30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

Session	Friday, October 2, 2020 Residents, MS Students, and Undergraduate Students Please click this URL to join: https://msu.zoom.us/j/92498840931 Passcode: CVMPZD Moderator: Dr. Sri Sreevatsan
8:15-8:30	**Elliot Majlessi[4]** Benchmarking Computational Approaches for the Identification of Diagnostic sRNA Targets (Janani Ravi)
8:30-8:45	**Julie Pfeifer[2]** Gastric or Duodenal Perforation and Secondary Septic Peritonitis Following NSAID Administration (Matthew Beal)
8:45-9:00	**Julie Pfiefer[2]** Retrospective Evaluation of Bupivacaine Liposome Injectable Suspension in Canine Caesarean Section (Nyssa Levy)
9:00-9:15	**Cristian Rendon-Mora[5]** PIEZO1 Activation Inhibits Adipogenesis and Lipogenesis in PVAT Preadipocytes (Andres Contreras)
9:15-9:30	**Bridie McClusky[4]** Spermatogenesis in Mexican Wolf Testis of Differing Ages (Dalen Agnew)
9:30-9:45	**Philip Calhoun[4]** Identifying Diagnostic sRNA Targets in Infected Hosts Using Computational Approaches (Janani Ravi)
9:45-10:00	**Heather Sayles[4]** The Clinical Significance of Hemorrhage and Congestion in the Uterus of Mexican Wolves (Dalen Agnew)
10:00-10:15	**Renato May Rossi[2]** Association of Metabolic Status During Late Gestation and Volume of Colostrum in Dairy Cows (Angel Abuelo)
10:15-10:30	**Brooke Boger[4]** Equine Beta Defensins as Biomarkers of Septic Arthritis in Horses (Jane Manfredi)

*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

EQUINE BETA DEFENSINS AS BIOMARKERS OF SEPTIC ARTHRITIS IN HORSES

Brooke L Boger[1], Carrie A Jacobs[2], Patty Sue D Weber[1], Jane M Manfredi[1] 1) College of Veterinary Medicine Michigan State University 2) College of Veterinary Medicine North Carolina State University

Diagnosis of septic arthritis in horses is not straightforward. Increasing time between onset, diagnosis, and treatment can have significant consequences for quality of life. Defensins are used in diagnosis of joint infection in people. The presence of beta defensins (BD) in equine synovial fluid and their utility as a biomarker of sepsis has not been investigated. The objective was to compare protein expression of BD from normal, aseptically inflamed, and septic joints. We hypothesized that BD would be higher in septic joints when compared to other groups. Beta defensin expression was measured from synovial fluid samples from equine joints with: no disease, aseptic inflammation, and septic inflammation using a commercial ELISA and analyzed with a Kruskal-Wallis test (significant at $P<0.05$). Joints with aseptic inflammation had higher expression of BD-1 as compared to septic joints and higher expression of BD-3 when compared to normal and septic joints. No differences in expression of BD-2 were seen. The differential expression pattern between the specific BD proteins was not expected. In humans, BD-3 was increased in infected bone and synovium. Reasons for these differences may include variation among species in defensin function, variation among clinical cases, and samples from synovial fluid versus synovium, bone, or cartilage. Low case numbers and different types of cases in the aseptic inflammation group were main limitations. Differential expression of BD-3 between joint conditions was identified and could provide a new area for diagnosis and therapeutic intervention in aseptic inflammatory equine joint disease, though further study is warranted.

IDENTIFYING DIAGNOSTIC SRNA TARGETS IN INFECTED HOSTS USING COMPUTATIONAL APPROACHES

Philip Andrew Calhoun[1,2], Elliot Majlessi[2,3], Srinand Sreevatsan[2], Janani Ravi[2*] 1.) College of Natural Science, Michigan State University; 2.) Dept. of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University; 3.) Lyman Briggs College, Michigan State University

Early diagnosis of many agriculturally relevant zoonotic diseases has always been problematic since the hosts often remain asymptomatic until it's too late. The second main problem has been with accurate and sensitive diagnosis often confounded by closely-related and environmental bacteria. We, therefore, focus on developing a computational workflow to identify pathogenspecific diagnostic targets that can be detected in infected hosts. We will use sRNA as a diagnostic target molecule due to the changes that occur in host and pathogenic sRNA upon infection. We are interested in identifying bacterial sRNAs that are pathogen-specific, making them excellent candidates for diagnostic biomarkers. Although intra-and extracellular sRNAs have been used extensively in cancer detection (host miRNA), few studies have addressed sRNA as biomarkers for detecting pathogens within the host. Here, we propose computational approaches to detect pathogenic sRNA in infected host samples. The computational workflow will be used to identify pathogenic sRNA in sequenced sera from white-tail deer (*Odocoileus virginianus*) infected with bovine tuberculosis (*Mycobacterium tuberculosis bovis*). We will use existing sRNA databases, host and pathogen reference genomes, measure differential expression, and benchmark against existing sRNA pipelines to help us discern uniquely bacterial sRNA that can be isolated and detected in infected host samples. Taken together, our approach will help us identify sRNA signatures unique to *M. tuberculosis* variant bovis in Odocoileus virginianus, and any combination of pathogen-infected hosts, and facilitate early diagnosis of bacteremia.

