sighted off campus and in urban habitats. The number of cat photographs recorded on trail cameras was significantly correlated with the density of food resources in the area. Free-roaming cats were significantly more active during the night than during daylight hours. The results of this study indicate that the trail camera method was preferable to the line transect method for studying free-roaming cat populations in a mixed-urban environment because it detected a higher number of cats in a wider variety of habitat types and also allowed for the detection of other wildlife species. Communities planning to implement a population control program for free-roaming cats should choose the trail camera method to gather baseline data and should consider programs to decrease available food resources.

Keywords: Free-roaming cat populations, study design, field research, urban, trail camera

**CHANGE, WIPE, REPLACE, REPEAT: AN EVALUATION OF CLEANING WATER VALVES.** G. Voros, D. Harrison, J. Petty, S. Beck and J. Hickman-Davis. University Laboratory Animal Resources

*The Guide for the Care and Use of Laboratory Animals (The Guide)* states watering devices, such as drinking tubes and automated water delivery systems should be checked frequently to ensure appropriate maintenance, cleanliness and operation. To determine the cleaning method and sanitation frequency for detachable water valves on individually ventilated cage (IVC) racks, we utilized available disinfectants and cage washer systems with standard husbandry practices. Mice were housed in IVCs on corncob bedding according to The Guide standard for cage density, and were changed at 2-week intervals. Water valves were wiped with a paper Wypall® soaked in Spor-Klenz® or Opti-Cide3® or were removed and processed in a cage washer at the time of cage change. Water valves were tested before and after cleaning using a combination of ATP monitoring system (SystemSURE Plus™ Luminometer and UltraSnap swabs) ATP luminometer and sterile swabs for bacterial culture. Wiping with Spor-Klenz® or processing in a cage washer provided “pass” (<17 RLU) ATP levels for 26 weeks and had no impact on animal health or breeding. Volunteer animal care staff (n=15) trained on the disinfection process using Spor-Klenz® were monitored at the time of cage change for quality assurance (QA) of water valve disinfection. The average time for cleaning of water valves during cage change was  $3.2 \pm 0.16s$  (n=116), and decreased ATP RLU from  $106 \pm 22$  to  $7 \pm 1$  (n=60). QA testing of water valves attached to the rack sanitized in a rack washer after 6 months was negative for ATP and bacterial culture (n=10 water valves per rack, 176 racks tested). These data indicate that detachable water valves can be maintained on the rack for 6 months using disinfection with Spor-Klenz® during cage change out, and water valves do not need to be detached from the rack for sanitation using a standard rack washer.

Keywords: Water valve, Disinfection, Health surveillance, Husbandry, Mouse, Regulations

**CONTAMINATION OF PORTABLE AGRICULTURAL FAIR OBJECTS WITH INFLUENZA A VIRUS.** C. M. Wright, M. Zentkovich, J. Nolting, A. S. Bowman.  
Department of Veterinary Preventive Medicine

Agricultural fairs provide adequate opportunities to efficiently transmit pathogens both within and between species. Influenza A virus spreads rapidly in this setting due to multiple close contact swine-swine and swine-human interactions. In addition, influenza A virus can survive outside of a host for days to weeks, and the amount of potential fomites at an agricultural fair further risks facilitating viral dissemination. Our hypothesis is that portable objects used at fairs are harboring influenza A virus. The present study sampled 20 inanimate surfaces at 20 agricultural fairs using cotton gauze to sample objects in the swine barn (feeders, waterers, tack boxes, sort panels, and chairs). Samples were then placed into brain heart infusion broth and frozen until RRT-PCR could be performed. Influenza A virus RNA was detected on 75/400 (18.75%) environmental surfaces, of which interestingly 14.67% were not in direct contact with swine during time of sampling. Influenza A virus was isolated from 7/400 (1.75%) environmental surfaces. These results and the zoonotic potential influenza A virus has highlights the importance of biosecurity measures at agricultural fairs, such as disinfection and hand hygiene, that mitigate pathogen spread.

Keywords: Influenza A virus, agricultural fairs, biosecurity, public health, viral isolation

**IMMUNOLOGY  
AND  
INFECTIOUS DISEASES**

**DEVELOPMENT OF GENETIC RISK PROFILE FOR EPSTEIN-BARR VIRUS ASSOCIATED POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE. E.**

Ahmed, F. Jeney, C. Hale, S. Sloan, S. Schlotter, P. Smith, G. Ozer, R. Baiocchi and M. Caligiuri. Dept. of Veterinary Biosciences, Comprehensive Cancer Center, Biomedical Informatics and Internal Medicine

Post-transplant lymphoproliferative disease (PTLD) is a serious complication of organ transplant and often has poor patient outcome. Most PTLD cases are associated with the Epstein-Barr virus (EBV), an oncogenic herpes virus that infects more than 90% of the world's population. Despite its ubiquitous nature, less than 20% of transplant patients develop EBV related PTLD after undergoing an immunosuppressive regimen. Such clinical heterogeneity implies that host factors exist which predispose individuals to PTLD. The preclinical mouse model of EBV-driven lymphoproliferative disease (EBV-LPD) utilizes severe combined immune deficient (SCID) mice which are engrafted with peripheral blood leukocytes from EBV seropositive individuals.

In our model, approximately 20% of EBV+ donors consistently develop EBV-LPD in all engrafted mice (HIGH incidence donors), while 20% of EBV+ donors never develop LPD (NO incidence donors). Currently there is no mechanism to predict which individuals are at risk for EBV-driven diseases. We are utilizing the Hu-PBL-SCID mouse model to identify biomarkers of at-risk individuals for EBV-LPD. Moreover, immuno-phenotypic analysis of T-cell subsets shows that HIGH incidence donors have higher basal levels of T follicular helper cells (Tfh) and regulatory T cells (Treg). Tfh cells are essential for germinal center (GC) formation, the primary site of B lymphocyte affinity maturation, whereas Tregs are immunosuppressive and prevent memory cytotoxic T cells from killing EBV tumor cells. RNA Transcriptome analysis demonstrated distinct gene expression signatures associated with Tfh and Treg activity in HIGH incidence donors. To evaluate the effect of Tfh and Treg cells in EBV-LPD *in-vivo*, we engrafted SCID mice with Tfh depleted, Treg depleted or Tfh/Treg depleted PBL from HIGH incidence donors.

This work will provide rationale for prospective clinical trials evaluating PTLD risk profiles of patients who undergo solid organ transplantation. Results from this work may be applicable to individuals with primary (genetic) or acquired (HIV) immune deficiency.

Keywords: Epstein-Barr virus (EBV), Post-transplant lymphoproliferative disease, mouse model



## IMID - 2

**SAMHD1 SUPPRESSES HIV-1 GENE EXPRESSION AND REACTIVATION OF VIRAL LATENCY IN CD4+ T-CELLS.** JM Antonucci<sup>1,2,3</sup>, AA Duchon<sup>1,2,4</sup>, O Buzovetsky<sup>5</sup>, K Knecht<sup>5</sup>, Y Xiong<sup>5</sup>, K Musier-Forsyth<sup>1,2,4</sup>, L Wu<sup>1,2,3</sup>

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The cellular dNTP hydrolase SAMHD1 restricts HIV-1 replication in non-dividing cells by degrading intracellular dNTPs to a level that limits efficient viral reverse transcription. Recombinant SAMHD1 binds HIV-1 DNA and RNA fragments *in vitro*, but the functional significance of the binding remains unclear. SAMHD1 is highly expressed in cell types that contribute to HIV-1 latent reservoirs, such as resting CD4+ T cells and myeloid cells. Transcriptional suppression of proviral DNA gene expression contributes to HIV-1 latency. However, it is unknown whether SAMHD1 regulates HIV-1 proviral gene expression in latently infected cells. Here, we investigate the effect of SAMHD1 on HIV-1 gene expression and the underlying mechanisms. We found that overexpression of SAMHD1 in HEK293T cells suppressed HIV-1 LTR promoter-driven luciferase expression in a dose-dependent manner at the level of transcription. We hypothesize that SAMHD1 may bind to the HIV-1 LTR to transcriptionally suppress viral gene expression in latently infected CD4+ T-cells. To study the effect of SAMHD1 on gene expression of integrated HIV-1 proviral DNA, we utilized the HIV-1 latently infected J-Lat cell line. We expressed exogenous SAMHD1 in J-Lat cells and observed that SAMHD1 reduced reactivation of HIV-1 gene expression. Additionally, a chromatin immunoprecipitation assay followed by qPCR revealed that SAMHD1 binds preferentially to the LTR in J-Lat cells, though it also binds to other HIV-1 gene sequences such as *gag*, *rev*, and *vpr*. We further investigated the *in vitro* binding affinity of recombinant SAMHD1 to single-stranded HIV-1 LTR and *gag* DNA fragments by fluorescence anisotropy. Binding assays performed over a range of salt concentrations are consistent with more specific binding of SAMHD1 to the LTR-derived DNA fragment relative to the *gag* fragment. Our data suggest that SAMHD1-mediated suppression of HIV-1 gene expression likely contributes to viral latency in CD4+ T-cells.

Keywords: HIV-1, SAMHD1, Restriction factor, Viral Latency

### IMID - 3

**A VACCINE SUPPLEMENTATION APPROACH FOR INDUCTION OF MUCOSAL IGA BY INJECTED VACCINES.** Z. Attia<sup>1,2</sup>, E. Kim<sup>1</sup>, J. C. Rowe<sup>1</sup>, H. E. Steiner<sup>1</sup>, E. Cormet-Boyaka<sup>1</sup>, and P. N. Boyaka<sup>1</sup>.

