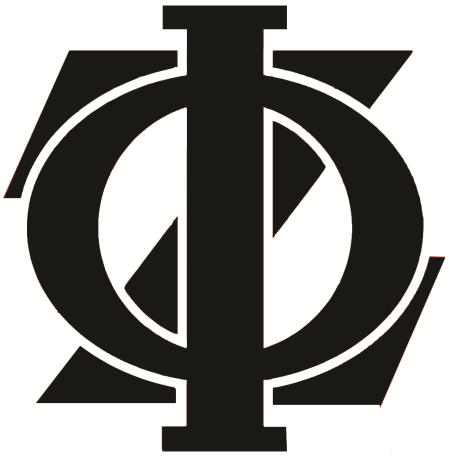
PROCEEDINGS

The 31st Annual

Phi Zeta Research Day

MICHIGAN STATE
UNIVERSITY

COLLEGE OF VETERINARY MEDICINE



Friday, October 1, 2021

The 2021 Phi Zeta Day is dedicated to the memory of Dr. Lorraine Sordillo, Meadow Brook Chair in Farm Animal Health and Wellbeing and Professor in Large Animal Clinical Sciences. Lorraine was a pillar in the CVM research community and a mentor to many students during her almost 18 years at MSU. We will miss her dearly.

JAQUA FOUNDATION SYMPOSIUM
IN MEMORY OF DR. SAMUEL POLLOCK

The keynote speaker this year is sponsored by the Jaqua Foundation Symposium in Memory of Dr. Samuel Pollock, which honors the name and career of an esteemed alumnus of Michigan State University's College of Veterinary Medicine, Samuel Pollock, DVM.

In 1996, MSU conferred the Distinguished Veterinary Alumnus Award upon Dr. Pollock, citing his remarkably productive career.

Upon graduation in 1941, Dr. Pollock served with the Bureau of Animal Industry as a cattle tester. After spending nearly four years stationed in the desert region of Ahwaz during World War II, he returned home and established the South Orange Animal Hospital in South Orange, New Jersey. He practiced there for more than 40 years, ably assisted by his beloved wife, Madalyn.

In 1950, he and a group of like-minded individuals established the Metropolitan New Jersey Veterinary Medical Association, which eventually became the largest active group in the state. Dr. Pollock was a strong advocate of continuing education, attending hundreds of such courses himself and developing new and exciting programs for other veterinarians. Among other things, he became keenly interested in molecular genetics.

The monthly meetings of the veterinary association provided him with the stimulus for documenting challenging cases, and he eventually published 35 papers. One such paper led to his appointment as veterinary consultant for research facilities at the Newark Beth Israel Medical Center, a relationship that lasted for 20 years.

Dr. Pollock's activities also earned him the 1972 Practitioner of the Year Award from the New Jersey State Veterinary Medical Association and the 1973 AVMA Practitioner's Research Award. A photographic essay he published won a medal at the XXI World Veterinary Congress in 1979.

His interest in the humane care of research animals attracted the attention of the Jaqua Foundation, a philanthropic organization, which named him as a trustee in 1982. From this position, he helped carry out the founder's wishes to benefit the veterinary profession and animals.

To honor his memory, the Jaqua Foundation established an endowment at MSU to sponsor symposia that feature leaders, scientists, and scholars from the veterinary profession and the biomedical community. Dr. Kim Dodd, DVM, MS, PhD and Director of the Michigan State University Veterinary Diagnostic Laboratory will present *"Veterinary Diagnostic Laboratories: Leaders in One Health"* as our Jaqua Foundation Symposium Speaker in Memory of Dr. Samuel Pollock.

2020 Phi Zeta Research Day Award Recipients

Judging Category	Best Presentation
DVM Professional Student	Antonia Langfeldt, Kaitlyn Bailey
Resident, Intern, or MS Graduate Student	Julie Pfeifer
PhD Graduate Student	Devika Bahal
Undergraduate Student	Philip Calhoun

31st ANNUAL PHI ZETA RESEARCH DAY

College of Veterinary Medicine
Michigan State University
Veterinary Medical Center

October 1, 2021

PROGRAM

Time	Event	Location
8:00 – 12:30	**Assembling of posters**	G-205 (Anatomy Lab)
8:15-10:30	**RIMS Student Oral Presentations** (see detailed presentations schedule)	A-214 (Buchanan Room)
8:30-11:45	**DVM Student Oral Presentations** (see detailed presentations schedule)	A-213 (Cafeteria)
8:15-11:45	**PhD Student Oral Presentations** (see detailed presentations schedule)	G-201 (Reading Room)

11:45- 12:30	Boxed lunches will be available outside of G-150.

1:30 – 2:30 POSTER VIEWING in G-205 (Anatomy Lab)

Zoetis Award and Keynote Address
G-150 Vet Med Ctr

12:05 – 12:30 **G-150**

Presentation of Zoetis Award
for Veterinary Research Excellence

TBA

12:30-1:30 **G-150**

JAQUA FOUNDATION SYMPOSIUM KEYNOTE SPEAKER

"Veterinary Diagnostic Laboratories: Leaders in One Health"

Dr. Kim Dodd, DVM, MS, PhD, Director of the
MSU Veterinary Diagnostic Laboratory

3:00-3:15 **G-150**

Presentation of Phi Zeta Student Research Day Awards

31st ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Buchanan Room (A-214 VMC) **Residents, MS Students & Post-Docs** **Moderator:** **Dr. Dodd Sledge**
8:15-8:30	**Julianne White[2]** Phenylbutazone, Firocoxib, and Dipyrone all Diminish Furosemide Diuresis in Horses (Harold Schott)
8:30-8:45	**Sara Taylor[2]** Core vs. Peripheral Heat Loss in Horses under General Anesthesia (Kirk Munoz)
8:45-9:00	**Mayra Tsoi[2]** Quantitative Gene Expression Patterns of TYR, CD34, and CALD1 Accurately Differentiate Canine Oral Melanomas from Soft Tissue Sarcomas (Matti Kiupel)
9:00-9:15	**Ryan Yanez[2]** SARS-CoV-2 Infection in Two Farmed Minks (Neovision Vision) (Dodd Sledge)
9:15-9:30	**Caitlin Thorn[2]** Bispectral Index (BIS) Monitoring of Standing Sedation in Horses (William Horne)
9:30-9:45	**Allison Gerras[2]** Immunophenotyping of Iridociliary Epithelial Neoplasms in 21 Dogs (Dodd Sledge)
9:45-10:00	**Sarad Patel[5]** Whole Genome Sequencing for Genomic Epidemiological Studies of Tuberculosis in Asian Elephants of Nepal (Srinand Sreevatsan)
10:00-10:15	**Carine Holz[5]** Comparative Analysis of RT-QUIC, ELISA, and IHC for Chronic Wasting Disease Diagnostics (Srinand Sreevatsan)
10:15-10:30	**Natalia Duque-Wilckens[5]** Mast cell-specific inactivation of *Fosb* exacerbates release of pro-inflammatory mediators in models of systemic anaphylaxis and lipopolysaccharide-induced sepsis (Adam Moeser)

31st ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Cafeteria (A-213 VMC) **DVM Students** **Moderator:** **Dr. Sue Ewart**
8:30-8:45	**Megan Allen[1]** Differences in Physiologic Parameters in Sled Dogs and Shelter Dogs Undergoing Anesthesia (Sarah Shull)
8:45-9:00	**Ilissa Chasnick[1]** Evaluation of Veterinary Medical Student Retention of Anatomy and Physiology Concepts with Various Experiential Learning Methods (Nyssa Levy)
9:00-9:15	**Brendan Nagler[1]** ATP7A, ATP7B, and RETN Genotypes in Labradors with and without Copper Associated Hepatitis (Vilma Yuzbasiyan-Gurkan)
9:15-9:30	**Antonia Langfeldt[1]** Per- and Polyfluoroalkyl Substances (PFAS) and Animals: A Review (Melinda Wilkins)
9:30-9:45	**Jessica Hynes[1]** Safety and Efficacy of a Novel Anti-DEK Aptamer Treatment for Osteoarthritis Using a Canine Model (Jane Manfredi)
9:45-10:00	**Elizabeth Jackson[1]** Low-Dose Radiation Therapy as a Modality for the Management of Feline Chronic Rhinitis (Leanne Magestro)
10:00-10:15	**BREAK** **Moderator: Dr. Jane Manfredi**
10:15-10:30	**Brooke Kahn[1]** Platelet Inhibition by Metabolites of Clopidogrel (Adam Lauver)
10:30-10:45	**Siana Stanton[1]** Forces Exerted on the Back During Jumping in Horses (Jane Manfredi)
10:45-11:00	**Hunter Ferchaw[1]** Hematology Reference Intervals for Zoo-housed Neonatal Giraffe Calves (*Giraffa camelopardalis*) (Kimberly Thompson)
11:00-11:15	**Megan Crawford[1]** Relationship Between Parity and Reproductive Disease in Managed African Painted Dogs (Dalen Agnew)
11:15-11:30	**Hunter Wojtas[1]** Mosquito Diversity, Abundance, and Virus Associations of Binder Park Zoo (Edward Walker)
11:30-11:45	**Lauren Phillip[1]** The Effects of Castration and Early Weaning on Long-Term Immune Responses (Adam Moeser)

31st ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Reading Room (G-201) **PhD Students** **Moderator:** **Dr. Gisela Hussey**
8:15-8:30	**Ursula Abou-Rjeileh[3]** Oleic Acid Improves Insulin Sensitivity in Adipose Tissue of Periparturient Dairy Cows (Andres Contreras)
8:30-8:45	**Husnain Ahmed[3]** Lactobacillus Activates AH Receptor to Attenuate Pro-Inflammatory Response in CACO-2 Cells (Linda Mansfield)
8:45-9:00	**Devika Bahal[3]** 2B4: A Novel Checkpoint Receptor for INKT Cell Functions (Rupali Das)
9:00-9:15	**Glorián Berríos-Vázquez[3]** Immortalized Feline Respiratory Epithelial Cells Preserve Immunological and Morphological Characteristics of Feline Respiratory Epithelial Cells and Can Be Used to Study Feline Herpesvirus-1 (Gisela Hussey)
9:15-9:30	**Miguel Chirivi[3]** Endotoxin Activates Lipolysis in Bovine Adipocytes (Andres Contreras)
9:30-9:45	**Hinako Terauchi[3]** Gut Microbiome Composition at 3 Months of Age Associated with Eczema Diagnosis in Infants (Linda Mansfield)
9:45-10:00	**Zimu (Christine) Wei [3]** Thrombin Controls Fibrin(ogen) Solubility Dynamics in Early Acetaminophen Hepatotoxicity (James Luyendyk)
10:00-10:15	**BREAK** **Moderator: Dr. Andres Contreras**
10:15-10:30	**Cristian Rendon-Mora[3]** Mechanosensor Piezo1 Alters Adipogenesis in PVAT Preadipocytes via Calcium Signaling (Andres Contreras)
10:30-10:45	**Azam Sher[3]** Plasmid-Mediated Spread or Antibiotic Resistance Genes Among Bacteria in the Human Gut Microbiome (Linda Mansfield)
10:45-11:00	**Dawn Kusynski[3]** Clopidogrel Treatment Inhibits P2Y2-mediated Constriction in the Rabbit Middle Cerebral Artery (Adam Lauver)
11:00-11:15	**Kyan Thelen[3]** Mast Cell Histamine Mediates the Intestinal Immune Response to Early Weaning in Piglets via Histamine 2 Receptor (h2r) (Adam Moeser)

31st ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Reading Room (G-201) PhD Students
11:15-11:30	**Vishvapali Kobbekaduwa[3]** Invasion of the blacklegged tick (*Ixodes scapularis*) and the agent of Lyme disease Borrelia burgdorferi across Michigan from 2017-2019 (Jean Tsao)
11:30-11:45	**Ashley Putman[3]** Isoprostanes Increase Raw 264.7 Cell Viability and ROS Production in a Model of Inflammation (Andres Contreras)

2021 Phi Zeta Poster Presentations

Presenter's Last Name	Presenter's First Name	Poster #	Category*	Mentor	Title
Acevedo	Stephanie	1	1	Jacquelyn Del Valle	Effect of a Warmed Environment and Supplemental Heat on Mouse Behavior, Breeding and Temperature
Gallego	Alondra	2	1	Aimee Colbath	The Effects of Losartan Use in Dogs on Stifle Joint Pain and Progressive Osteoarthritis Following Tibial Plateau Leveling Osteotomy
García	Carmen-María	3	1	Adam Moeser	Impact of Early Weaning on Intestinal Histamine Receptor Expression and Localization in the Piglet Small Intestine
Hamlin	Nicole	4	1	Valerie Johnson	Phenotypic and Functional Characterization of Mesenchymal Stem Cells in Exotic Species
Hipkiss	Hannah	5	1	Srinand Sreevatsan	Identification of Novel miRNA Isolated from Serum-Derived Exosomes as a Diagnostic Indicator of Bovine TB Infection
Howard	Cory	6	1	Bonnie Harrington	Characterization of $CD3^{+}$/$CD20^{+}$ Large Cell Canine Lymphoma
Maresca-Fichter	Hailey	7	1	Jane Manfredi	Dynamic Insulin Response to Acclimation of a High Carbohydrate Diet in Horses
Myers	Madison	8	1	Andres Contreras	Adipose Tissue Adipogenesis and Lipogenesis are Modulated by Cannabinoids in Dairy Cows
Rafique	Sonia	9	1	Linda Mansfield	Developing a Rapid Plasmid DNA Extraction Method
Skalecki	Shannon	10	1	Valerie Johnson	Assessment of Anti-viral and Anti-inflammatory Effects of Mesenchymal Stem Cells in Herpesviral Infections
Spilker	Eric	11	1	Linda Mansfield	Effect of Chemotherapy on the Diversity of the Fecal Microbiome of Canine Lymphoma Patients
Vollmer	Shelby	12	1	Jane Manfredi	Saddle Fit During a Long Distance Endurance Ride
Winn	Emily	13	1	Margaret Petroff	The Molecular Clock Genes, *CLOCK* and *PER3*, in the Human Placenta
Cortes	Daniela	14	2	Andres Contreras	Lipolysis Inhibition Improves Clinical Outcomes in the Treatment of Ketosis in Dairy Cows
Brenner	Evan	15	3	Srinand Sreevatsan	Genetic Fingerprints of Host Specialization in the *Mycobacterium tuberculosis* Complex
Moya-Uribe	Ivon	16	3	Linda Mansfield	Human Microbiota Decreased Lung Function in a Mouse Asthma Model
Whitehead-Tillery	Charles	17	3	Linda Mansfield	Characterization of the Extended Spectrum ß-lactamase *E.coli* Plasmid CTX-41