BENCHMARKING COMPUTATIONAL APPROACHES FOR THE IDENTIFICATION OF DIAGNOSTIC SRNA TARGETS

Elliot Majlessi[1,2], Philip Andrew Calhoun[2,3], Janani Ravi[2*], 1) Lyman Briggs College, Michigan State University; 2) Dept. of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University; 3) College of Natural Science, Michigan State University. *Corresponding author

Small RNAs (sRNA) are short non-coding RNA molecules that regulate gene expression and play a key role in early diagnosis of infectious diseases. Due to the lack of clinical recognition, the similarity between pathogens, and the variability of common symptoms, early diagnosis in zoonotic hosts is often difficult. This has created an imminent need for computational approaches to produce early and sensitive diagnostic tests. Currently, there is rising interest surrounding the use of sRNA detection in pathogens and microRNA (miRNA) in hosts, as diagnostic tools which can be attributed to unique biomarkers that become deregulated in host samples (miRNA) and bacterial sRNA once infection occurs. To date, several computational methods and workflows have been developed using sRNA as a diagnostic tool. However, short read lengths, variability across platforms, and the inability to identify pathogen-specific diagnostic targets, still pose significant challenges. Therefore, we propose a novel comprehensive computational workflow which will: (i) identify pathogenic sRNA (host miRNA) that is significantly up or downregulated, (ii) analyze mixed host-pathogen samples, and (iii) determine if the quantification is sufficiently accurate and efficient for diagnosis. Thus far, we have been able to successfully detect unique pathogenic sRNA in cows *(Bos taurus)* infected with *Mycobacterium avium paratuberculosis*, a pathogenic bacterium responsible for economic loss and morbidity in sheep and cattle, using our workflow and Oasis 2.0. Through the continuation of our benchmarking analysis we hope to present the merits of our novel approach in light of current research and techniques which have already been developed.

ASSOCIATION OF METABOLIC STATUS DURING LATE GESTATION AND VOLUME OF COLOSTRUM IN DAIRY COWS

Renato May Rossi[1], Faith Cullens[2], Paola Bacigalupo[2], Lorraine Sordillo[1], Angel Abuelo[1], 1) Large Animal Clinical Sciences, College of Veterinary Medicine, 2) MSU Extension; Michigan State University

In this study we compared the metabolic status of dairy cows during colostrogenesis based on the volume of colostrum produced. Healthy Holstein cows were randomly selected from 2 commercial dairy herds in Michigan. Two cohorts of 21 cows each were enrolled per farm with similar parity distribution. Cows were blood sampled weekly during the last 6 weeks of gestation, and biomarkers of nutrient utilization, oxidant status, and inflammation were quantified in serum. Cows were milked within 6 h of calving and grouped into high colostrum producer (HCP) or low colostrum producer (LCP) using a arbitrary cut-off of 6 L. For this study, only the data from 63 cows producing colostrum with IgG > 50 g/L were considered to avoid confounding due to poor IgG content. Data were analyzed statistically through mixed models with repeated measures. The HCP cows had higher β-hydroxybutyrate, blood urea nitrogen, magnesium, and lower glucose concentrations throughout the study compared to LCP cows. Furthermore, HCP cows showed higher concentration of reactive oxygen species, resulting in higher oxidant status index values despite no changes in antioxidant potential. No differences were found for haptoglobin, a biomarker of inflammation. Collectively, these data show HCP cows had greater utilization of nutrients throughout the study. Thus, indicating greater metabolism activity, which might explain the elevation in markers of oxidant status. Nevertheless, these differences did not result in changes in biomarkers of inflammation or lipid mobilization, suggesting that physiological homeostasis was not disrupted in HCP cows.

SPERMATOGENESIS IN MEXICAN WOLF TESTIS OF DIFFERING AGES

Bridie McClusky, College of Natural Science Michigan State University

Mexican wolves are one of the most endangered subspecies in North America. From 1976 to 1998 the species was considered extinct in the wild until their reintroduction in Arizona and New Mexico. Due to their small population size, inbreeding has become necessary for the species to survive. Past studies have found evidence that the inbreeding in the male Mexican wolf population has reduced sperm quality compared to the generic grey wolf. The purpose of this study is to examine the quality of spermatogenesis in Mexican wolves, allowing population managers to determine the optimum age for breeding success. After puberty, we expect to see a decrease in spermatogenesis as age increases. Differentiation techniques using a digital imaging analysis program (Image Pro ®) will be used to establish the ratio of seminiferous tubules to leydig cells. Image Pro ® will also be used to measure the surface area of seminiferous tubules, including and excluding the lumen, in microscopic sections. These techniques will provide a morphological proxy for the quality of spermatogenesis in Mexican wolf testes.

RETROSPECTIVE EVALUATION OF BUPIVACAINE LIPOSOME INJECTABLE SUSPENSION IN CANINE CAESAREAN SECTION

Julie Pfeifer, Nyssa Levy, Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Perioperative analgesia for canine dystocia presents a challenge due to risk of drugs crossing the blood-placenta and blood-milk barriers. Bupivacaine liposome injectable suspension, brand name Nocita®, a local anesthetic, has been shown to improve pain control for up to 72 hours. The objective of this study was to report on complication rate and puppy mortality following use of Nocita® in dogs undergoing caesarean section.

Medical records were searched from January 1, 2015 – March 31, 2020 for dogs receiving incisional Nocita® following caesarean section. Age-matched controls were identified. Cases were excluded if medical records were incomplete, owners were unable to provide case information, or if epidural anesthesia was used. Owners were queried regarding survival of puppies, nursing behavior, and post-operative complications. Due to small sample size and nonparametric data, descriptive statistics and Mann-Whitney U testing were utilized.