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Most current vaccines are injected vaccines that contain alum as adjuvant. These vaccines promote good antibody with predominantly Th2 responses in the bloodstream and they have helped limit many infectious diseases worldwide. Since, most infectious agents enter the host via mucosal surface of the gastrointestinal, respiratory or genito-urinary tracts, induction of secretory (SIgA) in these sites could provide a first line of defense. Previous work in the laboratory has demonstrated an inverse relationship between the ability of a sublingual vaccine to recruit neutrophils and induction of SIgA. Since alum was shown to recruit Gr1+ cells secreting IL-4, we examined whether inhibition a neutrophil function could help alum-based vaccines induce serum IgA responses and perhaps, SIgA in mucosal tissues. For this purpose, groups of mice were immunized IP with antigen alone (Ovalbumin + protective antigen of anthrax), antigen plus alum, or antigen plus alum and a neutrophil elastase inhibitor (NEI). Our results show that addition of NEI enhances the magnitude of antigen-specific serum IgG1, but also IgG2a responses. This profile of serum IgG responses was consistent with increased frequency of IFN $\gamma$  CD4<sup>+</sup> and IL-17<sup>+</sup>CD4<sup>+</sup> T cells in the spleen of mice that received NEI. We also found that addition of NEI enhances promoted antigen-specific serum IgA responses, as well as SIgA in mucosal secretion of the gastrointestinal tract, but not in the genito-urinary tract. Taken together our results show that addition of NEI broaden the immune responses induced by alum as adjuvant and represent a potential supplementation approach for induction of SIgA by injected vaccines.

Keywords: Vaccine, Adjuvants, Alum, mucosal IgA, Neutrophil elastase inhibitors, T helper cells.

## IMID - 4

### **RANDOM MUTAGENESIS OF *EHRlichia* SP. HF STRAIN FOR IDENTIFICATION OF VIRULENCE GENES.** H. Bekebrede, M. Lin, Y. Rikihisa. Dept. of Veterinary Biosciences

*Ehrlichia* spp. (*E. canis*, *E. ruminantium*, *E. ewingii*, and *E. chaffeensis*) are tick-borne obligatory intracellular bacteria that infect variety of mammals including dogs, ruminants, deer, and human, causing severe and sometimes fatal systemic disease. Research to identify virulence factors of *Ehrlichia* spp. is hampered by the lack of small laboratory animal models. The Rikihisa laboratory isolated a novel *Ehrlichia* species named "HF strain" from ticks in Japan. The HF strain is most closely related to *E. chaffeensis* human isolates, and kills laboratory mice in 10 days. The Rikihisa laboratory also recently completed whole genome sequencing of the HF strain. My research seeks to analyze gene function of the HF strain using Himar transposon mutagenesis. A random mutant HF strain library will be generated in canine macrophage DH82 cells. Mutant HF strains will be cloned, and genomic loci of transposon insertion will be identified by semi random two step PCR (ST-PCR). Isolated mutants that can disrupt the promoter region or open reading frame will be confirmed by RT-PCR for the lack of mRNA. Ten distinct mutants will be selected to determine effects of the mutant HF strain on mice pathogenesis. I have so far isolated six stable mutants expressing mCherry fluorescence. Intergenic insertion sites were identified between EHF\_0098 and EHF\_0097, between EHF0332 and EHF0333. Intragenic insertion sites were identified in EHF\_0231 and in EHF\_0522. EHF\_0231 mutants lack EHF\_0231 mRNA, but there is no difference in mouse pathogenesis compared to wild type. Currently, we are seeking to obtain more mutants suitable for *in vitro* and *in vivo* pathogenesis analysis with optimal transformation methods. We expect these studies to elucidate virulence factors of the HF strain. Because *Ehrlichia* spp. share homologous genes, the proposed study will help understanding virulence factors of other *Ehrlichia* spp. as well.

Keywords: Ehrlichia, HF strain, obligate intracellular bacteria, virulence, mutagenesis

## IMID - 5

**ACUTELY LETHAL H1N1 INFLUENZA A VIRUS INFECTION IMPACTS ALVEOLAR TYPE II CELL MITOCHONDRIAL STRUCTURE AND FUNCTION IN MICE.** L. M. Doolittle, E. P. Calomeni, and I. C. Davis. Depts. of Veterinary Biosciences and Pathology.

**Rationale:** Alveolar type II (ATII) cells are the primary site of influenza A virus (IAV) replication in the distal lung. ATII cells also perform a number of processes critical for normal lung function, including synthesis and secretion of surfactant phospholipids and proteins, and vectorial ion transport. These processes depend upon ATP, which is primarily generated by mitochondrial oxidative phosphorylation. Recent lipidomics studies indicated that levels of several phospholipids that are important components of mitochondrial membranes (DPPC, DPPG, and phosphatidylethanolamine) are significantly decreased in ATII cells following IAV infection. We hypothesized that, by impairing phospholipid synthesis, IAV infection alters ATII cell mitochondrial structure and function.

**Methods:** C57BL/6 mice (n=5-6/group) were inoculated intranasally with 10,000 p.f.u./mouse influenza A/WSN/33 (H1N1), which induces severe ARDS in mice by 6 days post-inoculation (d.p.i.). Controls were mock-infected with virus diluent. Whole lungs were isolated at 6 d.p.i. and glutaraldehyde fixed for transmission electron microscopy and image analysis using ImageJ software. ATII cells were isolated by a standard lung digestion protocol at 6 d.p.i.. Energy metabolism was measured immediately following isolation using a Seahorse XFe Analyzer. Expression of mitochondrial electron transport chain (ETC) complexes involved in oxidative phosphorylation was analyzed by Western blotting.

**Results:** Relative to mock-infected controls, ATII cells from infected mice contained fewer mitochondria at 6 d.p.i., and those visible were electron-dense with disordered membranes. Availability of substrates for mitochondrial ATP generation was dysregulated at 6 d.p.i. Basal rates of glycolysis (extracellular acidification) and mitochondrial oxidative phosphorylation (oxygen consumption) decreased at 6 d.p.i. Additionally, ATII cells did not express Complex II of the ETC (succinate dehydrogenase) at 6 d.p.i.

**Conclusions:** IAV infection alters mitochondrial morphology and ATP production in ATII cells. Decreased ATP availability may impair ATII cell function and contribute to development of ARDS in IAV-infected mice.

**Keywords:** Influenza A virus, alveolar type II cells, mouse, mitochondria, lipidomics, oxidative phosphorylation

**ESTROGEN PROTECTS PROGESTIN-TREATED MICE AGAINST INTRAVAGINAL TRANSMISSION OF CELL-ASSOCIATED HIV-1.** M. Glick<sup>1</sup>, N. E. Quispe Calla<sup>2</sup>, R. D. Vicetti Miguel<sup>2</sup>, J. Kwiek<sup>3</sup>, J. Gabriel<sup>4</sup>, T. L. Cherpes<sup>2</sup>. <sup>1</sup>The Ohio State University (OSU) College of Veterinary Medicine,<sup>2</sup>Stanford University School of Medicine, Department of Comparative Medicine, <sup>3</sup>OSU College of Arts and Sciences Department of Microbiology, and <sup>4</sup>OSU College of Medicine, Department of Microbial Infection and Immunity

We recently found that treatment of female mice with the hormonal contraceptive depot-medroxyprogesterone acetate (DMPA) increases their susceptibility to genital herpes simplex virus type 2 infection, whereas treatment with estrogen reverses this effect. Herein, we sought to extend these findings to susceptibility to cell-associated HIV-1 infection in a humanized mouse model. In our studies, female humanized NOD *scid* gamma (NSG) mice were reconstituted with human HIV-negative peripheral blood mononuclear cells (hPBMC). Mice were randomized to groups that received DMPA or DMPA and a commercially available intravaginal estrogen cream 5 days before infection. 14 days after hPBMC reconstitution, mice were intravaginally inoculated with HIV-1-infected hPBMCs. At 10 days post infection, mice were euthanized, blood was collected and spleens processed into single cell suspensions to determine HIV-infection status. Specifically, splenocyte cultures were used to determine HIV infection status via a luciferase reporter gene assay in TZM-BL cells and serum HIV load was quantified using Abbott's RT-PCR on stored peripheral blood samples. These studies showed that 100% of DMPA-only treated mice were infected with cell-associated HIV-1, whereas all mice treated with DMPA and estrogen cream were protected against infection. In addition to these assays, vaginal tissues from euthanized mice were processed to determine between-group differences in expression of a cell-cell adhesion molecule, desmoglein-1 $\alpha$  (DSG-1 $\alpha$ ) by RT-PCR. These studies showed that DMPA treatment alone significantly decreased DSG-1 $\alpha$  expression. Such findings indicate that DMPA increases susceptibility to cell-associated HIV-1 infection by decreasing vaginal epithelial integrity, whereas concomitant estrogen protects DMPA-treated mice from systemic HIV infection by restoring the epithelial barrier. These mouse model findings may therefore have implications for HIV susceptibility of women using DMPA for hormonal contraception.

Keywords: hormonal contraception, HIV-1, genital mucosa, humanized mouse

**CX3CR1 IS A RECEPTOR FOR RESPIRATORY SYNCYTIAL VIRUS *IN VIVO*.**

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Respiratory syncytial virus (RSV) is a leading cause of death among infants worldwide, yet there are no effective vaccines or antivirals available. It has recently been demonstrated that RSV uses CX3CR1 as a receptor on primary human airway epithelial cell cultures. To further evaluate CX3CR1 as a receptor for RSV *in vivo*, we used the cotton rat animal model, which is more permissive to RSV infection than other small animal models, and the cellular tropism of RSV is the same in the cotton rat as it is in the human. Sequencing and cloning of cotton rat CX3CR1 revealed 91% amino acid similarity to human CX3CR1. RSV binds to CX3CR1 via its attachment (G) protein. To determine whether CX3CR1-G protein interaction is necessary for RSV infection, RSVs containing mutations in the CX3CR1 binding site of the G protein were tested in cotton rats. All virus mutants grew well in cell culture, but in contrast to wild-type virus which grew well, the G protein mutants were not detectable at four days post-infection. In a similar experiment, RSV was incubated with an antibody which prevents G protein binding to CX3CR1. Subsequent intranasal inoculation into cotton rats resulted in undetectable levels of RSV in the lungs at four days post-infection. If RSV was incubated with heparan sulfate (the receptor for RSV on immortalized cell lines) before intranasal inoculation into cotton rats, viral replication was not affected. Additionally, when peptide-conjugated morpholinos were used to decrease CX3CR1 expression in the cotton rat prior to RSV inoculation, it resulted in a reduction of RSV titers at four days post-infection. Together, these results indicate that CX3CR1 functions as a receptor for RSV in cotton rats and, in combination with data from human airway epithelial cell cultures, strongly suggest that CX3CR1 is a primary receptor for RSV *in vivo*.