*Category: [1]DVM professional student; [2]Resident, intern, or MS graduate student; [3]PhD graduate student; [4]Undergraduate student; [5]Other member of the CVM Community

Presenter's Last Name	Presenter's First Name	Poster #	Category*	Mentor	Title
Van Allen	Mia	18	4	Linda Mansfield	Plasmid-Mediated Transfer of Antibiotic Resistance Genes Between Bacteria
Fardisi	Mahsa	19	5	Adam Moeser	Early Weaning Stress Shapes Long-Term Immune Responses in a Sex-Specific Manner in Pigs

*Category: [1]DVM professional student; [2]Resident, intern, or MS graduate student; [3]PhD graduate student; [4]Undergraduate student; [5]Other member of the CVM Community

OLEIC ACID IMPROVES INSULIN SENSITIVITY IN ADIPOSE TISSUE OF PERIPARTURIENT DAIRY COWS

U. Abou-Rjeileh, M. Chirivi, J. Parales-Giron, J. M. Dos Santos Neto, C. M. Prom, J. Laguna, A. L. Lock, and G. A. Contreras. Michigan State University, East Lansing, MI

Excessive adipose tissue (AT) lipolysis around parturition in dairy cows is associated with impaired AT insulin sensitivity (IS) and increased incidence of metabolic diseases. We demonstrated that supplementing oleic acid (OA) can promote lipid accumulation in AT. In the liver, OA promotes lipid droplet formation by activating PPARα and perilipin 5 (PLIN5), however it is unknown if this mechanism occurs in AT. We hypothesize that OA regulates lipogenesis and IS in AT of periparturient dairy cows through the activation of PPARα and its key target protein PLIN5. Twelve multiparous Holstein cows were infused abomasally with 60 g/d of a vehicle (CON) or OA for 14d after calving. Subcutaneous AT samples were obtained at 11±3.6d before calving (PreP), and 6±1d (PP1) and 13±1.4d (PP2) after parturition. Intravenous glucose tolerance test was performed on d14 after calving. Isoproterenol (ISO) stimulated lipolysis and insulin inhibition of ISO was determined using a short-term in vitro explant culture by measuring glycerol release. PPARα and PLIN5 expression was analyzed using Simple Wes. Statistical analyses were performed using a mixed effect linear model in JMP. CON cows had a higher plasma glucose concentration peak than OA cows (188 vs 173±5.8, P<0.001) and higher clearance rate (2.06 vs 1.89±0.04, P<0.01). Moreover, at PP2, AT from OA cows had a lower response to ISO and were more sensitive to insulin compared with CON (P<0.01). OA cows had higher PPARα content (0.006 vs 0.002±0.001; P<0.10). Additionally, OA infusion increased PLIN5 protein expression at PP2 compared with CON (0.007 vs 0.002±0.001; P<0.05). Our results show that OA limits lipolysis by improving IS and demonstrated that lipogenesis in AT may be enhanced through PLIN5 activation in a PPARα dependent manner.

EFFECT OF A WARMED ENVIRONMENT AND SUPPLEMENTAL HEAT ON MOUSE BEHAVIOR, BREEDING AND TEMPERATURE

Stephanie Acevedo[1], Jacquelyn M Del Valle[2], F. Claire Hankenson[1,2] 1) College of Veterinary Medicine, 2) Campus Animal Resources, Michigan State University

It is essential to minimize variables related to laboratory mice for study success and experimental reproducibility. One extrinsic variable is the “cold-stress” endured by mice in standard housing conditions with room temperatures of 20-26°C, which are much cooler than the mouse thermoneutral zone (TNZ), the preferred temperature range (29-34°C) in which mice can maintain normal body temperatures with little energy expenditure. Studies have noted impacts of chronic “cold-stress”, the resulting physiological variance in mice, and its potential to affect this animal model in emulating human physiology in disease states. In this pilot study, we evaluated mouse behavior, breeding success, and body temperature when ambient room temperature (Ta) was increased (29.4°C) and further supplemental heat (37°C) was provided to a portion of the individually ventilated cage (IVC) floor via a digitally controlled heat source. Mice were divided by sex (n=4 per group) with continuous access to two interconnected IVC cages; one of these cages received supplemental heat. Mice were then placed in breeding pairs (n=4 pairs) in single IVC cages, two with heat plate access. Cage-side videography was utilized for data collection. With increased Ta, we expected that animals would engage in more sedentary behaviors during the light cycle, as “cold-stress” encourages hyperactivity, and suspected a decline in nest quality and pup size/weight associated with decreased demand for heat conservation. Preliminary results indicate that mice with supplemental heat have decreased nest scores and occupy non-heated cage quadrants. Providing a range of temperatures close to the TNZ within the mouse microenvironment allows rodents to self-select preferred temperatures throughout the circadian cycle.

LACTOBACILLUS ACTIVATES AH RECEPTOR TO ATTENUATE PRO-INFLAMMATORY RESPONSE IN CACO-2 CELLS.

Husnain Ahmed[3], Julia Bell[1,3], and Linda Mansfield[1,2,3] 1) Large Animal Clinical Sciences, 2) Microbiology and Molecular Genetics, 3) College of Veterinary Medicine, Michigan State University.

Previously we isolated and characterized a *Lactobacillus murinus* strain from C57BL/6 IL-10-/- mice protected from colitis when challenged with colitogenic isolate of *Campylobacter jejuni*. We hypothesized that *L. murinus* will activate the Aryl hydrocarbon receptor (Ah receptor) to attenuate the proinflammatory response in the human intestinal epithelial cells *invitro*. We developed a model of human intestinal epithelial barrier using Caco-2 cells (human intestinal epithelial cells) grown on Transwell inserts. Transepithelial Electrical Resistance (TEER) was measured to evaluate the formation of tight junctions. Once TEER reached 500 ohms per cm2, the cells were considered to have formed tight junctions. To simulate *C. jejuni* infection conditions, we treated the cells with increasing doses of TNFalpha. TNF-alpha decreased TEER and induced the secretion of IL-8 (pro-inflammatory cytokine) in a dose-dependent manner. We found that Ah receptor was activated by treating the Caco-2 cells with *L. murinus* (MOI 1:100). Two defined ligands for Ah receptor were used as controls i.e., 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 6-formylindolo[3,2-b] carbazole (FICZ). After activation of the Ah receptor, the Caco-2 cells were then treated with TNF-alpha (10 ng/ml). We found that activation of the Ah receptor attenuated the disruption of epithelial barrier integrity and decreased the secretion of IL-8 as compared to the positive controls. Our results indicate that Ah receptor can be a novel drug target for treating gut bacterial pathogens such as *C. jejuni*..

DIFFERENCES IN PHYSIOLOGIC PARAMETERS IN SLED DOGS AND SHELTER DOGS UNDERGOING ANESTHESIA

Megan M. Allen[1], Kirk A. Muñoz[2], Jane M. Manfredi[3], Sarah K. Rich[4], Sarah A. Shull[5], 1) College of Veterinary Medicine 2,5) Small Animal Clinical Sciences 3) Pathobiology and Diagnostic Investigation, Michigan State University 5) Banfield Pet Hospital

Anesthetic monitoring parameters consider differences such as species, body weight, and age, but little is known about the influences of physical fitness on the established normal ranges. The hypotheses of this study are that conditioned sled dogs will have a 5% lower heart rate (HR), 10% less HR variation, and a 10% lower mean arterial pressure (MAP) from induction to 15 minutes post induction when compared to shelter dogs. Anesthetic reports from sterilization procedures completed by veterinary students at one institution (n=166) were evaluated. Sled dogs (n=62) from one kennel were compared with shelter dogs (n=104). Dogs were paired based on sex, age, weight, and pre-medication protocol. Pre-operative parameters studied included body weight, body condition score (BCS), temperature (TEMP), HR and respiratory rate (RR), with TEMP, HR, RR, and MAP recorded from induction to end of anesthesia. Data were analyzed using descriptive statistics and independent t-tests with significance at P<0.05. The BCS of sled dogs was lower than shelter dogs (P=0.02). There were no significant differences between groups in HR, HR variation, RR, or MAP from induction to 15 minutes post induction. Sled dogs had a significantly lower TEMP pre-operatively (P=0.03) and a higher TEMP post- operatively (P=0.001). Based on the results of this study, current anesthetic monitoring parameters are adequate to ensure patient safety of conditioned patients. Future studies are recommended with different anesthetic protocols. Autonomous continuous anesthetic monitoring equipment would be recommended, which would ensure that all parameters would be measured and to limit human error and variation.

2B4: A NOVEL CHECKPOINT RECEPTOR FOR INKT CELL FUNCTIONS

Devika Bahal[1], Hyun Hee Lee[2], Rupali Das[2], 1) Comparative Medicine and Integrative Biology Program, College of Veterinary Medicine, Michigan State University, 2) Department of Physiology, College of Natural Science, Michigan State University

Invariant natural killer T cells (iNKTs) are innate T lymphocytes that express an "invariant" T cell receptor (TCR) that recognize glycolipid antigens when presented in complex with the MHC class I molecule CD1d. Following TCR engagement, iNKTs rapidly secrete cytokines and up-regulate the expression of co- stimulatory molecules. This rapid effect can modulate both innate and adaptive immunity and is important in influencing host immune responses to cancer. Several studies have established that iNKTs mount potent cytotoxic responses to numerous CD1d+ tumors *in vitro* and *in vivo*. Although TCR-CD1d interactions are generally required for iNKT cell cytotoxicity, the receptors and signaling mechanisms that co-operate with the TCR to promote maximal anti-tumor responses are poorly understood.The receptor 2B4 is a member of the immunoglobulin superfamily that binds to CD48 on target cells and modulates immune activation by facilitating cell-cell interactions and transducing intracellular signals. We recently observed that 2B4-deficient (*2b4−/−*) iNKTs have enhanced cytolytic activity where as those stably expressing full-length 2B4 fail to kill cancer cells. Importantly, resting iNKTs do not express 2B4 but following activation they express an inhibitory form of this receptor. Thus, 2B4 is not only a negative regulator but also a novel checkpoint molecule for iNKT cell function.Further elucidation of the mechanisms by which 2B4 regulates TCR-induced iNKT cell anti-tumor activity is of significant scientific and clinical importance as these studies will provide insights into how iNKT cell cytotoxic responses can be further enhanced to improve the treatment of cancer patients.

IMMORTALIZED FELINE RESPIRATORY EPITHELIAL CELLS PRESERVE IMMUNOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF FELINE RESPIRATORY EPITHELIAL CELLS AND CAN BE USED TO STUDY FELINE HERPESVIRUS-1

Glorián Berríos-Vázquez[1,2] Yao Lee[1,2], Gisela Soboll Hussey[1,2]
1. Pathobiology and Diagnostic Investigation, 2. College of Veterinary Medicine, Michigan State University

Feline herpesvirus 1 (FHV-1) is the leading cause of respiratory and ocular disease in cats. Previous *in vitro* studies have used primary feline respiratory epithelial cells (FREC) to understand the immunomodulation and growth kinetics of FHV-1. However, cell yields limit the number of experiments we can perform. To further reduce the number of cats and tissues needed for experiments, we immortalized FRECs to generate a sustainable cell line for perpetual use. We hypothesized that immortalized feline respiratory epithelial cells (iFRECs) retain morphological and immunological characteristics and could be used to study FHV-1 and other respiratory pathogens. iFRECs were grown and compared to primary FRECs morphologically and immunologically using microscopy and conventional PCR. In addition, FRECs and iFRECs were inoculated with FHV-1, and viral growth kinetics were determined by virus titration and real- time quantitative PCR (qPCR). Finally, cytokine expression in response to viral inoculation was compared by qPCR. iFRECS and FRECs showed no morphological or immunological difference, but iFRECS showed less variability when expressing immunological receptors. Infection of iFRECs was readily achieved and viral growth kinetics were comparable to FRECS. In addition, IFNα, IL-10, IL-1β, GM-CSF, TLR9, and TNFα mRNA expression was increased in iFRECS at 72 hpi with FHV-1, which was similar to what has been observed following inoculation of FRECs. Our results highlight the potential of using iFRECs for studying FHV-1-host interactions while reducing the number of animals needed for future studies.

GENETIC FINGERPRINTS OF HOST SPECIALIZATION IN THE *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

Evan P. Brenner, Srinand Sreevatsan; Pathobiology & Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

The *Mycobacterium tuberculosis* complex (MTBC) contains a closely related set of notorious pathogens that plague a staggering host range of animals and humans worldwide. Distinguishing these infections from each other, as well as from mycobacteria outside the complex, can be a challenge with lifeor-death consequences. With commonplace whole-genome sequencing and advances in computational power, it is now feasible to analyze data from thousands of genomes, and extract single nucleotide polymorphisms (SNPs) that reliably separate lineages, as demonstrated with Coll *et al.*'s tuberculosis SNP barcode. With the conservation of genes both in and outside the MTBC, we can also use this increased resolution to search for SNPs linked to host range instead. We will collect Illuminasequenced FASTQ reads from *Mycobacterium tuberculosis* sensu stricto and *M. tuberculosis* variant bovis cases in humans and animals around the world, process them through the snippy SNP-calling pipeline, and identify SNPs that correlate with variant, as well as those that instead correlate with host species. Finally, a larger dataset including non-MTBC mycobacteria will be queried to determine specificity of differentiating SNPs, and to assess if host specialization markers extend to other mycobacteria like *M. kansasii* as well. A library of differentiating SNPs will be useful for diagnostic purposes, but may also yield insights into fine specialization of mycobacterial pathogenesis.