Forty-four dogs met inclusion criteria: 20 dogs received Nocita, and 24 did not. The Nocita® and control groups were similar in total puppies delivered (median 4 [1-11] and 5 [2-12], respectively). Post-operative puppy deaths were not significantly different ($p = 0.27$) between dogs receiving Nocita® and control dogs (0 [0-4] and 0 [0-3], respectively). Nocita® and control groups were also similar in frequency of nursing (85% and 83.3%, respectively). Incisional complication rates were not significantly different ($p = 0.68$) between dogs receiving Nocita® (5% [1/20]) and control dogs (12.5% [3/24]). The use of Nocita for canine caesarean section was not associated with increased post-operative puppy mortality or incisional complications. Prospective evaluation is needed.

GASTRIC OR DUODENAL PERFORATION AND SECONDARY SEPTIC PERITONITIS FOLLOWING NSAID ADMINISTRATION

Julie Pfeifer, Matthew Beal, Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is common in both veterinary and human medicine. However, NSAID administration has been associated with gastroduodenal perforation (GDP). The goal of this study was to determine which NSAIDs were associated with GDP in dogs and the relative frequency of prescription of these medications.

Medical records were searched from January 2007- March 2020 for dogs diagnosed with GDP following NSAID administration. Included dogs had received NSAIDs within 7 days of perforation that was based on surgical or necropsy findings. A survey was distributed to Michigan State's referring veterinarians to determine the frequency of specific NSAIDs prescribed in their practice during the study period.

Thirty-two dogs were included. Five of 32 dogs were administered more than one NSAID, 4/32 dogs received a combination of an NSAID and a corticosteroid, and 1/32 dog received two NSAIDs and a corticosteroid within 7 days of GDP. Six of 32 dogs received an overdose of an NSAID. Eighteen of 32 dogs received only one NSAID at the recommended dose. Meloxicam was administered in 44.4%, firocoxib in 27.8%, deracoxib in 16.7%, and piroxicam in 11.1% of included dogs. One hundred sixty surveys were returned. Carprofen was the most commonly prescribed NSAID (70.6%), followed by meloxicam (10.6%), deracoxib (8.4%), firacoxib (7.8%), aspirin (1.5%), and other (0.9%).

NSAIDs remain a risk factor for GDP. Despite being the most frequently prescribed NSAID over the study period, carprofen was not associated with any GDP when administered alone. Prospective evaluation is needed to verify these findings.

PIEZO1 ACTIVATION INHIBITS ADIPOGENESIS AND LIPOGENESIS IN PVAT PREADIPOCYTES

Cristian Rendon[1], Emma Flood[2], Janice Thompson[2], Stephanie Watts[2], and Andres Contreras[1]. 1) Large Animal Clinical Sciences, 2) Pharmacology and Toxicology, Michigan State University.

During adipogenesis, perivascular adipose tissue (PVAT) preadipocytes turn into adipocytes. In non-PVAT preadipocytes, mechanical forces affect the commitment and lipogenic stages of adipogenesis. The mechanism may involve PIEZO1, a mechanosensor, that boosts differentiation of progenitor cells towards osteogenic and fibroblastic lineages. Since hypertension causes changes in the vascular forces that could affect adipogenesis in PVAT, our objective was to evaluate PIEZO1 expression in PVAT and the effects of PIEZO1 activation on the adipogenic potential of preadipocytes. We hypothesize that PIEZO1 activation limits the adipogenic potential of PVAT preadipocytes. PVAT from the thoracic aorta (APVAT) was collected from male Sprague Dawley rats at 10 weeks of age. Stromal vascular fraction (SVF) were obtained by Liberase digestion. Preadipocytes, CD34+PDGFRα+, were harvested from SVF by MACS. Piezo1 expression was similar in SVF and preadipocytes, the knockdown of *Piezo1* with siRNA reduced its gene (PCR) and protein expression (IF). SVF was induced to differentiate in standard media (CON) or in the presence of PIEZO1 agonist Yoda and inhibitor Dooku during days 0-2 (commitment), and 3-14 (lipogenesis). Triglyceride was reduced by Yoda and Dooku during 0-2 d compared to CON. Yoda for 12 d reduced triglyceride accumulation compared to Dooku and CON. A similar response was observed in preadipocytes during commitment. The expression of PIEZO1 in PVAT fractions suggests mechanosensitivity. PIEZO1 activation during adipogenesis commitment impaired adipocyte maturation. These data provide evidence for mechanosensation in PVAT preadipocytes and its impact on adipogenesis.

THE CLINICAL SIGNIFICANCE OF HEMORRHAGE AND CONGESTION IN THE UTERUS OF MEXICAN WOLVES

Heather Sayles, Department of Pathobiology and Diagnostic Investigation, Michigan State University College of Veterinary Medicine

Mexican wolves are carefully monitored and their reproduction controlled because of their endangered status. The genetic variability in this species is low due to inbreeding, which lowers their reproductive success and overall population health. To better understand the reproductive issues plaguing this species, uteri and ovaries from Mexican wolves after death or elective sterilization and evaluated grossly and microscopically in order to identify abnormalities. Vascular changes such as vasodilation and hemorrhage in the uteri and ovaries were common and several criteria were then examined in greater detail. We hypothesized that increased blood flow and hemorrhage were associated with the stage of the estrous cycle. The degree of vasodilation was scored based on the size of the blood vessels. Other parameters recorded include presence and location of hemorrhaging and stage of the estrous cycle (based on ovarian structures). Comparisons were made between canid uteri with and without vasodilation or hemorrhaging to identify other possible correlations with age, parity, and cause of surgery. By understanding the role and possible causes of vasodilation and hemorrhaging in Mexican wolves, we can determine the significance of this finding in poorly reproducing animals in the Mexican wolf population.