Keywords: respiratory syncytial virus, viral entry, receptor

**EPIDEMIOLOGY AND GENOMIC CHARACTERIZATION OF MDR *SALMONELLA* FROM DOMESTIC ANIMALS AND WILDLIFE.** B. Jourdan, L. Binkley, T. Eguale, W. Gebreyes, J. O'Quin, A. Archawakulathep. Department of Veterinary Preventative Medicine

As human populations expand, the gap between the natural environment and anthropogenic landscapes is disappearing, bringing wildlife and humans into closer contact. This is true for Ethiopia (the study site) and other developing regions. Resistant *Salmonella* strains have been identified in Ethiopian livestock and are known to spread to humans via consumption of contaminated water and food. Resistant *Salmonella* strains may similarly be transmitted between wildlife and domestic animals. To date, research on distribution of *Salmonella* in Ethiopia has primarily focused on food animals. Further, the occurrence and prevalence of multi-drug resistant (MDR) *Salmonella* strains among Ethiopian wildlife populations has not been investigated. In addition, the role of wildlife in transmission of MDR *Salmonella* to humans remains unknown. In order to identify *Salmonella*, fecal samples from wildlife carcasses were collected in a variety of Ethiopian regions. Using conventional cultural methods, *Salmonella* was isolated from two of the seven samples. These isolates will be tested for MDR against a panel of 12 selected antimicrobial drugs using Kirby-Bauer disk diffusion method. Currently, Whole Genome Sequencing of these isolates is underway. The findings will be compared to previously collected isolates from domestic and wild animals as well as isolates of human origin to determine the extent of phenotypic and genotypic similarity and understand pathogen transmission among these species. The findings of this pilot study will help inform future research and ultimately improve our understanding of MDR *Salmonella* transmission among these populations. Such information will be vital for the development of management strategies that can interrupt the transmission of such MDR strains when animal and human health could be at risk.

Keywords: *Salmonella*, multi-drug resistance, zoonotic, Ethiopia

**DIFFERENTIAL ROLE OF OXIDATIVE STRESS SIGNALING PATHWAYS AND MICROBIOTA IN THE DEVELOPMENT OF ALLERGY FOLLOWING CHRONIC INGESTION OF CADMIUM.** E. Kim<sup>1</sup>, M. M. Lember<sup>1</sup>, S. O. Opiyo<sup>2</sup>, Z. Attia<sup>1</sup>, John C. Rowe<sup>1</sup>, Haley E. Steiner<sup>1</sup>, E. Cormet-Boyaka<sup>1</sup>, and P. N. Boyaka<sup>1</sup>

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Environmental pollutants are believed to contribute to the increased incidence of allergy diseases. Chronic ingestion of low dose of cadmium is becoming a major public health issue due to the presence of this heavy metal in contaminated water and its accumulation in leafy vegetables, fish and grains. Unlike high doses of cadmium, ingestion of low subtoxic doses of cadmium has been poorly studied. We found that C57BL/6 mice chronically exposed to low doses of cadmium (<10 ppm) through drinking water develop higher titer of allergen-specific IgE responses upon oral sensitization. Cadmium-treated mice also exhibited higher IL-17 and Th1 responses in the airways and displayed more severe signs of airway allergic responses, upon nasal antigen challenge. Analysis of innate responses in the intestine revealed that ingestion of low doses of cadmium activated both the canonical and the non-canonical NF- $\kappa$ B pathway and promoted proinflammatory cytokine and antimicrobial responses through activation of oxidative signaling pathways including Duox2 and Nrf2. Chronic ingestion of low doses of cadmium also resulted in a dysbiosis of the intestinal microbial community, which persisted long after exposure was interrupted. The commensal microbes played a central role in the IgE promoting effect of cadmium since germ-free C57BL/6 mice exposed to the same doses of cadmium exhibited not sign of gut inflammatory responses or increased allergic sensitization. Finally, naïve C57BL/6 mice recipient of fecal microbiome transplantation from cadmium mice enhanced allergen-specific IgE responses. Furthermore, when transferred into cadmium-treated mice, the fecal microbe of naïve mice restored the allergen-specific IgE responses in the recipients confirming the central role the dysbiosis in the induction of allergic responses.

Keywords: Environmental pollutant, Cadmium, Allergic sensitization, Gut inflammation, Dysbiosis



**ORAL VITAMIN A SUPPLEMENTATION OF PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)-INFECTED GILTS ENHANCES THE GUT-MAMMARY GLAND-SECRETORY IGA AXIS AND PASSIVE PROTECTION IN NURSING PIGLETS**

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Stimulation of lactogenic immunity in gilts via the gut-mammary gland-secretory IgA (sIgA) axis is critical for passive protection of nursing piglets against PEDV. Vitamin A (VitA) imprints gut homing of B and T cells and enhances mediators for differentiation of IgA antibody-secreting cells (ASC). We hypothesized that VitA supplementation of gilts would enhance both mucosal immune responses and the gut-mammary-sIgA axis to boost lactogenic immunity and passive protection of nursing piglets against PEDV challenge. Gilts received oral retinol palmitate daily (30,000IU) from gestation day 76 throughout lactation. At 3-4 wks pre-partum, both VitA-supplemented (PEDV+VitA, n=3) and non-supplemented (PEDV, n=4) gilts were orally inoculated with PEDV; non-supplemented gilts received MEM (Mock, n=4). All piglets were PEDV-challenged at 3-5 days post-partum. The mortality rate of PEDV-challenged piglets of PEDV+VitA gilts was 6.25% compared with 33.3% and 94.3% for PEDV and Mock litters, respectively. Piglets born to PEDV+VitA gilts had lower diarrhea scores at multiple post-challenge days (PCD). PEDV-specific IgA ASC appeared earlier in peripheral blood [post-inoculation day (PID) 6-8] of PEDV+VitA gilts compared with PEDV gilts (PID 12-17) and PEDV+VitA gilts had higher frequencies of IgA<sup>+</sup> and IgA<sup>+</sup>β7<sup>+</sup> mononuclear cells in blood at PID 6-8. In colostrum, milk and serum, PEDV+VitA gilts had higher mean PEDV neutralizing antibody titers and milk PEDV-specific IgA ASC compared with PEDV gilts at various piglet PCDs. This innovative approach and our findings suggest that oral VitA supplementation stimulates mucosal immunity and the gut-mammary-sIgA axis in gilts resulting in increased lactogenic immunity and protection in neonatal piglets.

Keywords: lactogenic immunity, gut-mammary gland-secretory IgA axis, porcine epidemic diarrhea virus, vitamin A

**LENTIVIRAL NEF PROTEINS ANTAGONIZE TIM-MEDIATED INHIBITION OF VIRAL RELEASE.** M. Li<sup>1, 3</sup>, E. O. Freed<sup>2</sup>, S.L. Liu<sup>1, 2, 3</sup>

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We recently reported that the T cell immunoglobulin and mucin domain (TIM) proteins inhibit release of HIV-1 and other enveloped viruses by interacting with virion- and cell-associated phosphatidylserine (PS) (Li et al, PNAS 111, 2014). In this study, we demonstrate that the Nef proteins of HIV-1 and other lentiviruses antagonize TIM-mediated restriction. We show that TIM-1 exhibits stronger inhibition of the release of Nef-deficient relative to Nef-expressing HIV-1 particles and that ectopic expression of Nef relieves this restriction. Consistent with this finding, knockdown of endogenous TIM-3 in human PBMCs effectively enhances the production of Nef-deficient HIV-1 particles. HIV-1 Nef does not appear to downregulate TIM-1 expression on the cell surface, nor does it disrupt TIM-1 incorporation into HIV-1 virions. Interestingly, we observed that coexpression of SERINC3 and SERINC5 potentiates TIM-1 inhibition of HIV-1 release, and that depletion of SERINC proteins in viral-producer cells rescues TIM-mediated inhibition of HIV-1 release. These results suggest that SERINC proteins are involved in TIM-mediated restriction of HIV-1 release. In addition to HIV-1 Nef, the Nef proteins of simian immunodeficiency virus (SIV) strains and HIV-2 also antagonize the antiviral activity of TIM-1, suggesting an evolutionarily conserved role of the lentiviral *nef* gene in antagonizing TIMs. Collectively, our work reveals a new role for lentiviral Nef in antagonizing TIM, and highlights a complex interplay between lentiviral Nef and cellular restriction by TIMs and SERINC proteins.

Keywords: TIM, HIV-1 Nef, SERINC, Release

**EXPERIMENTAL MODELING OF THE NONSPECIFIC PROTECTIVE EFFECTS OF MEASLES VIRUS VACCINATION.** S. Linn and S. Niewiesk. Department of Veterinary Biosciences.