EVALUTION OF VETERINARY MEDICAL STUDENT RETENTION OF ANATOMY AND PHYSIOLOGY CONCEPTS WITH VARIOUS EXPERIENTIAL LEARNING METHODS

Ilissa Chasnick[1], Nyssa Levy[1,2], Michael Everett[3], Henry (Rique) Campa III[4], 1) College of Veterinary Medicine, 2) Small Animal Clinical Sciences, MSU, 3) Community Sustainability, MSU, 4) Fisheries and Wildlife, MSU

Many veterinary medical colleges have undergone curricular changes that have moved away from traditional lecture-based teaching in favor of evidence-based, experiential methods of instruction. Such a curricular reinvention occurred in 2018 at Michigan State University's College of Veterinary Medicine, with individual courses using numerous instructional and learning methods. In the present study, three courses were assessed, two of which used a method of experiential learning, and the other which utilized a traditional lecture approach. The purpose of this study was to determine if the method of instruction impacted exam grades, retention of content, and student perspective. Methods of teaching and learning were quantified for each course using the Classroom Observation Protocol for Undergraduate STEM. Following completion of each course, participants (n=27) re-took the same final examination and participated in a survey to evaluate their perspective five weeks later. Mean scores on the initial examinations in the experiential learning courses were significantly higher than the mean score of the traditional lecture course ($p=0.01$). However, mean retake examination scores were similar for all courses ($p=0.76$). Students reported feeling most comfortable with courses that used a discussion-based method of instruction, and the least comfortable in a course that was primarily lecture-based. Although true retention is difficult to assess in veterinary medicine due to many concurrent factors, evaluation of student perspective suggests that a combination of experiential instruction methods and lecture allow them to best understand material, and this approach should be considered for current and ongoing veterinary curricular changes.

ENDOTOXIN ACTIVATES LIPOLYSIS IN BOVINE ADIPOCYTES

Miguel Chirivi*, G. Andres Contreras; Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA.

Adipose tissue (AT) inflammation and intense lipolysis predispose periparturient cows to metabolic disorders. Stimulation of AT with endotoxin (ET) triggers lipolysis and insulin resistance. However, it is unknown if lipolysis is directly activated in adipocytes or if it is mediated by immune cells. We hypothesized that LPS directly triggers lipolysis and insulin resistance in bovine adipocytes. Subcutaneous AT explants were collected from 6 non-lactating non-gestating multiparous Holstein dairy cows to harvest stem cells-derived preadipocytes by the enzyme-free method. After expansion, preadipocytes were differentiated in standard adipogenic media for 7 d. Mature adipocytes were stimulated for 3 h with Lipopolysaccharide (LPS; O55:B5; 0.001, 0.01, 0.1, 1, and 10 µg/mL) or vehicle (BAS) to evaluate its lipolytic effects. The beta-adrenergic agonist isoproterenol (ISO, 1µM) was used as positive control. Concurrently, the anti-lipolytic response of insulin (IN=1µL/L) was evaluated in the presence of the endotoxin (LPS+INS). Lipolysis was determined by quantification of glycerol release. Compared to BAS, ISO increased glycerol release by 54.1±15% after 3 h (P<0.01). LPS increased glycerol release in a quadratic response (P<0.01). LPS at 1 µg/mL had the highest increase (46.1±15%) in glycerol released compared to BAS. IN inhibited LPS-induced glycerol release (LPS+IN= 3.4±15% compared to BAS). Collectively our results suggest that LPS induces lipolysis in adipocytes. Exposing adipocytes to LPS for 3h did not affect the antilipolytic effect of insulin. Future studies will determine if chronic exposure to LPS (>7 h) will alter insulin sensitivity in adipocytes. These results suggest that endotoxemia may enhance dairy cows' susceptibility to lipolysis dysregulation and metabolic diseases associated with dyslipidemia.

RELATIONSHIP BETWEEN PARITY AND REPRODUCTIVE DISEASE IN MANAGED AFRICAN PAINTED DOGS

Megan Crawford[1], Anneke Moresco[2], Monica McDonald[3], Dalen Agnew[1,2] ,1) Michigan State University, 2) Reproductive Health Surveillance Program, 3) Reproductive Management Center

The managed female African Painted Dog (APD) population has been found to have high prevalence of reproductive disease, including cystic endometrial hyperplasia (CEH), adenomyosis, and pyometra. These diseases can result in infertility and even death. It is important to have females that can reproduce in order to maintain genetic diversity and sustain the managed APD population. The purpose of this study was to identify potential risk factors (e.g., parity, age, contraceptive use), associated with different reproductive diseases in order to provide zoos with management recommendations for female APDs. After a female APD died or was ovariohysterectomized, tissues were fixed in 10% formalin and shipped to the Reproductive Health Surveillance Program (RHSP). Tissues were photographed, dissected, embedded, sectioned, and stained with hematoxylin & eosin. Microscopic evaluation identified reproductive lesions. Spearman's correlation coefficients were produced using GraphPad Prism®. Parity was not significantly correlated with cystic endometrial hyperplasia, adenomyosis, or pyometra. Age was positively correlated with presence of CEH (r=0.539, p<0.001), and adenomyosis (r=0.631, p<0.001). Time since last parturition was positively correlated with presence of CEH (r=0.696, p=0.002), adenomyosis (r=0.513, p=0.03), and endometritis (r=0.511, p=0.03). Preliminary data suggests that cystic endometrial hyperplasia and adenomyosis are, at least in part, degenerative changes. Data suggests that parturition could decrease the prevalence of CEH, adenomyosis, and endometritis. Breeding female APD at a younger age and breeding them frequently may allow them to reproduce before CEH and adenomyosis develop.

LIPOLYSIS INHIBITION IMPROVES CLINICAL OUTCOMES IN THE TREATMENT OF KETOSIS IN DAIRY COWS

Daniela Cortes, Miguel Chirivi, Annette O'Connor G, Andres Contreras; Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA.

Lipolysis dysregulation in adipose tissues (AT) is a major risk factor for lactating dairy cows' clinical ketosis (CK). Accepted treatment is oral propylene glycol (PG) which stimulates gluconeogenesis and provides glucose. However, PG does not reduce lipolysis. We hypothesized that inhibition of lipolysis with Niacin (NIA) and Flunixin Meglumine (FM) improves CK recovery. Multiparous Jersey cows (n=15, 7.2±2.8 DIM, and median parity 2.9±0.9) were selected from a commercial dairy. Inclusion parameters were CK symptoms and hyperketonemia (BHBA ≥1.2 mmol/L). Cows were randomly assigned to 1 of 3 treatments; T1) PG: 310g/PO/day for 5 d, T2) PG+NIA: 24g/PO/day for 3 d, and T3) PG+NIA+FM: 1.1 mg/Kg/IV/day for 3 d. Blood samples were collected before (D0) and after treatment [3, 7, and 14 days (D)]. Plasma BHBA, NEFA, glucose (GLU), and magnesium (Mg) were evaluated. At D0, all cows showed high BHBA (2.09±0.19 mmol/L) and NEFA (0.98±0.10 mmol/L). Compared to D0, all treatments reduced BHBA by -35.8%, -33.1%, and -23.2±9.4% at D3, D7, and D14, respectively. However, BHBA in T1 relapsed to 2.86±0.33 mmol/L by D14. Compared to D0, NEFA decreased -19.6%, -5.5% and +5.6±13.5% at D3, D7, and D14 respectively. Only T3 presented normal NEFA at D14 (0.65 mmol/L). In all cows, GLU increased +26.1%, +17.2%, and +16.4±5.8% at D3, D7, and D14, compared to D0. However, only T3 showed an increment in GLU (+25.7±6%). Mg levels were not affected by T1, while T2 and T3 increased 16.4% and 15.79±6.6%, respectively. These data suggest that including NIA and FM improves plasma biomarkers of CK. Drastic reduction of BHBA and NEFA suggests inhibition of lipolysis. Increment of GLU and Mg may indicate a recovered energy balance. Future studies will evaluate the effect of lipolysis inhibitors on AT's responses.

MAST CELL-SPECIFIC INACTIVTION OF *FOSB* EXACERBATES RELEASE OF PRO-INFLAMMATORY MEDIATORS IN MODELS OF SYSTEMIC ANAPHYLAXIS AND LIPOPOLYSACCHARIDE-INDUCED SEPSIS

Duque-Wilckens, N. Maradiaga, N., Szu-ying, Y., Nestler, E., Robison, A.J., Moeser, A.J.

Mast cells (MCs) are innate immune cells that can exert a diverse array of physiologic and pathologic functions thanks to their capacity to respond to an impressive variety of stimuli and selectively release bioactive molecules ranging from pro and anti-inflammatory cytokines to growth factors and neurotransmitters. This vast heterogeneity of functions implies that the transcriptional profile of MCs needs to be extensively regulated, but the underlying mechanisms are not understood. The MCs expression of FosB and ΔFosB, two transcription factors formed by alternative splicing of the *Fosb* gene, is increased in response to a variety of stimuli, including IGE-antigen activation and stress, but the specific role of FosB and ΔFosB-on MCS transcriptional regulation remains unexplored. Here we crossed the Mcpt5-Cre mouse line with the Cre-dependent *floxed FosB* mouse strain to assess the functional effects of mast cell-specific deletion of fosB expression. First, *in vitro* studies showed that bone marrow derived mast cells without functional *Fosb* show intact internal structure, but elevated IGE-antigen and lipopolysaccharide (LPS)-mediated release of proinflammatory mediators. Next, using a passive systemic anaphylaxis model and LPS injections *in vivo*, we found that animals lacking *Fosb* in their MCs display a more dramatic drop in rectal temperature, worse clinical outcomes, and elevated serum inflammatory mediators. Together, these results suggest that Fosb products exert an inhibitory effect on mast cell mediator storage and release, without affecting morphology. Ongoing studies are using CUT&RUN-sequencing to identify the down-stream targets of MCs FosB and ΔFosB.

EARLY WEANING STRESS SHAPES LONG-TERM IMMUNE RESPONSES IN A SEX-SPECIFIC MANNER IN PIGS

Mahsa Fardisi, Kyan Thelen, Andrew Roney, Lauren Phillip, Carmen Garcia, Gloria Yarandi, Adam Moeser, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Two major factors known to influence immune function in animals and people are early life stress and biological sex. How these two factors interact to shape long-term immune development is poorly understood. Here we conducted experiments in pigs to address the hypothesize that immune system development and responses to stressors later in life are shaped by both independent and interactive effects of early weaning and biological sex. Ten-week- old female (F), intact-male (IM) and castrated-male (CM) pigs that were previously early-weaned (EW) and later-weaned (LW) (at 15 or 28 d of age, respectively), were injected with either saline vehicle, or lipopolysaccharide (LPS) to induce a systemic inflammatory response. Blood samples were obtained at 0, 2, and 4h post LPS-challenge. Plasma cortisol and neutrophil (N) to lymphocyte (L) ratios (NLR) were assayed to assess the systemic inflammatory and HPA axis response to LPS. Early weaning affected later life LPS-induced elevations in cortisol levels only in EW-IM which exhibited greater ($P<0.05$) levels compared with LW-IM pigs. Blood NLR were greatest in EW-F pigs compared with other groups due to increased N numbers. EW-CM exhibited higher NLR compared with EW-IM pigs. In contrast to EW-F, increased NLR in CM were due to decreased L numbers which indicates that both EW and male gonadal status impact LPS-induced alterations in immune cell populations. In conclusion, these studies show that EW, biological sex, and male gonadal status have significant impacts on later life LPS-induced immune responses. Understanding how immune development is altered by early life stress and sex could reveal n w targets and strategies for optimizing lifetime immune function.

IMMUNOPHENOTYPING OF IRIDOCILIARY EPITHELIAL NEOPLASMS IN 21 DOGS

Allison Gerras[1], Sarah Coe[2], Matti Kiupel[1], and Dodd Sledge[3] 1) Pathobiology and Diagnostic Investigation, College of Veterinary Medicine 2) Charles River Laboratories, Mattawn, MI. 3) Michigan State University, Veterinary Diagnostic Laboratory

Neoplasms originating from iridociliary epithelium can be difficult to differentiate from other primary or metastatic intraocular neoplasms, especially if anaplastic. The paucity of studies evaluating expression of immunohistochemical markers in normal iridociliary epithelium and iridociliary neoplasms limits diagnostic use of immunophenotyping. The goal of this study was to evaluate the immunophenotype of iridociliary adenomas and adenocarcinomas using a panel of antibodies. A tissue microarray was created using five 0.6mm punches from each of 12 iridociliary adenomas, 9 adenocarcinomas, and 3 ciliary bodies from normal canine globes. Serial 5µm sections from the resulting microarray were immunohistochemically labeled for N- cadherin, P-cadherin, E-cadherin, vimentin, pancytokeratin [AE1/AE3], cytokeratin 7, cytokeratin 20, neuron specific enolase [NSE], S100, desmin, synaptophysin, glial fibrillary acidic protein [GFAP], vimentin, and Melan-A, and treated with the Periodic acid-Schiff reaction. 21/21 iridociliary neoplasms labeled with vimentin and had variably prominent PAS positive basement membranes. Within many neoplasms, there was heterogeneity of labeling for N- and E-cadherin, but 12/19 iridociliary neoplasms labeled predominately for E-cadherin and 8/20 for N-cadherin. 3/3 control ciliary bodies labeled for vimentin and N-cadherin while 0/3 labeled for E-cadherin. These findings suggest E-cadherin may help differentiate iridociliary neoplasms from other intraocular neoplasms. Additionally, these findings prompt investigation into normal protein expression of iridociliary epithelium.