30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

Session	Friday, October 9, 2020 PhD Students Please click this URL to join: https://msu.zoom.us/j/93907300324 Passcode: CVMPZD **Moderator: Dr. Sri Sreevatsan**
8:15-8:30	**Miguel Chirivi[3]** Short and Long Exposure to Lipopolysaccharide Induces Lipolysis and Insulin Resistance in Dariy Cows' Adipose Tissue (Andres Contreras)
8:30-8:45	**Elizabeth Haiderer[3]** Implicating an Alternative Sigma Factor in the Mycobacterial-Macrophage Interaction (Rob Abramovitch)
8:45-9:00	**Devika Bahal[3]** 2B4: A Novel Checkpoint Receptor for iNKT Cell Functions (Rupali Das)
9:00-9:15	**Vishvapali Kobbekaduwa[3]** Comparison of *Anaplasma phagocytophilum* Infections in the North-Central and Eastern USA in Blacklegged Ticks (Jean Tsao)
9:15-9:30	**Dawn Kuszynski[3]** Purinergic Receptor Function in the MCA from Clopidogrel-Treated Rabbits (Adam Lauver)
9:30-9:45	**Carsten Walker[3]** Endocannabinoid Concentrations and Receptor Expression in Cultured Bovine Endothelial Cells Challenged by Endotoxin (Lorraine Sordillo)
9:45-10:00	**BREAK** **Moderator: Dr. Gisela Hussey**
10:00-10:15	**Chima Maduka[3]** Glycolytic Reprogramming Underlies Inflammation and Fibrosis to Polylactide-Based Prosthetics (Christopher Contag)
10:15-10:30	**Jaimie Strickland[3]** Retinoic Acid Affects Barrier Integrity in Bovine Mammary Endothelial Cells (Lorraine Sordillo)
10:30-10:45	**Kyan Thelen[3]** Early Life Stress in Pigs Induces a Developmental Shift in Gut Epithelial Glucose Transporter Systems (Adam Moeser)
10:45-11:00	**Yajing Ji[3]** Transcriptomic Characterization of Pulmonary Arterial Hypertension to Identify Patient-Specific Therapeutics (Richard Neubig)

*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

30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

11:00-11:15	**Syeda Anum Hadi[3]** Discovery of a Predominant and Distinct Lineage of *Mycobacterium tuberculosis* in Brazilian Indigenous Population (Sri Sreevatsan)
11:15-11:30	**Ashley Putman[3]** Isoprostanes Reduce Reactive Oxygen Species and Apoptosis in a Bovine Model of Oxidative Stress (Lorraine Sordillo)

*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

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 costimulatory 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.

SHORT AND LONG EXPOSURE TO LIPOPOLYSACCHARIDE INDUCES LIPOLYSIS AND INSULIN RESISTANCE IN DAIRY COWS' ADIPOSE TISSUE

Miguel Chirivi[1], Madison Smith[1], Andres Contreras[1]; [1]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA

Excessive lipolysis and adipose tissue (AT) inflammation predisposes periparturient dairy cows to metabolic diseases, that often are comorbidities of inflammatory health events. We hypothesized that: lipopolysaccharide (LPS) exacerbates lipolysis and reduces insulin sensitivity in AT. Subcutaneous AT (SCAT) explants were collected from 24 healthy non-lactating nongestating Holstein cows at a local abattoir. Explants were incubated 3 and 7 hours in the presence of LPS (0 or 20µg/ml). The effect of LPS on stimulated lipolysis was determined using isoproterenol (ISO, 1µM) and LPS plus ISO (LPSISO). The impact of LPS on insulin sensitivity (1µL/L, LPS-IN) was determined by comparing it to the effect of insulin on lipolysis during ISO stimulation (ISO-IN). Lipolysis was determined by quantification of glycerol release. mRNA expression was quantified by RT-qPCR. Protein expression of pHSL and pAKT was determined by microcapillary Wes Simple assay. Compared to basal, LPS increased glycerol release from SCAT by 115±18% and 68,7±16%, at 3 and 7h respectively (P<0.05). The lipolytic effect of ISO was reduced by IN -70±3% (P<0.05), IN did not affect the lipolytic rates during LPS at 3 and 7h (45.2±22% and -16.3±16%). Compared to basal, LPS increased pHSL by 123±11% and compared to IN, it reduced the expression of pAKT by -90.2±12 (p<0.05). LPS increased mRNA expression of lipolytic (*LPL*, *LIPE*) and inflammatory (*IL6,* and *CCL2)* markers in AT (p<0.05). LPS triggers lipolysis and extended exposure increases insulin resistance in SCAT. Endotoxemic dairy cows may be more susceptible to periparturient lipolysis dysregulations and insulin resistance.