The administration of a vaccine can have non-specific protective effects against unrelated pathogens in an infant patient and can, therefore, be protective against pathogens for which currently no vaccines exist. Respiratory syncytial virus (RSV) and Streptococcus pneumoniae are two of the most common causes of acute respiratory tract infections in infants and children. Recent work in Denmark, however, demonstrated that children whose most recent vaccine was the live measles-mumps-rubella vaccine had a lower rate of RSV hospitalization compared to children who had inactivated DTaP-IPV-Hib3 as their most recent vaccine. It has also been shown that there is an epidemiological link between measles vaccination and reduction in S. pneumoniae load. The aim of this study is to provide an experimental model for the nonspecific protective effects of measles virus immunization against infection with RSV or S. pneumoniae. Three groups of cotton rats immunized with measles virus intranasally or subcutaneously and unvaccinated controls were established. After challenge with RSV, we measured viral titers in lung and nasal turbinate homogenates. After challenge with S. pneumoniae, we measured bacterial titers from nasopharyngeal washes. Measles vaccination did not influence RSV titers 1, 3, and 5 weeks or S. pneumoniae titers 1, 2 and 3 weeks post vaccination. Measles virus vaccination did, however, lower bacterial load 3-fold in animals colonized with S. pneumoniae indicating that cotton rats may be a model to investigate the unspecific effect of measles vaccination on bacterial colonization.

Keywords: vaccination, Measles Virus, immunology, animal modeling

**ROLE OF CHROMATIN INSULATOR CTCF IN HTLV-1 RETROVIRAL PATHOGENESIS.** M. Martinez<sup>1</sup>, J. Al-Saleem<sup>1</sup>, A. Panfil<sup>1</sup>, L. Ratner<sup>2</sup>, and P. Green<sup>1</sup>

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Human T-cell leukemia virus (HTLV-1) is a delta retrovirus endemic to the Caribbean and Japan. HTLV-1 is the etiologic agent of adult T-cell leukemia and the neurological disorder HAM/TSP. Approximately 5-10% of infected individuals will develop disease after a prolonged latency. The exact mechanisms through which this variable latency is regulated remains nebulous. CCCTC-binding factor (CTCF) is an 11-zinc finger transcriptional repressor that acts through the induction of conformational changes in chromatin structure and subsequent enhancer-blocking activity. A CTCF-binding site was recently identified within the HTLV-1 provirus and shown to affect the anti-sense derived *hbz* transcript. HBZ provides tumor maintenance function and supports viral persistence. Therefore, we propose to study the epigenetic effects of CTCF on HTLV-1-induced *in vitro* immortalization using short-term viral co-culture assays. Using an HTLV-1 proviral molecular clone, a  $\Delta$ CTCF mutant was generated using site-directed mutagenesis. The  $\Delta$ CTCF mutation prevents CTCF-binding via EMSA, but does not disrupt overlapping reading frames or splice sites. The  $\Delta$ CTCF mutant is transcriptionally similar to WT, as demonstrated by LTR-based reporter gene assays and viral gag release. Since efficient *in vitro* infection of naïve T-cells by HTLV-1 requires co-cultivation with infected cells, we next used stable transfection of the WT and  $\Delta$ CTCF proviral clones into a susceptible human B-cell line to produce consistent and quantifiable amounts of virus. Briefly, irradiated viral producer cells are co-cultured with freshly isolated peripheral blood lymphocytes (PBL). The initiation of transformation is apparent within 5-6 weeks following co-culture as detected by expansion of cells from the PBL cell population. Preliminary results indicate the  $\Delta$ CTCF mutant has similar immortalization potential compared to WT. Future experiments will examine the effect of the CTCF mutation on viral persistence using a rabbit model of infection. Ultimately, understanding epigenetic regulation of HTLV-1 latency could provide meaningful insights into mechanisms of immune evasion.

Keywords: CTCF, HTLV-1, ATL

**PULMONARY FUNCTION IN COTTON RATS AFTER RSV INFECTION**

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Human respiratory syncytial virus (RSV) is a leading cause of bronchiolitis and viral pneumonia in infants and young children worldwide, as well as a major cause of respiratory disease in the elderly. RSV is associated with increased airway resistance, decreased compliance, increased airway hyperresponsiveness and has been suspected to predispose infected infants to asthma later in life. The gold standard for measuring airway resistance, compliance and hyperresponsiveness is through forced oscillation technique (FOT), which has not been previously performed using the best small animal model for RSV infection, the cotton rat. Pulmonary edema can be assessed by measuring the lung wet:dry ratio, and mucus production can be quantified using PAS/Alcian blue special staining and Aperio software (color deconvolution algorithm). Our goal was to characterize pulmonary function in the cotton rat, as well as 2, 4, 6 and 8 days post-RSV infection, using these techniques. There was no difference in the airway resistance, compliance or hyperresponsiveness between genders in uninfected cotton rats; however during RSV infection, females had a significantly higher airway hyperresponsiveness and decreased compliance compared to uninfected females. There was a significant increase in pulmonary edema 2 days post-infection, although peak inflammatory infiltrates are observed histologically 5 days post-infection. There was no significant increase in mucus production after RSV infection with either RSV-A2 or RSV line-19F.

Keywords: Human respiratory syncytial virus, cotton rat, airway resistance, compliance, airway hyperresponsiveness

**IN VITRO CHANGES TO CANINE PACKED RED BLOOD CELLS FOLLOWING IRRADIATION AND STORAGE.** S. Press, E. Cooper, T. Archer, C. Fellman, E. Green and J. Stull. Veterinary Clinical Sciences.

Irradiation of blood prior to transfusion is required to prevent transfusion-associated graft-versus-host disease in patients undergoing hematopoietic stem cell transplant. Additional application for irradiated blood may exist in oncological surgery. The effect of irradiation on canine packed red blood cells (pRBCs) is unknown. Our aims were to characterize changes to *in vitro* lymphocyte viability, lymphocyte activation potential, electrolytes, acid-base variables and oxygen-carrying capacity in pRBCs following irradiation and storage.

Ten units of pRBCs were irradiated, and flow cytometric analysis of lymphocyte viability and activation was performed at days 0, 7, 10 and 17 of storage. Potassium, glucose, percentage free hemoglobin (fHb), p50, hemoglobin oxygen saturation (sO<sub>2</sub>), partial pressure of oxygen (pO<sub>2</sub>), lactate, pH, methemoglobin, white cell count (WCC) and lymphocyte count were measured at days 0, 7 and 17 post-irradiation.

There were significant differences in lymphocyte viability following irradiation, though not immediately. Additionally, irradiation induced activation of lymphocytes. A decrease in the percentage of viable lymphocytes over time occurred in both irradiated and unirradiated cells, similar to changes in WCC.

Significant changes occurred in pH, potassium, glucose, lactate, fHb, pO<sub>2</sub> and p50 value in irradiated and unirradiated units over time. Immediately following irradiation, pH, potassium, lactate and fHb were significantly different between irradiated units and controls. Small but significant differences were noted between irradiation status in pH, fHb, sO<sub>2</sub>, total oxygen content and p50 value 7 days post-irradiation.

In conclusion, irradiation and storage altered *in vitro* properties of pRBCs compared to unirradiated blood. Complete loss of viable lymphocytes was not seen immediately following irradiation, and presence of highly activated lymphocytes following irradiation warrants further investigation. The magnitude of the differences in electrolyte, acid-base and oxygen-carrying capacity was small. The clinical impact of irradiation on these variables may be negligible and is unlikely to preclude safe use of these products.

Keywords: Irradiation, erythrocyte, hematology, lymphocyte, dogs, T cells.

**ANTI-INFLAMMATORY DRUG SUPPLEMENTATION REGULATES IMMUNE RESPONSES TO SUBLINGUAL VACCINES.** Rowe J.C., Attia Z., Steiner H.E., Kim E., Cormet-Boyaka E., and Boyaka P.N. Department of Veterinary Biosciences

Since most infectious agents enter the host via mucosal surface, new vaccination strategies have focused on the induction of secretory IgA (SIgA), which coats the mucosal surface, in addition to systemic immunity. Previous research in our laboratory has shown that depletion of neutrophils improves the production of SIgA, and thus, demonstrated an inverse relationship between the ability of a vaccine to recruit activated neutrophils and induction of SIgA responses. C57BL/6 mice (n=5 per group) were sublingually immunized using two separate adjuvants, 3'3' cGAMP or anthrax edema toxin. While 3'3' cGAMP is not known to recruit neutrophils, anthrax edema toxin has a strong propensity to recruit neutrophils. The addition of the non-steroidal anti-inflammatory drug Alvelestat was used to correct for the negative effects of neutrophil activity on IgA responses. Serum antibody titers revealed significant differences in the balance of Th1 and Th2 modulated IgG subclass responses as well as systemic production of IgA. Fecal and vaginal antibody titers demonstrated significant differences in distal mucosal surface production of both IgA and IgG. Further, lasting protection afforded by the varying adjuvant and Alvelestat combinations was demonstrated through antigen neutralization assay. Overall, our data support the addition of Alvelestat to vaccinations typically adjuvanted by compounds that recruit IgA suppressing neutrophil activity.

Keywords: Sublingual Vaccination, Neutrophil, IgA, STING, Edema Toxin

**EOSINOPHIL ACTIVITY IS NOT ALTERED BY RESPIRATORY SYNCYTIAL VIRUS INFECTION IN THE COTTON RAT.** K. L. Smith, S. Niewiesk. Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH

Respiratory syncytial virus (RSV) is the most prevalent cause of infantile bronchiolitis and pneumonia worldwide. Although children are infected by age 2, infection does not induce long-term immunity and reinfection throughout life is common. Severe RSV infection as an infant significantly increases the likelihood to develop asthma. This connection is not well understood and has been studied in mice. However, RSV replicates only to low titers in lung tissue of mice. In the current study we used cotton rats which replicate RSV in the nose and the lung. Neonatal cotton rats, were infected with RSV and reinfected as young adults to evaluate lung and nasal remodeling. Neonatal lung remodeling was compared to animals whose first exposure occurred during adulthood. House dust mite antigen (HDM) was used to induce an allergic response in some animals to mimic natural allergen exposure in children. Allergic responses were measured by the number of eosinophils in bronchoalveolar lavage and secretion of eosinophil peroxidase. When cotton rats challenged with HDM were infected with RSV or influenza virus, the number of eosinophils in the lung was not significantly changed compared to an uninfected group. Influenza virus was used as a control because this viral infection is not known to result in asthmatic symptoms later in life. The amount of eosinophil peroxidase measured per eosinophil was not significantly different between any of the groups thus we conclude that eosinophil activity is not altered by RSV infection. The effects of neonatal infection on lung and nasal remodeling as compared to adult infection was not evident on histology.