HEMATOLOGY REFERENCE INTERVALS FOR ZOO-HOUSED NEONATAL GIRAFFE CALVES (*GIRAFFA CAMELOPARDALIS*)

Hunter Ferchaw[1], Jennifer Thomas[1,2], Ronan Eustace[1], Kimberly Thompson[1], (1) College of Veterinary Medicine, (2) Department of Pathobiology and Diagnostic Investigation, Michigan State University

Species-specific reference intervals are necessary for the proper interpretation of hematological values in zoo-housed species. Reference intervals may vary between neonates and adults. Thus, the aim of this study is to provide age-specific reference intervals for hematology values for neonatal zoo- housed giraffe (*Giraffa camelopardalis*). Complete blood count (CBC) results were obtained by performing a retrospective survey of zoological institutions throughout the United States. Inclusion criteria included: giraffe calves born between January 2016 and May 2021 which were deemed healthy by an attending veterinarian, had blood collected via jugular vein and placed into EDTA tubes, at the time of routine neonatal health exams, performed under manual restraint, when calves were less than 72 hours old. Reference intervals were calculated using the American Society for Veterinary Clinical Pathology's (ASVCP) consensus guidelines for determination of de novo reference intervals. The results of this study will enhance the ability of clinicians to interpret hematology values for neonatal giraffes.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF MESENCHYMAL STEM CELLS IN EXOTIC SPECIES

Nicole Hamlin, Valerie Johnson, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

Mesenchymal stem cells (MSC) have become a more widely used therapeutic agent in human and animal medicine with applications in inflammatory diseases and regenerative medicine. Currently, little work has been done to characterize MSC in exotic species. According to the International Society for Stem Cell Research (ISSCR) guidelines MSC must be plastic adherent with fibroblast like morphology and express certain surface markers with the absence of other surface markers. MSC are further characterized by their ability to undergo trilineage differentiation and suppress lymphocyte proliferation. In this study, we aimed to characterize MSC from Chilean and Lesser flamingos, polar bear, and tiger using the ISSCR criteria. Bone marrow-derived (BM-MSC) Chilean and Lesser flamingo and adipose-derived (Ad-MSC) polar bear and tiger MSC were cultured and expanded *in vitro*, and differentiated into adipocytes, chondrocytes, and osteocytes. Ability to suppress T cell proliferation was investigated by co-culturing lymphocytes isolated from peripheral blood with species-matched MSC. Cells were phenotyped using antibodies from species anticipated to cross-react such as human, mouse, rat, chicken, canine, and feline. Cross-reaction of the MSC negative surface markers was confirmed by positive staining of PBMC from each of these species with each antibody. All BM-MSC and Ad-MSC grown in culture were plastic-adherent and exhibited fibroblast like morphology. Under appropriate culture conditions the ability of these cells to undergo trilineage differentiation was confirmed utilizing appropriate stains. Characterization of surface markers and lymphocyte suppression assays were confirmed via flow cytometry.

IDENTIFICATION OF NOVEL MIRNA ISOLATED FROM SERUM-DERIVED EXOSOMES AS A DIAGNOSTIC INDICATOR OF BOVINE TB INFECTION.

Dr. Srinand Sreevatsan, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University. Hannah Hipkiss, Department of Pathiobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University.

Current diagnostic methods for the detection of *Mycobacterium tuberculosis* biovar *bovis*, the infectious zoonotic agent of bovine tuberculosis (bTB), are reviewed continuously to improve bTB surveillance and eradication programs. In the US, surveillance programs and milk pasteurization has greatly reduced the zoonotic risk of bTB infection in humans, however current methods of detection are time-insensitive, expensive, or are not capable of detecting latently infected cattle. Agricultural economic losses are primarily associated with eradication programming, but many costs such as herd depletion, market exclusion, or reputability of the producer are rarely quantified. Actiphage®, a phage-based assay created by Swift et al., has identified *M. bovis* in circulating peripheral blood monocytes of single comparative cervical intradermal tuberculin (SICCT)-positive cows with no evidence of granulomatous lesions. This challenges the current dogma that *M. bovis* bacteremia in cattle is rare. Given this insight, identification of circulating pathogen-derived biomarkers would be an attractive option given that it is minimally invasive, highly specific, and diagnostic of latent infection. miRNAs are 18-22nt non-coding RNAs that modify gene expression and transcription. During infection, miRNA can be packaged into exosomes to invade host cells and modify the host transcriptome, or serve a self-regulatory function and modify their own mRNA to optimize growth conditions in limiting environments. This study aimed to identify exosomal miRNA biomarkers specific to *M. bovis* based upon validated miRNA profiles in *Mycobacterium tuberculosis* biovar *tuberculosis.*

COMPARATIVE ANALYSIS OF RT-QUIC, ELISA, AND IHC FOR CHRONIC WASTING DISEASE DIAGNOSTICS

Carine Holz[1], Joseph Darish[1], Kelly Straka[2], Steven Bolin[1], Srinand Sreevatsan[1], 1) College of Veterinary Medicine, MSU 2) Michigan Department of Natural Resources

Chronic wasting disease (CWD) is a prion disease of cervids. The diagnostic for CWD involves screening brain or retropharyngeal lymph nodes (RLNs) with an ELISA followed by confirmation by immunohistochemistry (IHC). Despite their reliability, such methods have limited sensitivity in comparison to amplification methods, such as real-time quaking-induced conversion (RT-QuIC) assay. The RT-QuIC can detect minute amounts of prions in tissues and secretions of CWD-infected animals. In this study, ELISA, IHC and RT-QuIC were evaluated on RLNs (n= 1,257) from Michigan white-tailed deer collected during CWD surveillance from 2017 to 2021. Samples were screened for evidence of PrPCWD by ELISA and all ELISA- positive RLNs (n= 173) were also analyzed by IHC. An RT-QuIC assay was performed on all 1,257 RLNs and results were compared against those obtained by conventional ELISA and IHC. ELISA detected 173 RLNs positive for PrPCWD of 1,257 samples tested. From the 173 ELISA positive RLNs, 165 were also IHC positive for CWD. The RT-QuIC detected PrPCWD in 166 RLNs (of the 1,257 samples tested). There were eight discordant results when comparing RT-QuIC with ELISA and IHC. Of the eight discordant results, seven were RT-QuIC negative and ELISA positive (5 of the 7 with matched RT-QuIC and IHC results) and one was RT-QuIC positive (also ELISA positive) and IHC negative. The percentage of agreement and *kappa* value between RT-QuIC and ELISA were 99.4 % and 0.98, respectively and between RT-QuIC and IHC 99.7 % and 0.99, respectively. Taken together, RT-QuIC is comparable to ELISA and IHC for CWD detection. Further work is needed to assess RT-QuIC for the early-stage CWD diagnosis and CWD strain differentiation.

CHARACTERIZATION OF $CD3^{+}/CD20^{+}$ LARGE CELL CANINE LYMPHOMA

Cory M. Howard, Steffanie Anderson, and Bonnie Harrington Department of Pathobiology and Diagnostic Investigation, Michigan State University College of Veterinary Medicine

Immunophenotyping of canine non-Hodgkin's lymphoma for B- cell and T-cell surface antigens is commonly performed to better elucidate clinical outcome. Expression of CD3 is associated with T-cell malignancies while CD20 is expressed in B-cells. However, a small subset of canine non-Hodgkin lymphomas express both CD3 and CD20 (CD3+/CD20+). Currently, this form of non- Hodgkin lymphoma remains poorly defined at the molecular level. In this retrospective study, we aimed to better characterize the histogenesis of CD3+/CD20+ lymphoma. FFPE tissues from ten cases of CD3+/CD20+ large cell lymphoma and breed- matched controls of peripheral large T-cell lymphoma and diffuse large B-cell lymphoma were selected from the Michigan State University Veterinary Diagnostic Lab. Using PARR, we identified monoclonal T-cell receptor (TCR) rearrangements in all biphenotypic cases. In addition, three out of ten cases also exhibited monoclonal rearrangements in the immunoglobulin heavy chain (IgH), supportive of dual lineage rearrangement. There was no significant difference in frequency of antigen receptor rearrangement between CD3+/CD20+ and CD3+ cases. In comparison with CD20+ lymphomas, CD3+/CD20+ lymphoma exhibited TCR rearrangement more frequently and IgH rearrangement less frequently ($p=0.0007$ and 0.003, respectively). Immunohistochemical staining of the B-cell marker PAX5 was negative in all CD3+/CD20+ cases. Cases of canine CD3+/CD20+ demonstrate similar antigen receptor rearrangements and PAX5 staining properties when compared with CD3+ lymphomas, suggesting a similar histogenesis of these two subsets of lymphoma.

SAFETY AND EFFICACY OF A NOVEL ANTI-DEK APTAMER TREATMENT FOR OSTEOARTHRITIS USING A CANINE MODEL

Jessica Hynes[1], David M. Markovitz[2], Dalen Agnew[1], Hailey Maresca-Fichter[1], Joanna Acosta-Bencosme[1], Jane M. Manfredi[1] 1) Department of Pathobiology and Diagnostic Investigation College of Veterinary Medicine, Michigan State University, 2) Department of Internal Medicine University of Michigan

Osteoarthritis (OA) is the most common orthopedic disorder in many species, and current treatments are limited to pain management alone. Given the newly recognized inflammatory nature of OA, the objective of this study was to look at the efficacy of intra-articular treatment with a novel anti-inflammatory, anti-DEK aptamer, in a canine OA model. Sixteen beagles underwent surgical transection of the cranial cruciate ligament of a randomly chosen leg and were given an injection with either the anti-DEK aptamer or sterile saline; directly post-op and two weeks post-op. Serial data collection included: objective gait and stance analyses. Statistics included repeated measure ANOVAS and mixed effects analyses ($p<0.05$). Gait analysis showed, stance time ($p<0.03$) and maximum peak pressure ($p<0.009$) for the control (C) group were decreased over time, as was the maximum force for the treatment (T) group ($p<0.001$) directly after the second injection, and control group after both the first and second injection ($p<0.002$). Stance analysis revealed the T group had four instances of significantly decreased weight bearing ($p<0.05$) and the C group had eleven ($p<0.04$). Control treated dogs demonstrated more instances of significant decline in leg use and increased lameness post-operatively. These data support the finding that the anti-DEK aptamer had some efficacy in decreasing the inflammation and pain associated with surgically induced OA.

LOW-DOSE RADIATION THERAPY AS A MODALITY FOR THE MANAGEMENT OF FELINE CHRONIC RHINITIS

Elizabeth Jackson, Lauren Kustasz, Leanne Magestro, Stephan Carey; Michigan State University College of Veterinary Medicine

Chronic idiopathic rhinitis is a significant cause of morbidity in cats and is one of the most common nasal disorders encountered in the domestic feline population. Treatment strategies for this disease are limited to antimicrobial and anti-inflammatory therapies, which are met with variable success and carry the potential risks of chronic use of such therapies in cats. Furthermore, not all cats respond favorably to this approach, and many cats become resistant over time, leading to treatment failure. The purpose of this pilot study is to assess the safety and therapeutic efficacy of a low-dose radiation therapy (RT) protocol in cats with chronic rhinitis. Our overall hypothesis was that low dose RT is a safe and effective modality for the management of feline chronic rhinitis. Cats included in the study underwent three RT sessions under general anesthesia (2 Gy fractions once daily for three consecutive days). Clinical efficacy was evaluated via owner-reported assessment of symptoms using a severity of nasal inflammatory disease (SNIFLD) questionnaire modified for use in feline chronic rhinitis. Adverse effects were documented via daily examinations following each radiation treatment, owner follow-up, and recheck examinations at 1-, 3-, 6- and 12-month post radiation therapy. Three cats have been enrolled in the study to date. All three cats have successfully completed the RT protocol. None of the three cats exhibited acute adverse effects associated with treatment. Of the three of cats treated, two cats have shown improvement of owner-reported quality of life scores and clinical signs at 22- and 35-days post-treatment. The preliminary information gained from this study provides support for the development of a promising treatment modality for cats with refractory feline chronic rhinitis and will have a significant impact on the quality of life of cats with this condition.

THE INVASION OF THE BLACKLEGGED TICK (*IXODES SCAPULARIS*) AND THE AGENT OF LYME DISEASE, *BORRELIA BURGDORFERI* ACROSS MICHIGAN FROM 2017 – 2019

Vishvapali Kobbekaduwa[1*], William Miller[2] and Jean Tsao[3] • [1]Comparative Medicine and Integrative Biology, College of Veterinary Medicine, Michigan State University • [2] Department of Biology, Calvin College, Grand Rapids, MI • [3]Department of Fisheries and Wildlife, Department of Large Animal Clinical Sciences • Michigan State University, East Lansing, Michigan, USA • *kobbekad@msu.edu

Ixodes scapularis, is the primary vector for several pathogens including *Borrelia burgdorferi*. *I. scapularis* was first discovered in Menominee County in Michigan's Upper Peninsula in the 1980's, and later discovered in the southwestern region of the Lower Peninsula in the early 2000s. By 2010 it had spread more than 300 km northward along the coast of Lake Michigan; invasion eastwards occurred more slowly and mainly in southern Michigan. This study focused on monitoring the spread of *I. scapularis* and *B. burgdorferi* in Michigan. Drag sampling for questing ticks throughout Michigan was carried out from May to November 2017 – 2019 to better characterize the distribution of *I. scapularis* as well as that of B. burgdorferi. *Ixodes scapularis* was detected in 67 out of the 83 counties (80.7%) and 55 (66.2%) counties had established populations according to the CDC criteria. The density of nymphs ranged from 0.1 - 49 nymphs/1000 m2. *B. burgdorferi* infected ticks were found in 36 (81.8%) and 1 (7.7%) of counties with reported and established ticks populations respectively. The density of *B. burgdorferi* infected nymphs ranged between 0.08 - 8.4 nymphs/1000 m2. In the Upper Peninsula, ticks were rarely detected in the eastern region. In the Lower Peninsula, ticks had become established in several areas of eastern Michigan, including along the coast of Lake Huron. Interestingly, ticks were not detected in the central northern region of the Lower Peninsula. Modeling the invasion of ticks is important to predict where future ticks populations and associated pathogens would be.