DISCOVERY OF A PREDOMINANT AND DISTINCT LINEAGE OF *MYCOBACTERIUM TUBERCULOSIS* IN BRAZILIAN INDIGENOUS POPULATION

Hadi SA[1], Kolte IV[2], Brenner EP[1], Cunha EAT[3], Simonsen V[4], Ferrazoli L[4], Villela DAM[2], Santos RS[2], Ravi J[1], Sreevatsan S[1], Basta PC[2] . [1] Michigan State University, East Lansing, USA [2] Oswaldo Cruz Foundation, Rio de Janeiro, Brazil [3] Central Laboratory of Mato Grosso do Sul, Campo Grande, Brazil [4] Adolfo Lutz Institute, São Paulo, Brazil

Tuberculosis (TB) is one of the leading infectious disease in the world that causes 4109 deaths daily with at least 1.8 billion people being infected in the world according to WHO. It inflicts immense economic damage and perpetuates poverty. The Guarani-Kaiowá people are an indigenous population living in Paraguay and the Brazilian state of Mato Grosso do Sul. They experience severe poverty, marginalization along with three times the incidence rate of TB than the rest of the nation. Yet so far, the TB transmission dynamics have remained largely unstudied in their communities. In this study, 66 *Mycobacterium tuberculos* isolates from local clinics were whole genome sequenced. A population genetic framework was generated, along with analysis of multi-drug resistance associated genes. The phylogenetics showed that the *M. tuberculosis* isolates in the Guarani-Kaiowá people clustered far from selected global reference strains, which suggested divergence from these lineages as well as within population diversification. Most isolates clustered in a single group, which was further characterized as *M. tuberculosis* sublineage 4.3.3. Analysis of drug resistance-associated genes showed numerous variants, with several unique changes fixed across dominant subclusters. We report that local *M. tuberculosis* strains have acquired unique polymorphisms in the Guarani-Kaiowá people, and drug resistance characterization is urgently needed to inform public health to ensure proper care and avoid further evolution and spread of drug-resistant TB.

IMPLICATING AN ALTERNATIVE SIGMA FACTOR IN THE MYCOBACTERIAL-MACROPHAGE INTERACTION

Elizabeth Haiderer, John Williams, Chris Colvin, Robert Abramovitch; Microbiology & Molecular Genetics, Michigan State University

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis and remains the leading cause of death due to infectious disease in the world. Highly adapted to infect and survive for years within the human lung, Mtb arrests phagosome maturation to avoid the acidic, lethal environment of the lysosome. The mechanism for this Mtb-host cell interaction remains unknown. We have identified a sigma factor which appears to be at least partly responsible for alterations to the phagosome. In its absence, the Mtb transcriptome displays greater induction of the PhoPR acid response pathway, indicating that the environment is increasingly acidic which correlates with phagosome maturation. To study this sigma factor, we have created a genetic deletion mutant and complement which will be used to perform macrophage and mouse infection studies, allowing us to characterize the ability of these strains to survive in different environments and to study phagosome maturation to determine at what stage maturation arrest occurs. We will create genetic knock-downs of the acid response genes such as *phoP* in the sigma factor deletion mutant background and determine the viability of these strains in the macrophage. Finally, we will use chromatin immunoprecipitation and sequencing to determine binding sites, allowing us to define the regulon and examine its role in the Mtb-host cell interaction.

TRANSCRIPTOMIC CHARACTERIZATION OF PULMONARY ARTERIAL HYPERTENSION TO IDENTIFY PATIENT-SPECIFIC THERAPEUTICS

Yajing Ji, Richard R. Neubig M.D. PhD Department of Pharmacology and Toxicology, Michigan State University

Pulmonary arterial hypertension (PAH) is a severe and life-threatening disease characterized by elevated blood pressure in the pulmonary circulation. PAH can be categorized into different subgroups, such as idiopathic PAH (IPAH) and PAH associated with other diseases (APAH). While current treatments slow PAH disease progression, they are ultimately not curative. There have been several studies which have transcriptionally profiled and compared control and PAH samples using either gene expression microarrays or RNA Sequencing. I have pooled the available PAH gene expression profiles from the public database together to increase the statistical power and allow for more robust statistical analysis of the data.

The cause and severity of PAH differs from patient to patient, suggesting that personalized therapeutic strategies may be superior to a one-size-fits-all approach. Instead of the five subgroups of PAH commonly used in clinic, I propose to categorize the PAH samples based on their gene expression patterns. We can measure activity of the relevant signaling pathways in PAH by projecting gene expression signatures, which correspond to activation of these pathways, onto the expression profiles of PAH samples. Based on the activation of signaling pathways, I plan to cluster PAH samples to investigate phenotype substructures. Then I would cross-reference the identified transcriptional signatures with those in the connectivity map, which is an atlas of the transcriptional response of cells to thousands of compounds and gene deletions. Ultimately, this analysis let us to identify genes relevant in PAH pathogenesis or identify compounds that reverse gene expression signatures associated with PAH.

COMPARISON OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTIONS IN THE NORTH-CENTRAL AND EASTERN USA IN BLACKLEGGED TICKS

Vishvapali Kobbekaduwa[1] and Jean I. Tsao[2], [1]Comparative Medicine and Integrative Biology, College of Veterinary Medicine, Michigan State University, [2]Department of Fisheries and Wildlife, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA

Anaplasma phagocytophilum (Ap) is the causative agent of granulocytic anaplasmosis in humans, dogs, and cats. Ap is a bacterium vectored by the blacklegged tick (*Ixodes scapularis).* Two major strains of Ap circulate sympatrically, the human pathogenic strain (Ap-ha) and the deer strain (Ap-V1). The main objective of this study is to compare the infection prevalence, density of Ap-infected blacklegged ticks, and the strain prevalence among six sites located in three regions of the blacklegged tick's range where the incidence of human anaplasmosis varies: the Midwest (MW: Michigan and Wisconsin), the Northeast (NE: Massachusetts and Rhode Island) and the Southeast (SE: Florida Tennessee and North Carolina). Questing ticks were collected by drag cloth from 2010– 2012 from Wisconsin, Massachusetts, Rhode Island, Florida, Tennessee and North Carolina. For a separate project, ticks were dragged sampled from 2016 – 2018 from most counties in Michigan. Collected ticks were identified using dichotomous morphological keys. Total DNA was extracted, and a real-time PCR was performed to screen for Ap. A nested PCR was performed to confirm the Ap positive samples and sequenced to determine the strain of Ap. The infection prevalence in adults ranged between 20% in Wisconsin and 0.6% in North Carolina. While in nymphal infection prevalence ranged between 7.6% in Wisconsin and 2.3% in Massachusetts. As cases of human anaplasmosis increase this study strives to improve our knowledge about regional differences in Ap by understanding the strain diversity, and how juvenile tick phenology affects Ap disease risk.

PURINERGIC RECEPTOR FUNCTION IN THE MCA FROM CLOPIDOGREL-TREATED RABBITS

Dawn Kuszynski, Barbara Christian, Anne Dorrance, and Adam Lauver Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI

Patients at risk for thrombotic events are often prescribed a $P2Y_{12}$ antagonist. Clopidogrel is the most commonly prescribed $P2Y_{12}$ antagonist due to its high efficacy, however it is associated with adverse bleeding events like intracerebral hemorrhage. Clopidogrel induces bleeding at lower doses than required to inhibit platelet aggregation, making it unlikely that the clopidogrel-mediated bleeding events are solely due to platelet inhibition. Other purinergic receptors, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$, are expressed in cerebral arteries; they may provide an alternative explanation for the intracerebral hemorrhages associated with clopidogrel. We hypothesized that clopidogrel, acting at these other receptors, impairs artery function to increase the risk of bleeding. Our aim was to determine clopidogrel's effect on the arterial responses produced by $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor activation. Middle cerebral arteries (MCA) were used as they are commonly associated with bleeding events. Rabbits were pretreated with vehicle or clopidogrel for three days prior to MCA isolation and cannulation in a pressure myograph. Myogenic tone was allowed to develop before assessing the constriction response of the MCA to selective P2Y agonists. There was no change in vasoconstriction with $P2Y_1$, $P2Y_4$, or $P2Y_6$ activation. $P2Y_2$ receptor activation caused contraction in MCAs from control rabbits, this was significantly impaired by clopidogrel pretreatment. This suggests clopidogrel has effects on the $P2Y_2$ receptor in the MCA which may contribute the cerebral bleeding side effects observed clinically.

GLYCOLYTIC REPROGRAMMING UNDERLIES INFLAMMATION AND FIBROSIS TO POLYLACTIDE-BASED PROSTHETICS

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Despite increasing interest in polylactide (PLA) as a biodegradable alternative to metallic implant materials used in arthroplasty and fracture repair, the mechanism underlying PLA-induced inflammation and fibrosis remains unknown. After surgical implantation, PLA degrades by hydrolysis into D-, L- or meso-lactic acid, with crystalline PLA (cPLA) containing less D-lactide than amorphous PLA (aPLA). Accumulation of PLA breakdown products over several weeks or months results in prolonged inflammation and excessive fibrosis, and this impairs bone healing and osseointegration. Although the basis for immune activation by PLA breakdown products is speculated to be due to lowering of surrounding tissue pH, a clearly described mechanism has not been elucidated. We developed and validated a mammalian in-vitro bioenergetic model to study immune cell response to PLA breakdown products. Our findings reveal that prolonged exposure of macrophages and fibroblasts to cPLA and aPLA degradation products alters overall bioenergetic (ATP) levels and results in significantly elevated glycolytic flux (glycolytic reprogramming) in a pH-independent mechanism. The bioenergetic alterations and glycolytic reprogramming caused by PLA degradation products are similar to those caused by endotoxin (lipopolysaccharide) which activates immune cells into an inflammatory and fibrotic phenotype. Furthermore, after prolonged exposure to cPLA and aPLA breakdown products, macrophages and fibroblasts reveal higher lactate-linked proton efflux, suggesting a novel role for monocarboxylate transporters in immune response to PLA degradation. By targeting this altered physiology and normalizing this response, it may be possible to deploy large PLA-based prosthetics to enable bone replacement and regeneration.

ISOPROSTANES REDUCE REACTIVE OXYGEN SPECIES AND APOPTOSIS IN A BOVINE MODEL OF OXIDATIVE STRESS

Ashley Putman, Jeff Gandy, and Lorraine Sordillo; Department of Large Animal Clinical Sciences, Michigan State University College of Veterinary Medicine

Transition dairy cattle are prone to oxidative stress, which has been associated with several economically important diseases and results in tissue macromolecule damage. Isoprostanes (IsoP) are molecules generated from interactions between free radicals and membrane phospholipids, thus serving as excellent indicators of free radical-mediated lipid damage during oxidative stress. Indeed, concentrations of IsoP change during times that dairy cattle are susceptible to oxidative stress, such as the transition period. Although excellent biomarkers of oxidative stress, the physiological role of IsoP remains largely unknown. As the vascular endothelium is a primary target of lipid peroxidation during oxidative stress, this experiment aimed to determine the effect of the most extensively studied IsoP, 15-F2t-IsoP, on bovine endothelial cells during oxidative stress conditions. Bovine aortic endothelial cells (BAEC) were incubated in the presence of 10nM 15-F2t-IsoP alone and in combination with known oxidizers 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipopolysaccharide (LPS). Statistics were performed by one-way ANOVA with Tukey's adjustment and significance of $p<0.05$. After a 12 h incubation period, 15-F2t-IsoP decreased ROS production or apoptosis in BAEC incubated with AAPH or LPS, respectively, compared to cells incubated with the oxidizer alone. The results of this study indicate that 15-F2t-IsoP may have a cytoprotective role during times of oxidative stress. Future studies should be directed toward investigating if IsoP alter other factors associated with vascular damage during oxidative stress. This research advances animal health by indicating how a well-known biomarker of oxidative stress in dairy cattle may contribute to the pathophysiology of economically important diseases.