Keywords: eosinophil, respiratory syncytial virus, cotton rat, eosinophil peroxidase, asthma



**EXPLORING NEW ALTERNATIVES FOR RAPID *MYCOBACTERIUM BOVIS* DETECTION IN CATTLE USING POINT-OF-CARE TUBERCULOSIS LIONEX AND LAM TESTS IN NON-INVASIVE SAMPLES.** S. Waibel<sup>1</sup>, S. Sidiki<sup>2</sup>, H. Kelley, C. Tomatis-Souverbielle<sup>3</sup>, W. Gebreyes<sup>1</sup>, S. Wang<sup>4</sup>, and J.B. Torrelles<sup>2</sup>. 1. OSU Dept. of Preventative Medicine, College of Veterinary Medicine 2.OSU Dept. of Microbial infection and Immunity, College of Medicine, 3. Nationwide Children's Hospital, Pediatric Infectious Disease Division, 4. OSU Internal Medicine Dept., Infectious Disease Division, College of Medicine

Approximately 5-10% of all human tuberculosis cases are attributed to *Mycobacterium bovis* infection, the causal agent of bovine TB (BTB) in cattle. BTB has devastating consequences on animal and human health. Currently, testing BTB in cattle requires a significant investment of time, equipment, and labor to determine the disease status of an animal. Moreover, effective surveillance protocols are often non-existent in high BTB burden countries, which further hampers control measures. This has created a drive to find novel and cheaper methods for rapid BTB testing. The Lionex Animal TB Rapid Tuberculosis Test<sup>®</sup> (Lionex-test) is a new rapid TB test that uses serum or plasma to detect three unique mycobacterial antigens. The Alere Determine TB LAM Ag (LAM test) is another point-of-care rapid test that uses urine to detect active TB disease in humans. The objective of our research was to determine the efficacy of Lionex-test and LAM-test in milk and urine samples, respectively, from dairy cattle, and compare their results to accepted gold standards. Using milk samples (n=30) from *M. bovis* suspected infected cattle from a recent BTB outbreak in Michigan, the Lionex-test was positive in 26/30 milk samples and was negative 2/2 for the controls. Using urine samples (n=25) from the same cattle, the LAM-test detected *M. bovis* antigen in 15/25 samples tested and was negative 9/9 for the controls. These results were compared to culture/PCR and histology/necropsy using Cohen's kappa statistic and Pearson's p-values. The Lionex-test vs. histology/necropsy and Lionex-test vs. culture/PCR presented the most promising results with  $k = 0.2851$  ( $p = 0.0107$ ) and  $k = 0.3478$  ( $p = 0.0547$ ) respectively. Meanwhile LAM-test vs culture/PCR and LAM-test vs histology/necropsy were  $k = 0.0132$  ( $p = 0.9304$ ) and  $k = 0.0984$  ( $p = 0.5482$ ) respectively. These findings suggest that performing the Lionex-test in milk samples could become the new screening method for BTB.

Keywords: Lionex, LAM, bovine tuberculosis

**INTERFERON-INDUCIBLE LY6E PROTEIN PROMOTES HIV-1 INFECTION.**

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LY6E is a glycosylphosphatidylinositol (GPI)-anchored, interferon (IFN)-inducible protein that regulates T lymphocytes proliferation, differentiation and development. Single nucleotide polymorphism rs2572886 in LY6-family protein locus has been shown to associate with accelerated progression to AIDS. In this study, we show that LY6E promotes HIV-1 infection by enhancing viral entry and gene expression. Knockdown of LY6E in human PBMCs, SupT1, and THP-1 cells diminishes HIV-1 replication. Virion-cell and cell-cell fusion experiments revealed that LY6E promotes membrane fusion of the viral entry step. Interestingly, we find that the LTR-driven HIV-1 gene expression is also enhanced by LY6E, suggesting additional roles of LY6E in HIV-1 replication. HIV-1 infection induces LY6E expression in human PBMCs, concomitant with increased production of type I IFN and some classical IFN-stimulated genes (ISGs). Altogether, our results demonstrate that IFN-inducible LY6E promotes HIV-1 entry and replication and highlight a positive regulatory role of IFN-induced proteins in HIV-1 infection. Our work emphasizes the complexity of IFN-mediated signaling in HIV-host interaction and AIDS pathogenesis.

Keywords: LY6E, HIV-1, Entry



**MOLECULAR  
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## MCB - 1

**NOVEL *IN VITRO* MODEL SYSTEM OF CANINE DEGENERATIVE MYELOPATHY USING DIRECT CONVERSION OF SKIN FIBROBLASTS.** K Bartholomew, S Likhite, K Meyer, S Moore, B Kaspar. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH (Bartholomew, Moore); Center for Gene Therapy, Nationwide Children's Hospital, Columbus, OH (Likhite, Meyer, Kaspar)

Canine degenerative myelopathy (DM) is a multisystem, adult onset degenerative disease of the central and peripheral nervous system. Amyotrophic lateral sclerosis (ALS) and DM have similar disease progression culminating in paralysis and death, with no treatments available. Both diseases likely share similar pathogenic mechanisms involving non-cell autonomous toxicity exerted by mutant Superoxide Dismutase 1 (mtSOD1). To better understand these mechanisms, a direct conversion method was utilized to convert skin fibroblasts from DM-affected and control dogs into CNS cell types to create an *in vitro* model system. Dog skin fibroblasts were transduced with four retroviral vectors containing reprogramming factors causing conversion to induced neural progenitor cells (iNPCs). After conversion to iNPCs, further differentiation to astrocytes, neurons, and oligodendrocytes was accomplished using media specific to each cell type, and conversion was confirmed with PCR and IF. To establish the *in vitro* model system and to test the hypothesis that astrocytes promote neuronal degeneration in DM, skin-derived astrocytes were co-cultured with GFP-expressing wild-type mouse motor neurons. We expect that skin fibroblasts from DM-affected dogs can be readily converted to iNPCs and i-astrocytes. We expect that DM-derived astrocytes will affect the axonal growth and survival of motor neurons similar to ALS astrocytes from mice and humans. The focus of this work is to establish a fast and reliable *in vitro* model system to study disease pathogenesis and test potential therapeutic intervention. Additionally, establishment of a direct conversion protocol for canine fibroblasts will be helpful in understanding other canine neurological disorders.

Keywords: degenerative myelopathy, canine, reprogramming, *in vitro* model

**USING NATURE’S TRICK TO EFFECTIVELY DELIVER DNA FOR GENE THERAPY**

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*Ehrlichia chaffeensis* is a zoonotic bacterium that causes human monocytic ehrlichiosis when transmitted by the bite of infected ticks. Previous discoveries in the Rikihisa Laboratory have shown that *E. chaffeensis* binds and enters human blood cells using a specific bacterial surface protein, termed Entry triggering protein of *Ehrlichia* (EtpE), by directly binding to the mammalian cell surface protein, DNase X. Since DNase X is known to cleave DNA, we hypothesized that DNase X activity can be inhibited by EtpE-binding. Therefore, the purpose of this study was to examine whether EtpE can enhance gene delivery efficiency into mammalian cells. Mammalian RF/6A cells were incubated with recombinant C-terminal fragments of EtpE (rEtpE-C), and a recombinant *Ehrlichia*-secreted protein Etf-1 as a negative control. Cells were then transfected with a plasmid encoding green fluorescent protein (GFP). The amount of plasmid delivered into mammalian cells was determined by quantitative PCR (qPCR). Results showed that the plasmid amount was slightly increased in RF/6A cells incubated with rEtpE-C normalized with *Gapdh* gene. This suggests that EtpE might block the catalytic site of DNase X, allowing the stabilization of exogenous DNA; therefore enhancing its delivery and expression into mammalian cells. These findings can possibly improve the current method of gene therapy, which is often limited by DNA degradation.

Keywords: *Ehrlichia chaffeensis*, ehrlichiosis, DNase X, gene therapy

**NOVEL CANINE PROSTATIC CANCER CELL LINE (LUMA) WITH OSTEOBLASTIC BONE METASTASIS.** S. Elshafae, W. Dirksen, W. Supsavhad, N. Kohart, A. Demirer, B. Hassan, Z. Attia, P. Boyaka and T. Rosol. Dept. of Veterinary Biosciences, The Ohio State University.

Osteoblastic bone metastasis represents the most common complication in patients with prostate cancer (PCa). Throughout progression and bone metastasis, PCa cells acquire properties similar to bone cells in a phenomenon called osteomimicry, which promotes their ability to metastasize, proliferate and survive in the bone microenvironment. The mechanism of osteomimicry resulting in osteoblastic bone metastasis is unclear. In this study, we developed and characterized a novel canine prostatic cancer cell line (LuMa) that will be a useful tool in studying the relationship between osteoblastic bone metastasis and osteomimicry in PCa. The LuMa cell line was established from a primary prostate carcinoma of a 13-year-old mixed breed castrated male dog. Cell proliferation and gene expression of LuMa were measured and compared to three other canine prostatic cancer cell lines (Probasco, Ace-1 and Leo) in vitro. The effect of LuMa cells on calvaria and murine pre-osteoblastic (MC3T3-E1) cells was measured by qRT-PCR and alkaline phosphatase assay. LuMa cells were transduced with luciferase for monitoring in vivo tumor growth and metastasis using different inoculation routes (subcutaneous, intratibial, and intracardiac). Xenograft tumors and metastases were evaluated using radiography and histopathology.