PLATELET INHIBITION BY METABOLITES OF CLOPIDOGREL

Brooke Kahn[1], Dawn S. Kuszynski[2], Barbara D. Christian[2], D. Adam Lauver. 1. College of Veterinary Medicine, Michigan State University + Department of Pharmacology and Toxicology, College of Veterinary Medicine, Michigan State University

Clopidogrel is a prodrug used in veterinary and human health to prevent thrombosis. Clopidogrel is metabolized into the active metabolite (M4) by hepatic CYP450 enzymes. The M4 metabolite of clopidogrel prevents platelet activation by inhibiting ADP- induced P2Y12 receptor activation. However, there are 15+ additional metabolites that are derived from clopidogrel which have been assumed to be biologically inert. Our laboratory has previously developed a conjugate of the active metabolite, M4. Preliminary studies have demonstrated a reduction in bleeding time with the conjugate compared to clopidogrel when administered to animals. This suggests that at least one of the other clopidogrel metabolites disrupts hemostasis. Our objective was to determine whether the additional metabolites inhibit platelet activation. This may explain the difference in bleeding time.

To characterize the effects of these metabolites on platelet function, ex vivo platelet aggregation was performed on platelet- rich plasma that was incubated with the prodrug clopidogrel, the M4 conjugate, and two additional metabolites (M1 and M2). Platelet aggregation was not inhibited by the M1 and M2 metabolite or the prodrug clopidogrel. The M4 conjugate also did not significantly inhibit platelet aggregation however, this may have resulted from insufficient glutathione in the plasma which is required for the liberation of M4. The results suggest that platelet inhibition by other metabolites is not the mechanism by which clopidogrel increases bleeding. Future studies are currently underway to assess the effects of these metabolites on other aspects of the hemostatic system, specifically the vasculature.

CLOPIDOGREL TREATMENT INHIBITS P2Y2-MEDIATED CONSTRICTION IN THE RABBIT MIDDLE CEREBRAL ARTERY

Dawn S. Kuszynski[*+], Barbara D. Christian*, Anne M. Dorrance[#] and D. Adam Lauver*, *Dept of Pharm/Tox, CVM, MSU; +IIT, MSU; #Dept of Pharm/Tox, COM, MSU

Clopidogrel is an effective purinergic 2Y 12 receptor (P2Y12) antagonist used to prevent arterial thrombosis, but its use is associated with adverse bleeding. Clinical studies demonstrate that clopidogrel users have an increased risk of cerebral microbleeds and intracerebral hemorrhage. Our previous studies suggest that non-platelet mechanisms mediate these adverse bleeding events; we hypothesize that clopidogrel or one of its metabolites interacts with blood vessels directly to cause bleeding. Rabbits were treated orally with vehicle or clopidogrel (3 or 10mg/kg) for three days. On the fourth day rabbits were anesthetized for blood collection and then euthanized. The brain was collected, and the middle cerebral arteries were isolated. We used light transmission aggregometry and pressure myography to elucidate the mechanisms of the off-target effects associated with clopidogrel treatment. Inhibition of P2Y12 activation by clopidogrel inhibited ADP-induced platelet aggregation but had no impact on P2Y12-independent arachidonic acid- or collagen- induced platelet aggregation. Analysis of middle cerebral arteries (MCAs) from clopidogrel treated rabbits showed that clopidogrel did not affect P2Y4, P2Y6, and P2 Y14 receptor-mediated contraction but attenuated the contractile response after P2Y2 receptor activation. Further analysis determined P2Y2-mediated constriction was endothelium-dependent. Impaired vasoconstriction can prolong bleeding. These results suggest clopidogrel inhibits the endothelial P2Y2 receptor in the MCA, which provides a mechanistic explanation for the adverse cerebral bleeding associated with the drug.

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AND ANIMALS: A REVIEW

Antonia Langfeldt[1], Heather D. Brake[2], Melinda J. Wilkins[1] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University[1]. National Center for Environmental Health and Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention[2].

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals associated with adverse health effects in animals and humans. They are used globally in various manufacturing and industrial processes and are known environmental contaminants. Though nearly every human and animal around the world have been exposed, the impact of PFAS on health is not well understood. Many studies have focused on the public health consequences of PFAS exposures, however, there is a dearth of knowledge about these chemicals' effects on non-laboratory animals. The purpose of this review is to summarize current literature regarding PFAS exposures, bioaccumulation, toxicology, and health effects in livestock, wildlife, and pets. Most of what is known about health effects and toxicological processes of PFAS in animals stems from laboratory animal studies. Such experiments show that PFAS most commonly affect the liver, but other body systems including immune, endocrine, and reproductive systems can also be affected. Animals outside of the laboratory are most likely exposed through environmental contamination such as contaminated water, dust, or foods. The ability of PFAS to bioaccumulate is concerning, as they can build up in soils and plants that animals consume, and many apex predators have been noted to have remarkably elevated levels of PFAS in their blood. Future studies should focus on routes of exposure, health effects, and concentrations of PFAS in animals. As the public grows more aware about PFAS, it will be important for veterinarians to be aware of these contaminants and their implication for veterinary patients.

DYNAMIC INSULIN RESPONSE TO ACCLIMATION OF A HIGH CARBOHYDRATE DIET IN HORSES

Hailey Maresca-Fichter[1], Stephanie Valberg[2], Deborah Velez-Irizarry[2], Callum George Donnelly[3], Carrie Finno[3], Jane Manfredi[1], 1) Department of Pathobiology and Diagnostic Investigation, 2) Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University; 3) Center for Equine Health, University of California, Davis

Determining the relationship between acclimation to high carbohydrate diets and insulin dynamics in horses is clinically relevant due to the prevalence of metabolic disorders and frequency of dietary supplementation with carbohydrate rich grains. While it is recommended to generally avoid high starch diets, some previous work has shown that acclimating to carbohydrate rich diets improves insulin sensitivity. The aim of this study was to determine if there is an association between acclimation to high dietary carbohydrate load and improved insulin dynamics in normal and insulin dysregulated (ID) horses. Seventeen adult Quarter Horse mares were determined to be ID (n=9) or metabolically normal (n=8) prior to starting the study and were then fed a hay and sweet feed grain diet (8 g/kg BW of 25% NSC concentrate) for 4 weeks. A combined glucose insulin test (CGIT) (150 mg/kg glucose IV immediately followed by 0.1 IU/kg insulin IV) was performed followed by a high dose (0.45 ml/kg BW Karo Light Syrup) OST after a 4 day washout period to evaluate tissue level insulin sensitivity and incretin hormone responses. Data was analyzed using McNemar's Chi-squared test (significance of $P<0.05$). After dietary acclimation, 5 (29.4%) horses remained metabolically normal on both tests while 12 (70.5%) were ID on one or more. These findings show no insulin sensitizing benefit to horses already afflicted with ID and do not support acclimation to high carbohydrate diets as doing so can potentially induce ID in previously metabolically normal horses.

HUMAN MICROBIOTA DECREASED LUNG FUNCTION IN A MOUSE ASTHMA MODEL

Ivon Moya Uribe[1,2], Sanket Jantre[4], Susan Ewart[3], Julia Bell[1], Linda Mansfield[1,2,3] 1) Comparative Enteric Diseases Laboratory, 2)Comparative Medicine and Integrative Biology, 3)College of Veterinary Medicine, 4)Department of Statistics, Michigan State University

Recent literature suggests that gut microbiota impacts lung function and airway hyperresponsiveness (AHR) during allergic airway disease(AAD). To test this hypothesis, we transplanted germ free mice with microbiota from eczemic infants(risk), microbiota from non-eczemic infants(protective), or adult human microbiota. Transplanted microbiotas remained stable over two generations. Mice then were sensitized with house dust mite(HDM) to induce AAD. Data for central and smaller airway resistance, compliance, elastance and tissue stiffness were obtained using the forced oscillation technique at baseline and in response to a cholinergic agonist (Mch). Data were analyzed using a general linear mixed model to examine the effects of Mch concentration, microbiota group, and treatment on AHR. Mice carrying all of the human microbiotas had altered lung mechanics at baseline compared to mice carrying conventional mouse microbiota, including increased lung stiffness and airway resistance. Mice with all human-derived microbiotas showed a tendency to increased AHR compared to conventional mice; the group with protective microbiota showed the highest AHR and had a significant increase compared to conventional mice. Differences in AHR observed between HDM-treated mice with human microbiotas versus conventional microbiota did not correlate with higher levels of mucus or inflammation observed surrounding the airways, nor with higher numbers of specific inflammatory cells found in the bronchoalveolar lavage. Our study shows that microbiota has a major effect on respiratory mechanics and that early life microbiota composition directly affects lung function.

ADIPOSE TISSUE ADIPOGENESIS AND LIPOGENESIS ARE MODULATED BY CANNABINOIDS IN DAIRY COWS

Madison N. Myers[1], Miguel Chirivi[1], Ursula Abou-Rjeileh[1], Maya Zachut[2], Joseph Tam[3], G. Andres Contreras[1] 1) Dept. of Large Animal Clinical Sci., College of Vet. Med., Michigan State U. 2) Dept. of Ruminant Sci., Volcani Center 3) Obesity and Metabolism Lab., Hebrew U. of Jerusalem.

Increased adipose tissue (AT) lipolysis and suppressed adipogenesis and lipogenesis characterize the periparturient period of dairy cows. The intensity of these processes recedes with the progression of lactation; however, disease risk is exacerbated when dysregulation occurs. Cannabinoids (CBs) activate CB receptor 1 (CB1) in monogastric AT and enhance the energy-conserving capacity of adipocytes, yet their effects in dairy cow AT remain unknown. We determined the effects of the synthetic CB arachidonyl-2'-chloroethylamide (ACEA)±the CB1 antagonist rimonabant (RIM) in cultured dairy cow adipocytes. AT was collected from multiparous, nonlactating, non-gestating Holstein cows (n=8). Pre-adipocytes were isolated and induced to differentiate upon confluency for 6 (adipogenesis) or 14d (lipogenesis). Adipocytes were exposed to ACEA at 10μM (A10)±1h RIM pre-treatment (0.1μM). Viability, adipogenesis, and lipogenesis were evaluated using commercially available plate- based assays and the IncuCyte S3 system. Adipogenic efficiency was calculated as the number of cells with 1+ lipid droplet/total # of cells/well. Statistical analyses were performed in JMP. The viability of cells was unaffected by treatments. Adipogenesis was enhanced ($P<0.05$) by A10 (54.5±4.3%) vs. CON (44.23±4.3%). RIM reduced adipogenesis in ACEA-treated cells (46.25±4.1%) vs. CON (53.59±4.1%). Increased lipid accumulation (RFU/ng DNA±SEM) was observed in A10 (3115.43±278) vs. CON (1849.45). Lipid content was reduced in A10+RIM (2681.19), but not RIM (1840.40). Collectively, these findings suggest that adipogenesis and lipogenesis may be enhanced through activation of CB1 by CBs in dairy cow AT.

ATP7A, ATP7B, AND RETN GENOTYPES IN LABRADORS WITH AND WITHOUT COPPER ASSOCIATED HEPATITIS

Brendan Nagler[1],Ya-Ting Yang[1,3], Rebecca Smedley[2], Daniel Langlois[1], and Vilma Yuzbasiyan-Gurkan[1,3], 1) College of Veterinary Medicine, 2) Veterinary Diagnostic Laboratory, 3) Microbiology and Molecular Genetics, Michigan State University

Copper associated hepatitis (CAH) is the most common cause of chronic liver disease in dogs. A genetic mutation leading to CAH has been identified in the Bedlington terrier, but not in other predisposed breeds. Coding variants in the ATP7A and ATP7B genes have been associated with decreased or increased risk of CAH, respectively, in Dutch Labrador retrievers. Genetic testing for these variants is available, but the prevalence and significance of these variants in American Labrador retrievers is unknown. Recently, a variant in the RETN gene has also been associated with lower hepatic copper in Dutch Labrador retrievers. The aim of this study was to evaluate ATP7A, ATP7B, and RETN genotypes in American Labrador retrievers and identify potential associations with CAH. Formalin fixed paraffin embedded (FFPE) liver samples from Labrador retrievers (n = 91) collected between 2013-2021 were evaluated. Cases were classified into CAH, control, and intermediate populations based on histopathologic features and degree of copper accumulation. DNA extracted from FFPE tissues was genotyped for the reported variants. ATP7A and RETN variant allele frequencies were not different among populations. ATP7B variant allele frequency in the CAH population (0.36) was higher than in both control (0.13) and intermediate (0.18) populations ($P < 0.05$). Thirteen of 38 CAH dogs did not possess an ATP7B variant allele whereas 16 of 53 non-CAH dogs did possess an ATP7B variant allele. Our results support a contributory role for the ATP7B variant in the pathogenesis of CAH. However, genetic testing for these variants is of questionable value and could lead to erroneous clinical and breeding decisions given genotypic variability among populations.