RETINOIC ACID AFFECTS BARRIER INTEGRITY IN BOVINE MAMMARY ENDOTHELIAL CELLS

Jaimie Marie Strickland, Lorraine Marie Sordillo, Michigan State University, College of Veterinary Medicine

Reactive oxygen species (ROS) can cause tissue damage when countering antioxidant resources have been overwhelmed, such as during coliform mastitis, resulting more severe disease pathogenesis. All-trans retinoic acid (ATRA), the active form of vitamin A, has many important effects on inflammation such as repressing the damaging effects of oxidative stress in human and murine cell models. Previous studies showed that ATRA increased dramatically in the systemic circulation in dairy cows experimentally infected with coliform mastitis. However, it is unknown whether ATRA is cytoprotective or contributes to the pathogenesis of coliform mastitis. The objective of this research was to identify the effects of ATRA on bovine mammary endothelial cells (BMEC) viability and oxidative stress in-vitro. An in-vitro BMEC model of oxidative stress was used to evaluate cell viability. Cells were treated with 10 µM all-trans retinoic acid or the reactive oxygen producing compound, 2,2'-azobis-2-methylpropanimidamide, dihydrochloride (AAPH). Cell viability and reactive oxygen species were evaluated using fluorometric assays. Statistical analysis was performed with one- or two-way ANOVA with Graphpad Prism. The BMEC viability was unaffected by treatment with ATRA at 24 hours ($p>0.05$). ATRA reduced ROS production 75% when treated concurrently with AAPH (4.4 to 1.1 fold change compared to control, $p<0.0001$). Based on this data, ATRA has cytoprotective properties for BMEC in the face of oxidative stress. The precise method by which ATRA decreases oxidative stress in BMEC will need to be further investigated. This research indicates that ATRA may be protective against the pathologic effects of coliform mastitis.

EARLY LIFE STRESS IN PIGS INDUCES A DEVELOPMENTAL SHIFT IN GUT EPITHELIAL GLUCOSE TRANSPORTER SYSTEMS

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Early life adversity (ELA) is a significant risk factor for chronic inflammatory diseases into adulthood and has been linked to dysregulation of glucose homeostasis, which is recognized as a driver of inflammatory. The mechanisms by which ELA impacts glucose homeostasis and its implications for humans and animal disease risk remains poorly understood. Utilizing early weaning (EW) in piglets as a model of ELA, we tested that hypothesis that EW alters the normal developmental trajectory of intestinal epithelial glucose transporters. Yorkshire breed female piglets were weaned from their sows at 16 d of age (EW), or 28 d of age (LW, control LW). At 70 d of age, jejunum and ileum were collected and mounted on Ussing chambers to evaluate Na+-linked glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) function and expression. Jejunal and ileal SGLT1 transport, measured as the change in transepithelial short-circuit current (ΔIsc) after luminal glucose addition, was reduced ($P<0.05$) in EW pigs, compared with LW pigs. In contrast, intestinal GLUT2-mediated transport was increased ($P<0.05$) in EW pigs. Opposing effects of EW on SGLT1 and GLUT2 transport coincided with differential localization of SGLT1 and GLUT2 to the brush border membrane (BBM), suggesting altered intracellular trafficking in EW pigs. EW pigs exhibited elevated serum glucose and expression of GLUT in lymph node and spleen. Together, these data indicate that EW causes a developmental shift in intestinal glucose transport from SGLT1 towards GLUT2-mediated uptake. Given the increased transport efficiency of GLUT2, this shift may be a mechanism driving elevated serum glucose and increased tissue GLUT expression which in turn may contribute to increased lifetime risk for chronic inflammatory disease.

ENDOCANNABINOID CONCENTRATIONS AND RECEPTOR EXPRESSION IN CULTURED BOVINE ENDOTHELIAL CELLS CHALLENGED BY ENDOTOXIN

Carsten C F Walker[1] and Lorraine M Sordillo[1]
[1] Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Aberrant inflammation associated with coliform mastitis is a major contributor to the severity and potential lethality of systemic infections. The ability of the endocannabinoid (EC) system to modulate inflammation was shown in several non-bovine species. The EC system is comprised of the cannabinoid receptor 1 and 2 (CB1/CB2,respectively) and their ligands including fatty acid ethanol amides and glycerols. The EC arachidonoylethanolamide (AEA) was shown to possess anti-inflammatory properties through CB2 activation and can be metabolized by the cyclooxygenase-2 (COX-2) enzyme into prostamides. In contrast to pro-inflammatory prostaglandins also produced by COX2, several prostamides possess anti-inflammatory activities depending on cell type and species. Fluctuations of plasma EC concentrations were recorded in dairy cattle affected by coliform mastitis. However, the impact of EC expression on vascular endothelial cell function during lipopolysaccharide (LPS) challenge has not been elucidated. The purpose of this study was to elucidate changes in the EC system of cultured bovine aortic endothelial cells (BAEC) challenged with LPS. Taq-Man custom probes were used for QPCR. Quantification of EC was done by LC/MS. Receptor CB1 was more abundantly expressed after LPS treatment compared to media control, whereas CB2 was only expressed in LPS challenged cells, and was not detected in unchallenged cells. All EC concentrations increased with LPS exposure. Prostamide concentrations were only detectable after LPS treatment. Elevated AEA concentrations with LPS exposure and increased CB2 expression is indicative of possible involvement of the EC system in inflammatory regulation. Production of prostamides after LPS exposure may also be an inflammatory regulatory mechanism and warrants further investigation.