After left ventricular injection, LuMa cells metastasized to bone, brain and adrenal glands. Intratibial injections induced intramedullary new bone formation. LuMa cells had the highest levels of osteomimicry genes (*RUNX2*, *RANKL* and *Osteopontin*), *CD44*, *E-cadherin* and *MYOF* mRNA compared to Ace-1, Probasco and Leo cells. LuMa cells induced growth in calvaria defects and modulated gene expression in MC3T3-E1 cells. LuMa prostate cancer cells will serve as an excellent model for studying the mechanisms of osteomimicry and osteoblastic bone and brain metastasis in prostate cancer.

Keywords: Prostate cancer, Osteomimicry, Osteoblastic metastasis, Canine, Cell line

**EXPRESSION AND INHIBITION OF MONOCARBOXYLATE TRANSPORTERS IN CANINE OSTEOSARCOMA.** HL Gardner<sup>1</sup>, JM Fenger<sup>1</sup>, V Sandanayaka<sup>2</sup>, CA London<sup>1,3</sup>

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**Background:** A distinguishing feature of cancer cells is their ability to undergo aerobic glycolysis. This process generates lactic acid, which must be removed from the cell in order to maintain proliferation and survival. Monocarboxylate transporters (MCTs) move lactic acid across the plasma membrane, providing a mechanism for tumor cells to meet their bioenergetic needs in a variety of limiting microenvironments. The objective of this work is to determine the biologic effects of modulating cellular metabolism through inhibition of MCT1 and MCT4 in canine osteosarcoma (OS).

**Methods:** MCT1 and MCT4 expression was assessed in canine OS cell lines and primary canine OS tissues using qRT-PCR and western blotting. The effects of small molecule inhibitors (NGY066 and NGY008) and shRNA approaches targeting MCT1 and MCT4 on proliferation, survival and invasion of canine OS were examined using the CyQUANT Cell Proliferation, Caspase-3/7 and Matrigel invasion assays, respectively. The impact of MCT1/4 inhibition on cellular oxygen consumption was evaluated using the Seahorse Cell Mito Stress Test. Lastly, potential synergistic effects of combining metformin and doxorubicin with MCT1/MCT4 inhibition in canine OS cell lines were investigated using the CyQUANT Assay.

**Results:** MCT1 is expressed at higher levels relative to MCT4 in the canine OS cell lines. Small molecule inhibitors directed against MCT1/MCT4 did not significantly inhibit cell proliferation or induce apoptosis. However, downregulation of MCT1 expression in OS cells resulted in significantly decreased invasion and increased maximal respiratory capacity. Synergistic anti-proliferative activity was observed when OS cells were treated with doxorubicin in the presence of MCT1 inhibition.

**Conclusions:** MCT1 and MCT4 are expressed in canine OS. Downregulation of MCT1 inhibits cellular invasion and increases oxygen consumption in OS cell lines. Importantly, drug combinations with synergistic activity may be necessary to realize the full potential of MCT1/MCT4 inhibition.

Keywords: canine; metabolism; monocarboxylate transporter; osteosarcoma



**miR-146a IS AN ENDOGENOUS REGULATOR OF BOTH HEMATOPOIESIS AND BONE MASS.** J. Geisler, B. Hildreth III, J. Lee, A. de la Chapelle, P. Boyaka, M. Ostrowski, S. Sharma. Affiliations: College of Veterinary Medicine (Geisler, Boyaka) and Comprehensive Cancer Center (Hildreth, Lee, de la Chapelle, Ostrowski, Sharma, Geisler), The Ohio State University, Columbus, OH 43210

MicroRNAs (miRNAs), non-coding RNAs, regulate cellular activity by binding to protein-coding RNAs, suppressing translation or causing RNA degradation. One microRNA, miR-146a, is a key regulator of inflammation and is a physiologic break on immune activity. In the skeleton, osteoclasts share a common progenitor with macrophages of the myeloid lineage within the hematopoietic hierarchy. miR-146a has been shown to negatively regulate osteoclast differentiation and function by our laboratory and others in vitro. Because of this, we wanted to investigate the role of miR-146a in bone biology and hematopoiesis in vivo. Two transgenic mouse models were used for this purpose: a knock-out (KO) mouse model with global deletion of miR-146a and a knock-in (KI) mouse model overexpressing miR-146a. Male and female mice were aged 6-7 months and, at necropsy, tissue samples were collected for phenotyping. Spleen and liver weights were obtained, femurs isolated for radiographic evaluation, and blood, spleens, and bone marrow collected for flow cytometric evaluation. In both male and female KO mice and male KI mice there was a significant increase in spleen weight compared to wild-type controls. Female KO mice had significantly greater liver weights. These findings suggest altered hematopoietic cell number and/or cellularity. KO mice had decreased bone density and KI mice had increased density, suggesting that miR-146a also negatively regulates osteoclast function in vivo. These findings indicate that miR-146a regulates both hematopoiesis and bone mass. Further phenotyping is ongoing to provide insight into the role of miR-146a in these processes.

Keywords: Bone, hematopoiesis, osteoclasts, macrophage, microRNA, inflammation, mouse model

**EVALUATION OF A BIODEGRADABLE THERMOGEL POLYMER FOR INTRAOCULAR DELIVERY OF CYCLOSPORINE A TO PREVENT POSTERIOR CAPSULE OPACIFICATION.** KJ Gervais, HL Chandler, AJ Gemensky-Metzler, DA Wilkie, EJ Miller. Department of Veterinary Clinical Sciences; College of Optometry.

**Purpose.** To use a thermosensitive hydrogel (thermogel) polymer to achieve sustained intracapsular release of cyclosporine A (CsA) for reduction of *ex vivo* posterior capsule opacification (PCO). **Methods.** A PLGA-PEG-PLGA thermogel polymer was formulated to release CsA ([300µg/mL]) or vehicle (ethanol). Extracapsular cataract extraction and IOL placement were performed in 24 canine cadaver globes. Lens capsule explants with residual lens epithelial cells (LEC) were treated with 200µL of CsA-eluting (n=12) or vehicle-eluting (n=12) thermogel and maintained in culture. Posterior capsule coverage by LEC was graded following 7 (n=8), 14 (n=6), or 28 (n=10) days of treatment. LEC were quantified via light microscopy from capsules treated for 28 days. Differences in percent posterior capsule coverage and LEC counts were analyzed by student's t-test with Welch's correction. CsA concentration in culture media was quantified by LC-MS. **Results.** Posterior capsule coverage by LEC was significantly reduced in CsA-thermogel treated capsules compared to vehicle-treated. Percent posterior capsule coverage (mean ± SEM) for CsA-treated vs. vehicle-treated capsules was 0 vs. 33%±5.47 at day 7 (p<0.05), 13.3%±4.41 vs. 85%±3.87 at day 14 (p<0.0001), and 30%±6.35 vs. 96%±2.45 at day 28 (p<0.0001). Histologic LEC counts were significantly lower in CsA-thermogel treated capsules (88.38±28.65 cells) compared to vehicle-thermogel treated capsules (207.6±33.38 cells; p<0.05). Cumulative CsA release from the thermogel was greater than 10µg/mL over a minimum of 7 days. **Conclusions.** A CsA-eluting thermogel polymer may be a viable pharmacologic method for reducing PCO. Higher eluted CsA concentrations will likely be necessary to eliminate PCO formation entirely.

Keywords: lens, cyclosporine A, posterior capsule opacification, thermogel, cataract surgery

**EFFECTS OF ORAL *AKKERMANSIA MUCINIPHILA* SUPPLEMENTATION IN DOGS FOLLOWING ANTIBIOTIC ADMINISTRATION.** M. Jugan<sup>1</sup>, A. Rudinsky<sup>1</sup>, O. Paliy<sup>3</sup>, A. Gordon<sup>3</sup>, J. Daniels<sup>1</sup>, P. Boyaka<sup>2</sup>, C. Gilor<sup>1</sup>. <sup>1</sup>Depts. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University; <sup>2</sup>Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University; <sup>3</sup>Biochemistry and Molecular Biology, Wright State University.

Diarrhea and other sequelae of gastrointestinal hyperpermeability are common complications of antibiotic therapy. *Akkermansia muciniphila* is a mucin-degrading bacterium, positively associated with gastrointestinal epithelial health and decreased permeability. The objectives of this study were to measure effects of *Akkermansia* administration on systemic markers of gastrointestinal permeability and epithelial damage following antibiotic treatment.

Eight healthy, purpose-bred dogs were randomized to receive either *Akkermansia* ( $10^9$  CFU/kg; N=4) or vehicle (N=4) for 6 days following a 7-day course of metronidazole. After a 20-day washout, dogs were crossed-over to the alternate treatment. After an additional 20-day washout, the experiment was repeated with amoxicillin-clavulanate. Fecal *Akkermansia* qPCR and plasma concentrations (ELISA) of cytokeratin-18, lipopolysaccharide, and glucagon-like peptides (GLP-1, GLP-2) were measured at baseline (T0), post-antibiotic (T1), and post-treatment (vehicle or *Akkermansia*; T2). For each antibiotic, absolute or delta concentrations were compared between time-points using paired *t* tests.

*Akkermansia* was detected in feces in 7/8 dogs following supplementation (T2) but not at T0 or T1. Delta (T2-T1) cytokeratin-18 after metronidazole was significantly lower on vehicle (-0.27 ng/ml) versus *Akkermansia* (2.4 ng/ml;  $p=0.03$ ). Cytokeratin-18 concentrations tended to decrease from T0 to T1 on amoxicillin-clavulanate ( $p=0.05$ ). Post-prandial GLP-1 concentrations (38.2 pM) were higher than pre-prandial (15.5 pM) concentrations. Fecal score was higher following metronidazole vs amoxicillin-clavulanate ( $p<0.05$ ). No adverse side-effects or other significant biomarker alterations were noted.