THE EFFECTS OF CASTRATION AND EARLY WEANING ON LONG-TERM IMMUNE RESPONSES

Authors: Lauren Phillip[1], Mahsa Fardisi[2], Kyan M. Thelen[2], Adam J. Moeser[2]1) College of Veterinary Medicine 2) Department of Large Animal Clinical Sciences, Michigan State University

A sex-biased mortality exists in swine with significantly higher mortality rates in castrated males, compared with intact males and females. The reason for increased mortality in castrates remains unknown. In this study, we are investigating how castration and early weaning (EW), a common early life production practice, interact to impact their long-term immune system development which may underly increased disease risk. We hypothesize that EW and castration negatively impacts immune system development and response to later life immunological challenges. Male pigs were either castrated (MC) at 8-10d of age or left intact (MI) and then randomly assigned to either an EW group, where pigs were separated from the sow at 15 days, or a late weaning group (LW) where pigs were separated at 28 days. Pigs were vaccinated with a Circovirus vaccine at 49 days of age. Serum was collected for 5 weeks, and PCV2-specific-IgG titers were measured as an index of immune function. At 77 days of age, pigs were injected with Lipopolysaccharide (LPS, 25 µg/kg; intramuscular) or saline to evaluate inflammatory response. Rectal temperatures, clinical scores, and blood samples will be collected at basal (0h), 2h, and 4h after LPS challenge. Based on our preliminary results, we predict that EW and castration will have a deleterious impact on immune system development characterized by suppressed vaccine and LPS-induced immune responses. Findings from this study have the potential to provide insight into the enhanced susceptibility to disease in castrates thus having significant economic implications.

WHOLE GENOME SEQUENCING FOR GENOMIC EPIDEMIOLOGICAL STUDIES OF TUBERCULOSIS IN ASIAN ELEPHANTS OF NEPAL

Authors: Sarad Paudel[1], Evan P. Brenner[1], Syeda A. Hadi[1], Kamal Prasad Gairhe[2], Bhagwan Maharjan[3], Srinand Sreevatsan[1]Affiliations: 1) Pathobiology & Diagnostic Investigation Department, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA,
2) Department of National Parks and Wildlife Conservation, Chitwan National Park, Chitwan, Nepal, 3) German Nepal Tuberculosis Project, Kalimati, Kathmandu, Nepal

Tuberculosis (TB) is a devastating disease in elephants mostly caused by *M. tuberculosis* and sometimes by *M. bovis*. In addition to captive elephants, TB has also been detected in free ranging African and Asian elephants in range countries. *M.tb* complex bacteria were isolated from two captive Asian elephants from Chitwan National Park, Nepal. Both isolates were confirmed as *M. tuberculosis* by I S*6110*-RD4 multiplex PCR, *gyrB* sequencing, spoligotyping and multi-locus variable number of tandem repeat analysis (MLVA). Whole genome sequencing (WGS) was undertaken on the two *M.tb* isolates using Illumina NovaSeq 6000 platform and *de novo* genome assembly was conducted using shovill and annotated using PGAP. WGS analyses revealed the high genomic coverage (359x for isolate S1, 385x for isolate S3) for both isolates. This is first study of *M. tb* genomes from endangered Asian elephants in Nepal. It is expected that whole genome sequencing of TB isolates from Asian elephants will allow us to better understand the genomic epidemiology including outbreak investigation and occurrence of interspecies transmission that will eventually help in the conservation of this important endangered species.

ISOPROSTANES INCREASE RAW 264.7 CELL VIABILITY AND ROS PRODUCTION IN A MODEL OF INFLAMMATION

Ashley Putman, Lorraine Sordillo, and G. Andres Contreras; Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Dysregulated inflammation and oxidative stress contribute to the pathophysiology of several economically important diseases of dairy cattle that negatively impact animal health. A hallmark of these processes is the peroxidation of polyunsaturated fatty acids (PUFA) in cellular membranes. Peroxidation of PUFA leads to the generation of lipid mediators that are key to regulating the onset, progression, and resolution of inflammation. Macrophages are critical to the inflammatory response and are a primary source of the lipid peroxidation product, isoprostanes (IsoP), during inflammation. Although excellent indicators of lipid peroxidation, the role of IsoP remains poorly characterized. We hypothesized that IsoP will alter macrophage ROS production and viability. RAW 264.7 macrophages (n=4) were treated with 5 ng/mL lipopolysaccharide (LPS) or 3 mM 2,2'-azobis(2- amidinopropane) dihydrochloride (AAPH) followed by treatment of 10-500 nM omega-6- or omega-3-derived IsoP (15-F2t-IsoP and 15-F3t-IsoP, respectively). Commercial assays were used to determine cell viability and ROS production. Statistical analysis was performed with a one-way ANOVA followed by Tukey's HSD (α=0.05). Treatment of RAW cells with 500 nM 15-F 2t-IsoP resulted in 1.4-fold higher cell viability compared to LPS-treated cells (P=0.002). Both 15-F2t-IsoP and 15-F3t-IsoP resulted in 1.2-fold increases in ROS production compared to AAPH-treated cells (P=0.006 and P=0.0002, respectively). Our data suggests that IsoP increase cell viability and ROS production of RAW 264.7 macrophages in a dose-dependent manner, which may suggest a novel mechanism in which IsoP contribute to disease pathogenesis. Future studies should be directed towards further elucidating how IsoP impact inflammatory outcomes.

DEVELOPING A RAPID PLASMID DNA EXTRACTION METHOD

Sonia Rafique[1,2], Azam Ali Sher [1,3], Julia Bell [1,4], Lixin Zhang [4], Linda Mansfield [1,4] 1) Comparative Enteric Diseases Laboratory, 2) DVM program, College of Veterinary Medicine, Michigan State University, 3) Comparative Medicine and Integrative Biology Graduate Program, Michigan State University, 4) Department of Microbiology and Molecular Genetics, Michigan State University

Plasmid DNA are small extrachromosomal circular DNA strands, mainly found in bacteria, that can be transferred amongst each other during a process known as conjugation. This exchange of genetic material via plasmid transfer has allowed bacteria to acquire and share antibiotic resistance genes (ARGs), posing severe threats to both humans and animals. The isolation and characterization of plasmid ARGs in bacterial populations has always been challenging. We found that commercial plasmid extraction kits were not successful in extracting larger sized plasmids, and additional literature revealed that these kits were ineffective for complex samples such as soil or cecal samples. In our study, we hope to develop a rapid and cost-effective method for DNA plasmid extraction. This method will facilitate the isolation of plasmids from simple bacterial cultures to complex microbial samples such as sewage, soil, and feces. We plan to use this method to screen human isolates for the presence of conjugative plasmids carrying the Extended-Spectrum Beta-Lactamase (ESBL) enzyme, which is responsible for resistance to many types of antibiotics including penicillin and cephalosporins. We are currently testing both commercial plasmid DNA extraction kits and in-house methods on overnight bacterial cultures to isolate plasmids of known sizes with various resistance genes. The preliminary results show that tested methods have different abilities to extract plasmids from pure cultures of bacteria. The findings from this study will enable us to advance the screening process for multi-drug-resistant bacteria carrying ARGs on conjugative plasmids.

PLASMID-MEDIATED SPREAD OF ANTIBIOTIC RESISTANCE GENES AMONG BACTERIA IN THE HUMAN GUT MICROBIOME

Azam Sher [1,2], Ashley Peer [3], Julia Bell [4], Lixin Zhang [3], Linda Mansfield [3,4], Affiliation: 1) Comparative Medicine and Integrative Biology, 2) Environmental and Integrative Toxicological Sciences, 3) Department of Microbiology and Molecular Genetics, 4) Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University.

Antibiotic resistance (AR) in bacterial pathogens is a serious threat to public health. Conjugation, a plasmid-driven horizontal gene transfer (HGT) mechanism, can transfer genetic material from one cell to another, including antibiotic resistance genes (ARGs). This study investigates the spread of ARGs bearing plasmids in a complex, diverse population of bacteria in the human gut. Commensal donor bacteria mediate the transfer of ARGs to many different phylogenetic groups of bacteria through conjugation. We have made different combinations of donor bacterial strains and plasmids carrying different fluorescent and antibiotic resistance markers. Donor bacteria were orally fed to C57BL/6 mice transplanted with adult human microbiota having an undetectable level of Enterobacteriaceae. Fecal samples were collected every 24 hours and stored at -80°C. Colonization of donor strain was assessed by culturing fecal samples on a selective medium. Donor and transconjugant bacteria were recovered from fecal samples and sorted by FACS. Commensal donor strain colonized the mouse gut throughout the experiment, while the donor strain was not recovered after 48 hours. Flow cytometry-based sorting of the treatment group samples showed an increased spread of plasmid compared to fecal samples before the gavage. 16S sequencing will be performed on sorted cells to characterize transconjugants taxonomically. This work will provide a tractable mouse model to measure the transfer rate and drivers of HGT in a complex gut microbial community.

MECHANOSENSOR PIEZO1 ALTERS ADIPOGENESIS IN PVAT PREADIPOCYTES VIA CALCIUM SIGNALING

Rendon CJ[1], Flood ED[2], Thompson J[2], Chirivi M[1], Watts SW[2], Contreras GA[1]. 1. Dept of Large Animal Clinical Sciences, 2. Dept of Pharmacology and Toxicology. Michigan State University.

During hypertension, vascular remodeling allows the blood vessel to withstand increased blood pressure (BP). This process involves the media and intima layers of the vessel. In the perivascular adipose tissue (PVAT) there is evidence for fibrosis development during hypertension, but PVAT remodeling is poorly understood. In progenitors from non-PVAT depots, mechanical forces affect adipogenesis. The mechanism involves PIEZO1, a mechanosensor that boosts differentiation towards osteogenic and fibroblastic lineages. Our objective was to evaluate PIEZO1's role in the adipogenesis of preadipocytes. We hypothesize that activation of PIEZO1 reduces adipogenesis in PVAT preadipocytes. Aortic PVAT from male SD rats at 10 weeks of age (n=15) was digested (liberase) to harvest preadipocytes. Piezo1 activity was evaluated using a calcium flow marker. *Piezo1* expression was reduced using siRNA. Preadipocytes were differentiated for 4 d in adipogenic media containing Piezo1 agonist Yoda1 (10μM). Mechanical strain (MS) was applied with FlexCell at 12%, half-sine at 1 Hz for 4 d (MS+; MS-). Adipogenesis was assessed using RT-qPCR (adipogenic gene networks) and neutral lipid quantification (Bodipy, Oil Red O). Adipogenesis efficiency is reported as Adipocyte/Total cells. Yoda1 reduced adipogenesis by 33% compared with CON and as expected, increased cytoplasmic calcium. In si*Piezo1* cells, the anti-adipogenic effect of Yoda1 was reversed. MS+ reduced adipogenesis efficiency 15% compared with MS-. These data demonstrate that Piezo1 activation inhibits adipogenesis in PVAT and provides support for an adaptive mechanism that reduces PVAT adipocyte populations and possibly increases PVAT fibrosis.

ASSESSMENT OF ANTI-VIRAL AND ANTI-INFLAMMATORY EFFECTS OF MESENCHYMAL STEM CELLS IN HERPESVIRAL INFECTIONS

Shannon H. Skalecki[1] and Valerie Johnson[1] 1Michigan State University, Department of Clinical Sciences, College of Veterinary Medicine, East Lansing MI.

Elephant Endotheliotropic Herpesvirus (EEHV) is a major threat to both African Elephants (*Loxodonta africana*) and Asian Elephants (*Elephas maximus*) worldwide. This virus is especially threatening to young captive elephants that are being weaned from their mothers and accounts for most deaths of young elephants in captivity. Death from EEHV is a result of fatal hemorrhagic disease or systemic inflammation leading to organ failure. There currently is no vaccine and intensive supportive care is provided to animals as soon as they become viremic. Despite this, mortality remains approximately 50% in young elephants who contract this disease. Mesenchymal Stem Cells (MSCs) have been investigated as a therapy for sepsis and other infectious diseases due to their anti-inflammatory and anti- microbial properties. Few studies have been performed to assess the anti-viral effects of MSCs although studies investigating the anti-viral effect of MSCs in animal models of influenza suggest a beneficial effect. To determine if MSCs or factors secreted by MSCs (MSC-CM) have direct antiviral effects against herpesvirus equine cells will be infected with EHV-1 and then treated with MSCs, MSC-CM or untreated. Viral load will be assessed by qPCR. In addition to direct anti-viral effects MSCs could improve the outcome of this disease through reduction of the systemic inflammation that occurs in this disease process. Commercially available equine ELISAs have been demonstrated to cross react with elephant cytokines. Cytokine levels will be measured in banked serum samples from elephants with EEHV, with osteoarthritis (OA), and normal. Cytokine levels will be compared using a 2-way ANOVA to determine the levels in the elephants.

EFFECT OF CHEMOTHERAPY ON THE DIVERSITY OF THE FECAL MICROBIOME OF CANINE LYMPHOMA PATIENTS

Eric Spilker[1], Hinako Terauchi[2] and Linda Mansfield[1,2] , 1) College of Veterinary Medicine, 2) Microbiology and Molecular Genetics, Michigan State University

Chemotherapy-induced gastrointestinal disease (CIGD) has been associated with chemotherapy treatment resulting in symptoms of clinical nausea, vomiting and diarrhea. CIGD can decrease quality of life and is a common reason for discontinuation of potentially life-saving chemotherapy. We hypothesized that CHOP protocol chemotherapy agents either individually or synergistically decrease the diversity of the canine fecal microbiome and triggers dysbiosis of the gut microbial community gastrointestinal bacteria in dogs with lymphoma. Nine canine lymphoma patients undergoing cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy had fecal samples collected weekly before and throughout treatment. Vincristine, cyclophosphamide, doxorubicin and prednisone were administered weekly according to CHOP protocol. 16S rRNA gene sequencing analysis was performed on DNA from fecal samples to determine the composition and relative abundance of bacterial taxa. For each dog, 16S sequencing data from ten longitudinal samples were analyzed using QIME2 and bacterial taxa assignments made using the SILVA database. At specific intervals during CHOP treatment, changes in the relative abundance of bacterial taxa were observed. Plots provided evidence that CHOP protocol medication administration caused dysbiosis within the canine gastrointestinal tract. Analyses of 16S sequence analysis from 15 additional canine lymphoma patients is underway to determine if specific medications in the CHOP protocol create significant changes in the canine GI microbiome. Early results confirm CHOP chemotherapy was associated with dysbiosis and changes in bacterial abundance and decreases in bacterial diversity correlating with diagnosed CIGD in dogs with lymphoma.