*Akkermansia muciniphila* PCR detection suggested successful gastrointestinal transit following oral supplementation in dogs, with an effect on gastrointestinal epithelium based on plasma cytokeratin-18 alterations. Further study is needed to determine impact in dogs with naturally occurring disease.

Keywords: *Akkermansia muciniphila*, gastrointestinal permeability, canine, antibiotics, fecal score

**MECHANISMS OF SAMHD1-MEDIATED ANTI-PROLIFERATION IN CUTANEOUS T-CELL LYMPHOMA AND ACUTE MONOCYTIC LEUKEMIA CELLS.** K. M. Kodigepalli<sup>1</sup> and L. Wu<sup>1,2,3</sup>,

<sup>1</sup>Center of Retrovirus Research, Department of Veterinary Biosciences; <sup>2</sup>Comprehensive Cancer Center; <sup>3</sup>Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA

Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a mammalian dNTP hydrolase that regulates cellular dNTP homeostasis. The *SAMHD1* gene is mutated and its expression is downregulated in cancers including cutaneous T-cell lymphoma (CTCL) and leukemia. We reported that exogenous expression of SAMHD1 in CTCL-derived HuT78 CD4+ T-cells significantly reduces cell proliferation and increases apoptosis. We also reported that SAMHD1 knockout in acute monocytic leukemia (AML)-derived THP-1 cells causes increased cell growth and proliferation, but reduced apoptosis. These results suggest an anti-proliferative function of SAMHD1 in lymphoma and leukemia pathophysiology. The goal of this study is to investigate the mechanisms underlying SAMHD1-mediated anti-proliferation in lymphoma and leukemia cells. We found that exogenous SAMHD1 expression in HuT78 cells significantly reduced mRNA and protein levels of the short-form of cellular FLICE-inhibitory protein (cFLIP<sub>s</sub>), a key anti-apoptotic molecule. Reduced cFLIP<sub>s</sub> levels were partially due to enhanced proteasomal degradation, indicating that SAMHD1-induced effects in CTCL-derived HuT78 cells may be mediated via degradation of cFLIP<sub>s</sub>. In contrast, SAMHD1 knockout in AML-derived THP-1 cells did not alter cFLIP<sub>s</sub> expression, suggesting that SAMHD1-regulated cell proliferation and apoptosis in these cells is independent of cFLIP<sub>s</sub>. Interestingly, SAMHD1 knockout in THP-1 cells significantly increased the activation or expression of key mediators of PI3K and the downstream NF-κB pathway, including serine/threonine kinase Akt, and p100/p52. Inhibition of PI3K activity using specific inhibitor (LY294002) not only reduced p100/p52 levels, but also resulted in greater inhibition cell proliferation in THP-1 cells with SAMHD1 knockout. Together, our results suggest that SAMHD1 inhibits proliferation of CTCL and AML cells via different mechanisms involving cFLIP<sub>s</sub>, or PI3K and NF-κB pathways respectively. A better understanding the mechanisms of SAMHD1-mediated anti-proliferation in CTCL and AML cells will enhance our knowledge on pathogenesis of these cancers and can help to develop novel therapeutic strategies against them.

Keywords: SAMHD1, Lymphoma, Leukemia, PI3K, NF-κB, cFLIP.

**DIMINISHED MICRORNA-29B EXPRESSION IS ASSOCIATED WITH INCREASED BRD4-MEDIATED ACTIVATION OF TUMOR-ASSOCIATED GENES IN CUTANEOUS T-CELL LYMPHOMA.** R. Kohnken, J. Wen, B. Mundy-Bosse, M. Yano, L. Grinshpun, K. McConnell, A. Keiter, A. Hartlage, J. E. Bradner, M. A. Caligiuri, P. Porcu, and A. Mishra. Depts. of Veterinary Biosciences, Comprehensive Cancer Center, Dana-Farber Cancer Institute, and Internal Medicine.

Cutaneous T-cell lymphoma (CTCL) is a malignancy of skin-homing CD4<sup>+</sup> T-cells that initially presents in the skin but may progress and spread systemically. This cancer is universally fatal and effective treatments for advanced-stage patients are lacking. The molecular hallmarks of disease progression include altered expression of microRNAs (miRs) and epigenetic dysregulation of gene expression. In this study, we sought to characterize aberrant epigenetic modifications in malignant CD4<sup>+</sup> T-cells that contribute to CTCL development and progression. Using purified CD4<sup>+</sup> T-cells, we show significantly diminished levels of tumor suppressor microRNA-29b (miR-29b) in CTCL patients compared to healthy donors ( $0.007 \pm 0.002$ , n=9 vs  $1.008 \pm 0.052$ , n=6,  $p < 0.0001$ ). Alignment of the miR-29b seed sequence demonstrates complementarity with the 3' untranslated region of bromodomain and extra terminal (BET) protein BRD4, an epigenetic reader protein. Further analysis of patients' and miR-29b<sup>-/-</sup> mouse cells reveals that miR-29b regulates the expression of BRD4. We also found that genome-wide BRD4 binding occupancy at promoter and enhancer regions is increased in patient CD4<sup>+</sup> T-cells compared to healthy donors. The cumulative result of BRD4 binding is increased expression of lymphoma-associated proteins such as *NOTCH1* and *RBPJ*, as well as increased expression of all three components of the interleukin-15 (IL-15) receptor complex; the latter enhancing the IL-15 autocrine signaling loop that instigates progression of this incurable disease. Furthermore, we confirm the *in vivo* relevance of this pathway in our IL-15 transgenic mouse model of CTCL by showing that interference with BRD4-mediated signaling, either by restoring miR-29b levels via bortezomib treatment, or by direct inhibition of BRD4 binding at its target genes via JQ1 treatment, halts the progression of CTCL. These findings support a potential role for miR-29b and BRD4-targeted therapies in the treatment of CTCL.

Keywords: Epigenetics, lymphoma, microRNA, mouse model

**MIR-34A REGULATES THE INVASIVE CAPACITY OF CANINE OSTEOSARCOMA CELL LINES.** C. M. Lopez<sup>1</sup>, P. Y. Yu<sup>2</sup>, X. Zhang<sup>3</sup>, A. S. Yilmaz<sup>3</sup>, C. A. London<sup>4,5</sup>, and J. M. Fenger<sup>1</sup>.

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**Background:** Osteosarcoma (OSA) is the most common bone tumor in children and dogs; however, no substantial improvement in clinical outcome has occurred in either species over the past 30 years. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and play a fundamental role in cancer. The purpose of this study was to investigate the potential contribution of miR-34a loss to the biology of canine OSA, a well-established spontaneous model of the human disease.

**Methodology and Principal Findings:** Real-time PCR demonstrated that miR-34a expression levels were significantly reduced in primary canine OSA tumors and canine OSA cell lines as compared to normal canine osteoblasts. In canine OSA cell lines stably transduced with empty vector or pre-miR-34a lentiviral constructs, overexpression of miR-34a inhibited cellular invasion and migration but had no effect on cell proliferation or cell cycle distribution. Transcriptional profiling of canine OSA8 cells possessing enforced miR-34a expression demonstrated dysregulation of numerous genes, including significant down-regulation of multiple putative targets of miR-34a. Moreover, gene ontology analysis of down-regulated miR-34a target genes showed enrichment of several biological processes related to cell invasion and motility. Lastly, we validated changes in miR-34a target gene expression, including decreased expression of KLF4, SEM3A, and VEGFA transcripts in canine OSA cells overexpressing miR-34a. Concordant with these data, primary canine OSA tumor tissues demonstrated increased expression levels of putative miR-34a target genes.

**Conclusions:** These data demonstrate that miR-34a contributes to invasion and migration in canine OSA cells and suggest that loss of miR-34a may promote a pattern of gene expression contributing to the metastatic phenotype in canine OSA.

**Keywords:** microRNA, miR-34a, osteosarcoma, canine

**IL-6 RELATED SIGNALING IN SEPSIS-RELATED AND HYPERINSULINEMIC LAMINITIS.** M. Mironovich, M. Watts, K. Dern, and J. K. Belknap. Department of Clinical Sciences

Laminar failure in equine laminitis is characterized by dysregulation of cytoskeletal and cell adhesion dynamics in laminar epithelial cells, leading to structural failure of the laminae and a subsequent crippling displacement of the distal phalanx. Similarly, dysregulation of cytoskeletal and cell adhesion dynamics are two of the first events in epithelial cells undergoing transformation to less differentiated (e.g. tumor) cells (epithelial to mesenchymal transition/EMT). Specifically, these early events in epithelial tumorigenesis are reported to occur in intestinal epithelial cells (in inflammatory bowel disease) due to aberrant activation of STAT3 via IL-6-related signaling through the IL-6 receptor (IL-6R)/gp130 complex. Due to similarities between epithelial cellular events in laminitis and intestinal epithelial tumorigenesis, we hypothesized that laminar failure in laminitis is due to IL-6/IL6R gp130/STAT3 activation in the laminar epithelium induced by elevated levels of IL-6. We investigated laminar concentrations of IL-6, IL-6R and activated/phosphorylated STAT3 in models of sepsis-related (Oligofructose [OF] model) and endocrinopathic (Euglycemic Hyperinsulinemic Clamp [EHC] model) laminitis. Immunofluorescence was used to assess the laminar cell types undergoing STAT3 activation. Archived laminar samples from EHC and OF models were analyzed using real time quantitative PCR for assessment of laminar IL-6 mRNA concentrations, and immunoblotting for laminar concentrations of IL-6R and total and phosphorylated/activated STAT3. There were marked increases ( $p < 0.05$ ) in laminar concentrations of both IL-6 mRNA (200-2000-fold increase vs. control), and Phospho-STAT3 (two different moieties [S727 and Y705]) concentrations. STAT3 activation was localized to lamellar epithelial cells on immunofluorescence. The data obtained suggests that IL-6 activation of gp130/STAT3 signaling may play a significant role in dysregulation of the laminar epithelial cell leading to structural failure of the laminae in two types of laminitis, and needs to be further studied to determine its specific roles and the potential of this signaling for therapeutic targets for pharmaceutical intervention in laminitis.

Keywords: equine, laminitis, STAT3, IL-6, epithelial-mesenchymal transition

**CHONDROCYTE CO-CULTURE WITH SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLDS AND SYNOVIAL-DERIVED MESENCHYMAL STEM CELLS.**

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The use of decellularized extracellular matrices (ECM's) as a scaffold and Mesenchymal Stem Cells (MSCs) have become increasingly popular as a regenerative treatment. The ECM's retain the porosity of the native tissue, the collagen components, and many growth factors, which enhance cell seeding and survival and direct cell differentiation. Our laboratory has previously developed a novel synovial origin ECM (synECM) meeting specifications for transplantation of low DNA and cellularity, while maintaining tissue integrity; and successfully seeded the synECM with synovial MSCs (synMSC). The objective of this study was to investigate the potential synergistic effects of a synECM-synMSC-chondrocyte co-culture.

SynMSCs were cultured untreated or transduced to express human bone morphogenetic protein 2 (BMP2) using an adenoviral vector. SynECM was generated by decellularization of synovial sheets using 0.1% peracetic acid (PAA). Harvested chondrocytes were cultured in the well. SynECMs were infused with synMSC ( $0.5 \times 10^6$  cells/0.5 mL) using a 30% serum gradient and placed in the insert. Four experimental culture conditions were performed and maintained for 4, 7 or 14 days; chondrocytes alone, synECM co-cultured with chondrocytes, synECM seeded with synMSCs co-cultured with chondrocytes, and synECM seeded with BMP2 transduced synMSCs co-cultured with chondrocytes. Cell growth into the synECMs and differentiation were determined by the cell surface marker CD90, viability (flow cytometry) and histologic morphology of the chondrocytes. BMP2 expression, hyaluronan (HA) synthesis and proteoglycan (PG) production were determined. Data were statistically analyzed to determine the effect of experimental condition and time.

BMP2 concentration was greater ( $P < 0.001$ ) in transduced syngrafts over the testing period. Co-culture with synECM- synMSC-BMP2 had significantly higher morphology scores; increased number of cells, decreased number of dead or pyknotic nuclei. SynECM seeded with synMSCs showed a 3 - fold increase in live cells over the 14 days, after a 30% decrease on day 3. SynMSCs engrafted into the synECM showed a significant decreased CD90 expression, as well as increased HA, GAG, and, in transduced synMSCs, BMP2 and GFP production, more than seen without the chondrocyte co-culture ( $P < 0.05$ ), showing a synergy between synovium scaffold, synMSC, and chondrocytes, stimulated to even greater gene production and differentiation.

Keywords: extracellular matrix, mesenchymal stem cells, BMP-2, synovium



**CHARACTERIZATION OF EXOSOME MIR-9 EXPRESSION IN CANINE**

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and contribute to tumorigenesis. Exosomes are small (40-150 nm) cell membrane derived vesicles that are secreted into bodily fluids such as serum and urine. These vesicles function in intercellular communication as they transfer their contents, including miRNAs, between cells and may serve as non-invasive biomarkers in various malignancies. We identified miR-9 as being highly expressed in canine osteosarcoma (OSA) tumors and OSA cell lines and found that overexpression of miR-9 in normal osteoblasts and OSA cell lines promotes cell invasion *in vitro*. We hypothesize that high levels of miR-9 will be detected in exosomes derived from canine OSA cell lines. We further hypothesize that serum exosome miR-9 will be increased in dogs with OSA compared to healthy controls and that tumor-associated miR-9 levels will decrease following therapeutic intervention. Exosomes were isolated from conditioned media from cell lines or patient serum and analyzed using NanoSight™ imaging. qRTPCR demonstrated that exosome miR-9 expression is higher in canine OSA cells compared to normal osteoblasts. Data obtained from serum exosomes from healthy dogs and dogs with OSA found that miR-9 transcript is not detectable by qRTPCR; however, studies are underway to evaluate circulating miR-9 levels in an expanded cohort of dogs. Our findings demonstrate that miR-9 is detectable in canine OSA cell-derived exosomes and that exosome miR-9 expression is increased in canine OSA cell lines compared to normal osteoblasts. MiR-9 was not detectable in serum-derived exosomes from healthy dogs or dogs with OSA, suggesting that circulating miR-9 may not be a useful biomarker for OSA.

Keywords: osteosarcoma, miRNA, exosomes, translational oncology

**IMMORTALIZATION OF FELINE MESENCHYMAL STEM CELLS WITH FULL LENGTH FELINE TELOMERASE AND NOVEL SPLICE VARIANT ISOFORMS**

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Telomerase activity directly correlates with uncontrolled cellular proliferation in cancer. This increased activity has been reported in several feline studies including malignant tumors and feline mammary cancer cell lines. Several studies using human cells have examined telomerase immortalization activity in cancers as well as the function of alternative splicing to regulate activity, but the study of telomerase activity in feline cancers has been restricted by an incomplete feline telomerase (fTERT) cDNA sequence. fTERT cDNA was recently cloned in this lab, in addition to several novel and feline unique isoforms using RT-PCR of cDNA acquired from three feline oral squamous cell carcinoma (FOSCC) cell lines, three FOSCC tumors, normal cat oral tissues, and normal cat testis. We cloned four dominant isoforms into the mammalian expression vector pcDNA3.1 and will compare their telomerase activity to the wild type fTERT using an immortalization assay. fTERT is expected to immortalize feline mesenchymal stem cells in vitro based on previous studies of the behavior of TERT in cell proliferation, but the function of the isoforms is unknown. The fTERT wild-type clone and isoforms will be transfected into telomerase negative feline mesenchymal cells using Lipofectamine® 2000 Reagent and selected with G418 while monitoring survival. These results will confirm that telomerase is an important factor in feline cancer, and allow for the progress of cell-based therapies in telomerase positive cancers, including those in human.

Keywords: feline, squamous cell carcinoma, FOSCC, animal model, TERT, telomerase, alternative splicing, cloning, immortalize, mesenchymal

# **STRUCTURE/FUNCTION**

**CHARACTERIZATION OF A NOVEL ORTHOTOPIC MOUSE MODEL OF PANCREATIC CANCER-INDUCED MUSCLE WASTING**

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Pancreatic cancer is the 3<sup>rd</sup> leading cause of cancer death in the United States. It is associated with a dismal prognosis with a 5-year survival rate of less than 9% for all stages. Furthermore, cachexia, defined as severe weight loss due to depletion of muscle mass, is seen in 85% of pancreatic cancer patients. Cachexia contributes significantly to morbidity and mortality, as nearly one-third of pancreatic cancer deaths are due to cachexia rather than tumor burden. The mechanisms behind the development of cachexia are poorly understood. Therefore, there is an urgent need to better understand cachexia in pancreatic cancer in order to develop targeted therapeutics and improve clinical outcomes. Here, we describe the characterization of a novel mouse model of pancreatic cancer-induced cachexia. Athymic nude mice were injected orthotopically into the pancreas with  $1 \times 10^6$  AsPC-1 pancreatic tumor cells. Body weight and food consumption were measured two to three times weekly. Muscle function was analyzed by grip strength just prior to sacrifice. At 6 weeks post-injection, mice were euthanized and muscle was collected for qPCR and Western blot analysis of cachexia biomarkers. Compared to tumor free mice, AsPC-1 tumor bearing mice had a significantly decreased percent body weight loss at day 42. Muscle weight and adipose tissue were also significantly decreased. Food consumption was mildly, but not significantly, decreased. Grip strength was significantly decreased relative to control mice. qPCR analysis of gastrocnemius muscle showed upregulation of several cachexia-associated biomarkers, including E3 ligases Atrogin-1 and MuRF1, IL-6 receptor, Stat3, and Socs3. Increased expression of signaling pathways involved in muscle degradation, including phosphorylated Stat3 and phosphorylated NF- $\kappa$ B were observed through Western immunoblotting. These data indicate that the AsPC-1 orthotopic model recapitulates several aspects of cancer cachexia, and represents a useful model for further mechanistic and therapeutic development targeting pancreatic cancer-induced cachexia.

Keywords: pancreatic cancer, cachexia, mouse models

## **SUBCHONDRAL BONE PAIN IMPACT MODEL**

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**Background:** Osteochondral disease is a cartilage and subchondral bone injury due to bone fatigue and overloading. Soldiers and sports athletes are prone due to excessive impacts.

**Introduction:** Joint and bone pain occur from impact to weight-bearing joint surfaces that result in hemorrhage, edema, and microfracture of the subchondral bone. Bone necrosis and collapse of the overlying cartilage occurs resulting in osteoarthritis. This painful condition is termed traumatic osteochondral disease and can be seen early in MRI.

**Methods:** We defined a non-penetrating impact model using equine bone in a location that mimics this disease naturally in that species (palmar MCIII). We evaluated a range of psi and defined the injury by MRI, gross inspection and histology of the injury.

**Results:** Histologic evaluation of the impact sites show that collapse of calcified cartilage and subchondral bone occurred with minimal overlying cartilage injury at 40 psi. The injury mimicked that seen in natural disease at this location. Similar histologic sections using 120 and 80 psi showed evidence of complete shearing of the articular cartilage and fragmentation of the underlying subchondral bone, consistent with an overly aggressive model of trauma.

**Discussion:** Currently there is no treatment or cure for this syndrome that results in bone pain and early joint destruction. Several novel approaches are being suggested that may include direct bone infusions and decompressions. This model will be able to assess these therapies in a controlled experimental design and allow MRI to be correlated to histologic findings.

**Conclusion:** An animal model of high non-penetrating impact can induce bone injury similar to natural occurring osteochondral disease in soldiers and sports athletes.

**Keywords:** Osteochondral disease, impact injury, subchondral bone