CORE VS. PEROPHERAL HEAT LOSS IN HORSES UNDER GENERAL ANESTHESIA

Sara N Taylor[1], Aimee C Colbath[2], Jane M Manfredi[3], Kirk A Muñoz[1]
[1]Department of Small Animal Clinical Sciences
[2]Department of Large Animal Clinical Sciences
[3]Department of Pathobiology and Diagnostic Investigation. College of Veterinary Medicine, Michigan State University

Reduced body temperature has been linked to poor anesthetic recoveries in several species. During general anesthesia, horses experience potent vasodilation and an accelerated rate of heat loss from the inhalant anesthetics. Distal limbs are a major source of heat loss during anesthesia. Thus, measuring core and peripheral body temperatures may be a more effective way to monitor heat loss. This pilot study measured core and peripheral body temperatures in horses under general anesthesia. Four healthy client-owned horses presented for orthopedic procedures were sedated with xylazine, induced with ketamine and diazepam, and administered butorphanol and lidocaine for analgesia, as needed. Duration of general anesthesia was 70 minutes. Core body temperature was measured using rectal and intranasal thermometer probes, and peripheral body temperature was measured using thermographic imaging at the fetlock. Room temperature was measured using a digital thermometer. Statistics included repeated measures ANOVAs with Tukey's post-hoc comparison analysis, $p < 0.05$. No significant difference was detected in rectal (R_T), intranasal (N_T), and room (Ro_T) temperatures overtime. Fetlock temperatures (F_T) started significantly increasing 20 minutes post-induction. F_T was significantly lower than R_T and N_T at all timepoints, except at T60 ($p = 0.093$; $p = 0.075$). No difference was detected between R_T and N_T overtime. In conclusion, peripheral body temperature (F_T) changed faster and to a greater extent than core body temperature indicating that peripheral temperature may be a more sensitive technique for measuring total heat loss in horses under general anesthesia.

FORCES EXERTED ON THE BACK DURING JUMPING IN HORSES

Siana Stanton[1], Duncan Peters[2], Michael Lavignino[3], Lori Bidwell[2], Jane Manfredi[1], 1) College of Veterinary Medicine, Michigan State University, 2) East-West Equine Sports Medicine, 3) College of Engineering, Michigan State University

Back pain in hunter/jumper equine athletes can affect performance and welfare. The study's objective was to compare the pressure placed on a horse's back during the phases of takeoff, flight, and landing at various jump heights. We hypothesized that there would be greater forces exerted with increased jump height and during the landing phase. A Pliance-s mat collected pressure data from five horses and three riders at the canter and over obstacles of set heights (0.84 m and 1m) and was synchronized with video to analyze average mean or peak pressures from the whole, front, and back of the mat. Pressures during the phases were normalized to the canter baseline to control for differences between horse-rider combinations. Statistics included a repeated measures ANOVA with Šídák's multiple comparisons tests (significant at $P<0.05$). There was no significant difference in pressures seen between jump heights. The mean pressures during takeoff and landing were not significantly different from each other but were significantly greater than flight (all $P<0.05$). Peak pressure of the whole pad and mean pressures of both the front and back of the pad during takeoff and landing were greater than during flight, and landing peak pressure was greater than takeoff ($P<0.05$). Additionally, a peak pressure of 99.6 kPa was found in one instance of obstacle refusal. Relating jumping phases with quantitative pressure results may aid in further understanding of the cause and subsequent prevention of back--related performance issues.

GUT MICROBIOME COMPOSITION AT 3 MONTHS OF AGE ASSOCIATED WITH ECZEMA DIAGNOSIS IN INFANTS

Hinako Terauchi, Julia A. Bell, PhD, Phillip T. Brooks, PhD, , John W. Holloway, PhD, Wilfried Karmaus, MD, MPH, Susan L. Ewart, DVM, PhD, S. Hasan Arshad, MBBS, DM, FRCP, Linda S. Mansfield, MS, VMD, PhD

Asthma is a chronic disease with inflammation and constriction of airways, leading to wheeze, cough, and airway constriction. There are complex array of risk factors associated with asthma and allergy development, and gut microbiome has been suggested to be one of the major factors. It is widely accepted that a fetus has no, or very little, microbial colonization during gestation, making the first year post-birth a critical period for microbiome development. In addition to the initial development of their individual microbiome, the first year of infancy is also considered an important time for immune system development. We hypothesized that the gut microbiome during this early stage of life influences the susceptibility of infants to later develop asthma and allergy. To assess the gut microbiome compositions, fecal samples of infants at 3 months of age were collected. These infants were part of the Isle of Wight generational allergy study cohort. 16S rRNA sequences of bacterial microbiota were collected and analyzed for each sample via Illumina sequencing. The clinical assessments were performed for eczema, wheeze, and atopy, as these are allergic conditions often closely related to later development and diagnosis of asthma. As expected, any members of the gut microbiota were shared among the infants studied, while simultaneously, individuals had their own unique microbiota. The microbiota of the infants who were diagnosed with eczema at 3 months of age showed some variation when compared to those without the diagnosis at the same age. Based on Principal Component Analysis, several bacterial taxa were indicated to be the source of the variation between the groups, indicating that beyond the commonly shared members of the microbiome, there are specific members of bacterial taxa that are associated with development of eczema, which may lead to other allergic conditions in later years.

MAST-CELL HISTAMINE MEDIATES THE INTESTINAL IMMUNE RESPONSE TO EARLY WEANING IN PIGLETS VIA HISTAMINE 2 RECEPTOR (H2R)

Kyan Thelen[1], Neco Wilson[1], Mahsa Fardisi[1], Carmen-Maria Garcia[1], Adam J. Moeser[1]. 1)Department of Large Animal Clinical Sciences, Michigan State University

Mast cells are major mediators of stress-related disorders, especially that of the gastrointestinal (GI) tract. Previous studies investigating the impact of early weaning (EW) stress in piglets showed that histamine, a major mediator in mast cell granules, is released shortly after weaning and is followed by increased gene expression of histamine receptor 2 (H2R) in ileal, jejunal, and colonic mucosa. The precise contribution of histamine and histamine receptor subtypes to weaning stress-induced GI immune responses is unknown. Here we tested the hypothesis that EW induced intestinal immune activation is mediated by H2R. Fifteen-day-old Yorkshire female and male castrate piglets were administered either saline vehicle or the H2R antagonist, famotidine (10 mg/kg; intramuscular), 30 minutes prior to early weaning. At weaning, piglets were weaned from their dams and housed in nursery pens with ad libitum access to water. At 24 hours post-weaning, mid-jejunum was collected for qPCR gene expression of H2R. Markers of immune activation including myeloperoxidase (MPO), IL1β, TLR4, and β-integrin were measured by Western blot or ELISA. Unweaned control piglets remained with the sow and were collected at the same time as weaned piglets. Weaning increased gene expression for H2R at 3, 8, and 24 hours post-weaning. Compared with saline-treated controls, piglets administered famotidine had reduced jejunal MPO and IL1-β. Expression of β-integrin in the mesenteric lymph nodes was also reduced in famotidine-treated piglets. Together, these data demonstrate that histamine via H2R plays an important role in early weaning stress-induced intestinal immune responses. This provides a potential target for mediation of the stress response to early weaning practices.

QUANITATIVE GENE EXPRESSION PATTERNS OF *TYR*, *CD34*, and *CALD1* ACCURATELY DIFFERENTIATE CANINE ORAL MELANOMAS FROM SOFT TISSUE SARCOMAS

Mayra F. Tsoi[1], Tuddow Thaiwong[1], Rebecca C. Smedley[1], Erica Noland[1,2] and Matti Kiupel[1,2*]
[1]Veterinary Diagnostic Laboratory, Michigan State University
[2]Department of Pathobiology and Diagnostic Investigation, Michigan State University

Introduction: Melanoma is the most common malignant neoplasm in the oral cavity of dogs and can exhibit a wide variety of histologic phenotypes, including a spindloid variant. Microscopic differentiation of spindloid amelanotic melanomas from soft tissue sarcomas (STS) is very difficult in the absence of key diagnostic features, such as intraepithelial nests. Previously, immunohistochemistry (IHC) using an antibody cocktail that detects Melan-A, PNL2, TRP-1 and TRP-2 has been established as the gold standard for diagnosing oral melanomas (OM) with 100% specificity and 93.9% sensitivity. However, positive labelling is least common in spindloid amelanotic melanomas. **Objectives:** To establish protein and quantitative RNA expression profiles that accurately diagnose oral spindloid amelanotic neoplasms as OM or STS. **Methods:** Formalin-fixed, paraffin-embedded tissues from 20 OM, 20 STS, and 20 oral spindloid amelanotic neoplasms were selected and IHC for the antibody cocktail, SOX-10 and laminin, in parallel with RT-qPCR of *TYR, SOX10, CALD1, CD34, DES,* and *LAMA1*, was performed on all cases. **Results:** Quantitative expression of *TYR, CD34,* and *CALD1* differentiated OM from STS, with 100% specificity and 65%, 95%, and 60% sensitivity, respectively. All 20 OM were positive for SOX-10, however, two STS were also positive (100% sensitivity, 90% specificity). Surprisingly, none of the 20 oral spindloid amelanotic neoplasms had RNA expression profiles or IHC patterns consistent with a diagnosis of melanoma. **Conclusions:** Accurate diagnosis of oral spindloid neoplasms as either OM or STS is vital so that the appropriate treatment can be pursued. Results from this study, notably the identification of *TYR*, *CD34* and *CALD1* as differentially expressed genes by OM vs STS, will significantly improve our ability to accurately discriminate between these two entities.

BISPECTRAL INDEX (BIS) MONITORING OF STANDING SEDATION IN HORSES

Caitlin A Thorn[1], Sichao Wang[2], Dhruv Sharma[2], William A Horne[1]
[1]Department of Large Animal Clinical Sciences, Michigan State University Veterinary Medical Center
[2]Center for Statistical Training and Consulting, Michigan State University

Problem Addressed: Determine whether bispectral index (BIS) monitoring could detect deepening sedation levels in standing horses. **Hypothesis**: BIS can be used to discriminate between deepening sedation levels in standing horses caused by a combination of three drugs. **Methodology**: A longitudinal within subjects design study was performed. A total of 14 horses (450-560 kg) served in both treatment and control conditions with a washout period of at least two weeks. Treatment consisted of sequential thirty-minute constant-rate infusions (CRI) of detomidine (D), detomidine and butorphanol (DB), and detomidine, butorphanol, and midazolam (DBM). CRIs were preceded with an intravenous (IV) dosage of 0.01 mg kg^{-1} of the corresponding drug. Administration rates for detomidine, butorphanol, and midazolam were 0.01 mg kg^{-1} $hour^{-1}$, 0.02 mg kg^{-1} $hour^{-1}$, and 0.02 mg kg^{-1} $hour^{-1}$, respectively. Control horses were restrained for 90 minutes and administered lactated Ringer's solution (2.5 mL kg^{-1} $hour^{-1}$) only. BIS values were recorded continuously for 90 minutes in each group. Sedation score, heart rate, respiratory rate, SpO_2, $PE´CO_2$, blood pressure, and arterial blood gases were also recorded. Statistical analyses used linear mixed or multiple linear regression models as relevant. **Results**: Estimated mean BIS scores were: C, 93.5; D, 91.4; DB, 88.4; DBM, 84.5. *p*-values for D, DB, and DBM *versus* C were 0.2087, < 0.0001, and < 0.0001, respectively. *p*-values for treatment phase comparisons were: D *versus* DB, 0.003; and DB *versus* DBM, 0.0001.BIS scores corresponded to D, DB, and DBM visual sedation scores of 1.6, 3.2, and 4.5, respectively. With the exception of decreases in heart and respiratory rates relative to control horses, all physiological variables in treatment horses remained within normal limits throughout the study. **Conclusions and clinical relevance**: These results indicate that BIS monitoring can distinguish between moderate and deep sedation in standing horses.

PLASMID-MEDIATED TRANSFER OF ANTIBIOTIC RESISTANCE GENES BETWEEN BACTERIA

Mia Van Allen[1], Azam Ali Sher [3], Dr. Linda Mansfield [1,2] 1) Department of Microbiology and Molecular Genetics, Michigan State University 2) Large Animal Clinical Sciences 3) Comparative Medicine and Integrative Biology Graduate Program

Antibiotic resistant (AR) pathogens have become a major health problem: the CDC announced we are now existing in a post antibiotic era. Plasmids are the carriers of antibiotic resistance genes, and they spread between bacteria in the microbiome via a horizontal gene transfer mechanism called conjugation. During each conjugation event, plasmids enter a host cell and can express their antibiotic resistance genes, resulting in newly acquired antibiotic resistance for that cell. This process of unrestrained AR plasmid spread cultivates an evolving reservoir of antibiotic resistant pathogens and commensal bacteria in the human gut microbiome. This study aimed to replicate and observe the rate and patterns of transconjugant frequency of fluoro-tagged plasmids in combinations of commensal, pathogenic, and lab strain bacteria in vitro. Conjugation protocols that allowed for quantitating transconjugation events using both introduction and absence of antibiotic pressure for selection were created and employed. The transconjugant colonies were confirmed using colony PCR with primers selecting for presence of green fluorescent protein that exists within the plasmid. Fluorescent microscopy was used to observe the transconjugants plasmid directly. The transconjugant frequencies obtained from the individual combinations of donor and recipient bacteria could give insight into strain-specific features that affect transconjugant frequency and plasmid spread effect. Understanding plasmid spread between gut microbiota is crucial to gain insight on how potential treatments can be developed to combat the spread of antibiotic resistance genes.

SADDLE FIT DURING A LONG DISTANCE ENDURANCE RIDE

Shelby Vollmer [1], Dr. Melissa Esser [2], and Dr. Jane Manfredi [1], 1) Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, 2) Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Poor saddle fit can contribute to back pain and lameness especially in endurance distance rides where horses must bear the weight of the rider over varied terrain for 25-100 miles (2-23 hours ride time). There is a lack of knowledge on how saddle fit changes over time during a distance ride. The hypothesis was that saddles would fit well pre-ride, having mean and peak forces on the horses back of less than 20 kPa, and that post-ride they would not fit well due to weight loss and/or back shape changes resulting in higher forces. Horses were assessed for weight, and saddle fit (palpation, back tracing (N=14)), including seven horses with forces measured by a Pliance-s pressure mat before and after a 25 mile ride. Statistics included Student's t-tests (significant at $P<0.05$). There was a trend towards weight loss post-ride (mean=16 kg; $P=0.06$). Subjectively, post-ride two more saddles had an acceptable fit (N=9) as compared to pre-ride. Common fit issues included too: far forward, long, and tight in the bars. Back tracings showed seven horses got wider and seven narrower post-ride. Mean and peak forces weren't different pre- vs. post-ride; however, front of the saddle mean and peak were always significantly greater than back of the saddle ($P<0.01$). All horses had peaks above 20 kPa. Limitations included a small number of horses and one distance. Poor saddle fit, particularly high peak pressures at the bars/pommel, could contribute to pain.Saddle fit should be individually assessed in horses after distance rides.

PHENYLBUTAZONE, FIROCOXIB, AND DIPYRONE ALL DIMINISH FUROSEMIDE DIURESIS IN HORSES

Julianne M. White[1], Harold C. Schott II[1], Aimee Colbath[1], [1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly administered to horses to control pain and inflammation, via inhibition of cyclooxygenase (COX). Unfortunately, these drugs can have adverse effects on the gastrointestinal (GI) tract and kidneys. Both COX-1 and COX-2 regulate renal blood flow and treatment with phenylbutazone (a non-selective COX inhibitor) decreases the diuretic effect of furosemide by nearly 30%. The effect of COX-2 selective inhibitors (firocoxib) and atypical NSAIDs (dipyrone) on the diuretic response to furosemide in horses has not been studied. Hypothesis: Furosemide-induced diuresis after pre-treatment with firocoxib or dipyrone are diminished less than after pre-treatment with phenylbutazone. Methods: Eight mares received four treatments in a replicated Latin square design: furosemide alone (F), furosemide and firocoxib (FF), furosemide and dipyrone (FD), and furosemide and phenylbutazone (FP). After 2 days of NSAID treatment at recommended dosages, urine was collected continuously via bilateral ureteral catheters. After a 30-minute baseline collection period, furosemide (1.0 mg/kg, IV) was administered and urine was collected for 4 hours. Urine volume was assessed by one-way repeated measures ANOVA. Results: 4 hour urine volume decreased ($p<0.001$) 25- 30% after pretreatment with all NSAIDs, as compared to F, and there were no differences between FF, FD, or FP. Further, variability in individual responses to furosemide administration after pre-treatment with different NSAIDs was observed. Conclusions: NSAIDs remain important drugs for alleviation of pain and inflammation in horses. However, beneficial effects can be accompanied by adverse effects. In people, COX- 2 selective NSAIDs have been demonstrated to have similar analgesic and anti-inflammatory effects as non-selective COX inhibitors with less adverse GI effects. However, COX-2 selective NSAIDs have not been found to be "renoprotective". In horses, COX-2 selective NSAIDs also have less adverse GI effects, but our data suggest there are minimal differences in effects on renal function with use of COX-2 selective NSAIDs.

THROMBIN CONTROLS FIBRIN(OGEN) SOLUBILITY DYNAMICS IN EARLY ACETAMINOPHEN HEPATOTOXICITY.

Zimu (Christine) Wei[1], Kevin Baker[1], Dafna Groeneveld[1], Lauren Poole[1], Holly Cline-Fedewa[1], Matthew Flick[2], James Luyendyk[1] [1]MSU. [2]UNC.

Acetaminophen (APAP) hepatotoxicity is associated with rapid activation of the coagulation protease thrombin and hepatic accumulation of its primary substrate fibrinogen. Inhibition of thrombin-mediated fibrin clot formation enhanced peak APAP hepatotoxicity in mice. Surprisingly, the magnitude and timing of traditional fibrin formation in the APAP-injured liver are not fully understood. In fact, recent studies suggest at peak liver injury, the majority of fibrin(ogen) in the APAP-injured liver accumulates through a unique thrombin-independent mechanism. We tested the hypothesis that traditional thrombin-mediated fibrin clot formation occurs early after APAP challenge in mice. FibrinogenAEK (FibAEK) mice, which express mutant fibrinogen that cannot support thrombin-mediated fibrin formation, and wild- type mice were challenged with a hepatotoxic dose of APAP (300 mg/kg) and liver and plasma samples collected 6 hours later. APAP-induced hepatic injury was similar in wild-type and FibAEK mice, indicated by serum alanine aminotransferase activity and area of hepatic necrosis. Immunohistochemistry revealed similar fibrin(ogen) accumulation within areas of necrosis in both wild-type and FibAEK mice challenged with APAP. Western blotting revealed robust fibrinogen accumulation in the insoluble protein fraction in livers of APAP-challenged wild- type mice. In contrast, fibrinogen in livers of APAP-challenged FibAEK mice did not partition to the insoluble fraction and was instead retained in the soluble protein fraction. The results indicate the rapid formation of insoluble fibrin in the APAP-injured liver does not contribute to APAP hepatotoxicity. Alongside prior studies, the results imply early thrombin-mediated fibrin polymer formation precedes and pairs with thrombin-independent pathways to drive fibrinogen accumulation in the injured liver.

CHARACTERIZATION OF THE EXTENDED SPECTRUM ß-lactamase *E. COLI* PLASMID CTX-41

Charles Whitehead-Tillery[1,2], Dr. Lixin Zhang[1,2], Dr. Linda Mansfield [1,2] 1) College of Natural Science 2) Department of Microbiology & Molecular Genetics, Michigan State University

Antibiotic resistance is one of the biggest threats to global health, and food security. In the gut microbiome bacterial organisms can become antimicrobial resistant through a mechanism called conjugation. Conjugation can be used by microbial pathogens to transfer antibiotic resistant genes to other commensal bacteria by forming a F pilus. Therefore, we hypothesized that CTX-41 can be used as a donor to transfer its antibiotic resistant genes to recipient bacteria giving them the ability to become donors *in vitro* & *in vivo. Escherichia coli* plasmid CTX-41 was isolated from a patient with a urinary tract infection and tested for resistance to Cefotaxime (CTX). An *in vitro* conjugation protocol helped us determine if recipient bacteria can uptake the plasmid as well as the antibiotic resistance gene. *E.coli* DNA was isolated, sequenced by Illumina and Nanopore methods. Sequence was assembled using bioinformatic tools and analyzed identify antibiotic resistant genes encoded by the plasmid. DNA kits were used to isolate plasmid DNA and to size the plasmid using gel electrophoresis. Our results showed CTX-41 is resistant to the antibiotic cefotaxime. CTX-41 can conjugate with DH5a *E.coli* to produce transconjugant colonies that are resistant to cefotaxime. Annotation of sequence data showed indication of *tra* genes, and CTXR which are responsible for conjugation transfer. We obtained the best results using Qiagen kits for isolation of plasmid DNA. Our next steps would be to clone a green fluorescent protein (GFP) into CTX-41 to track conjugation in the gut microbiome *in vivo*. Overall, understanding how conjugation works *in vitro* can allow us to establish a mouse model that can give us a better idea of conjugation works in the human microbiome.

THE MOLECULAR CLOCK GENES, CLOCK AND PER3, IN THE HUMAN PLACENTA

Winn E.,[1] Ahn S.,[1] Duong T.,[3] Zhou, G., [4] Petroff M.G.,[1,2] Hoffmann H.[3] Departments of [1]Pathobiology & Diagnostic Investigation, [2]Microbiology & Molecular Genetics, College of Veterinary Medicine, [3]Animal Science, College of Agriculture and Natural Sciences, [4]Clinical & Translational Sciences Institute

Preeclampsia is a maternal vascular disease of pregnancy caused by malperfusion of the placenta, and manifests as life- threatening hypertension. Increasing evidence suggests that the body's internal time-keeping system, represented by circadian clock genes, helps regulate placental function and the timing and duration of labor. Disruption of these circadian rhythms can lead to reproductive pathologies suggesting an important role of molecular clock genes in healthy pregnancy. However, we do not fully understand the role of circadian rhythms in the placenta during normal and pathologic pregnancies. To address this, we explored the expression of the molecular clock genes *CLOCK* and *PER3* in normal and preeclamptic placentas. Using publicly available microarray data, we found reduced *CLOCK* and *PER3* gene expression in placentas from preeclamptic women. To validate this, we analyzed CLOCK protein in placentas from preeclamptic (n=10) and control (n=10) pregnancies by Western blot. Although we were unable to identify a correlation between CLOCK protein in a gestation stage-specific manner in placentas from preeclamptic pregnancies, a non-significant trend towards reduced CLOCK was observed in these placentas. This study shows for the first time correlation between reduced *CLOCK* and *PER3* expression in placentas of preeclamptic patients. Future studies will investigate the role of these genes in placental function and pathology.

SARS-COV-2 INFECTION IN TWO FARMED MINKS (NEOVISON VISON)

Ryan Yanez, Dodd Sledge, Matti Kiupel Department of Pathobiology and Diagnostic Investigation, Michigan State University Veterinary Diagnostic Laboratory, 4125 Beaumont Rd., Michigan State University, Lansing, MI, 48910, USA

A facility with 17,000 mink (Neovison vison) had 12 mink die on 9/27/2020 and 7 die on 9/28/2020. The facility owner reported coughing, anorexia, and bloody nasal discharge for several weeks in a large percentage of the group prior to death of these animals. Two male minks were submitted for necropsy to Michigan State University Veterinary Diagnostic Laboratory. Gross findings were unremarkable. Histologically, the nasal epithelium was eroded to ulcerated and had a lymphoplasmacytic to suppurative infiltrate. The turbinates were coated by mucus, hemorrhage and numerous degenerate neutrophils and foamy macrophages. In the lungs, large to small sized veins and arteries had prominent lymphoplasmacytic cuffing with vasculitis and fibrinoid necrosis. Extensive loss of terminal bronchiolar epithelium was a prominent feature. The alveolar interstitium contained lymphoplasmacytic to neutrophilic infiltrates and fibrin thrombi were within alveolar capillaries. Hyaline membranes were not observed. PCR for SARS-CoV-2 on nasal turbinate and lung samples from both minks was positive and confirmatory testing performed by the National Veterinary Services Laboratory (NVSL) in Ames, Iowa was also positive. Immunohistochemistry for the SARS-CoV-2 nucleoprotein antigen showed strong cytoplasmic immunoreactivity within mononuclear inflammatory cells, the nasal epithelium, tracheal epithelium, and bronchial and terminal bronchiolar epithelium. Rarely, there was cytoplasmic immunoreactivity of endothelial cells in the subepithelial stroma of the turbinates. This report documents unique histologic lesions and positive endothelial immunohistochemical labeling in farmed minks diagnosed with SARS-CoV-2.

MOSQUITO DIVERSITY, ABUNDANCE, AND VIRUS ASSOCIATIONS OF BINDER PARK ZOO

Hunter E. Wojtas[1], Morgan E. Ramsdell[2], John B. Keven[2], and Edward D. Walker[3] 1) College of Veterinary Medicine, 2) Entomology, 3) Microbiology and Molecular Genetics, Michigan State University

Mosquitoes are vectors of pathogens to both people and animals. In Michigan, three mosquito-borne viruses of concern are Eastern Equine Encephalitis virus (EEEV), West Nile virus (WNV), and Jamestown Canyon virus (JCV). One location of interest for virus presence is Binder Park Zoo in Battle Creek, Michigan, where, in 2019, two Mexican gray wolf pups contracted EEEV. The goal of this project was to initiate virus surveillance at the zoo and quantify mosquito abundance, diversity, and virus associations of the mosquito community there. Zoos present a unique environment to study the nexus of people, animals, and mosquito-borne pathogens. Mosquitoes have the potential to spread pathogens between zoo employees, patrons, wild and exotic animals, and pets and people of neighboring communities. By analyzing blood meals, we can illuminate the relationships between mosquito-borne viruses, people, and animals. We sampled mosquito populations at eight sites on zoo property weekly from June through July, using three methods (light traps, gravid traps, and resting boxes). After retrieving samples, we identified species, pooled mosquitoes by species, and tested them for virus infection in collaboration with the Michigan Department of Health and Human Services. Results showed that the mosquito community at Binder Park Zoo was rich with species and dynamic in space and time, species dominance was dependent on precipitation, and CDC light traps caught the most mosquitoes with the highest diversity. Additionally, one pool of *Culex pipiens/restuans* tested positive for West Nile virus. Blood meal analyses are ongoing to assess host utilization. Ultimately, information from this study will help prevent the spread of viruses effectively by targeting specific mosquito species.