30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

Session	Friday, October 16, 2020 DVM Students **Please click this URL to join:** https://msu.zoom.us/j/92124588436 **Passcode: CVMPZD** **Moderator: Dr. Sri Sreevatsan**
10:30-10:45	**Kaitlyn Bailey[1]** Active Warming and Surgical Draping for Perioperatiave Thermal Support in Laboratory Mice (Claire Hankenson)
10:45-11:00	**Peter Fowler [1,3]** Antimicrobial Resistant Non-typhoidal *Salmonella enterica* Prevalence Among Poultry farms and Slaughterhouses in Chitwan, Nepal (Melinda Wilkins)
11:00-11:15	**Shaun Goulet[1]** The *Alouatta pigra* Diet: The Influence of Age on Diets (Dalen Agnew)
11:15-11:30	**Taylr Wells[1]** Assessment of Plasma 25-hydroxyvitamin d and Ionized Calcium Concentrations in Two Collections of Captive Magellanic Penguins (Ronan Eustace)

*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

30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

Session	Friday, October 16, 2020 DVM Students Please click this URL to join: https://msu.zoom.us/j/92213006640 Passcode: CVMPZD **Moderator: Dr. Jane Manfredi**
12:15-12:30	**Haley Abbott[1]** The Prevalence of Enthesophytes in Equine Tarsal Radiographs (Jane Manfredi)
12:30-12:45	**William Pochal[1]** Spontaneous Background Arrhythmia Incidence in Canine, Swine, and Non-Human Primates. (Adam Lauver)
12:45-1:00	**Sierra McClain[1]** SARS-CoV-2 Phylogenetic Evolution S Gene in Human and Animal Isolates Using GISAID (Sri Sreevatsan)
1:00-1:15	**Anna Mukhina[1]** Genetic Mechanisms of Resistance to BTK Inhibition in Canine Diffuse Large B-Cell Lymphoma (Bonnie Harrington)
1:15-1:30	**Jessica Zenchak Petersen[1]** Examining Differences in Equine Serum and Whole Blood Selenium Reference Intervals (John Buchweitz)
1:30-1:45	**Ryan Hopper[1]** Use of Neuroprotective Therapies by Veterinary Ophthalmologists for Canine Retina and Optic Nerve Diseases (András Komáromy)
1:45-2:00	**Antonia Langfeldt[1]** Comparing the Effectiveness of Manual Tools to Perform Femoral Head and Neck Excision (Jane Manfredi)
2:00-2:15	**Shelbey Todd[1]** *M. kansasii*: Evolution, Diversity, and Diagnostics (Sri Sreevatsan)
2:15-2:30	**Kimberly Guzmán[1]** Systematic Review Investigating the Comparative Efficacy of Regenerative Therapies in Horses with Tendonitis and Desmitis (Aimee Colbath)
2:30-2:45	**BREAK** **Moderator: Dr. Sri Sreevatsan**
2:45-3:00	**Zachary Woloszyk[1]** Down Under in the Land Down Under: Monotreme Reproduction (Dalen Agnew)
3:00-3:15	**Eric Spilker[1]** Effect of Chemotherapy Agents on Fecal Microbiome of Canine Lymphoma Patients (Linda Mansfield)

*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

30th ANNUAL PHI ZETA RESEARCH DAYS

Presentations Schedule

3:15-3:30	**Olivia Child[1]** Assessment of Companion Animals for Novel Coronavirus (SARS-CoV-2) Infection (Daniel Langlois)
3:30-3:45	**Jessica Hynes[1]** Impact and Prevalence of Cranial Cruciate Ligament Disease in Field Trial Labrador Retrievers (Sarah Shull)
3:45-4:00	**Hailey Penticoff[1]** Survey of Amyloidosis Cases among Different Wildlife and Zoo Species (Jessica Fortin)
4:00-4:15	**Carmen-Maria Garcia[1]** Impact of Early Life Stress on the Activation of Gastrointestinal Mast Cells (Adam Moeser)
4:15-4:30	**Lindsay Woods[1]** Equine Herpes-Virus 1 and 9 Infection in Addra Gazelle (Nanger *dama ruficollis*) (Kimberly Thompson)
4:30-4:45	**Megan Allen[1]** COVID-19 and its Effects on the Human Animal Interaction (Kirk Munoz)
4:45-5:00	**Hannah Hipkiss[1]** Propensity of Serum Amyloid A to Misfold and Aggregate in Zoo, Exotic and Wildlife Animals(Jessica Fortin)

*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

THE PREVALENCE OF ENTESOPHYTES IN EQUINE TARSAL RADIOGRAPHS

Haley Yule Abbott[1], Jody Lawver[2], Jose M. Casillas[3], Jane M. Manfredi[1] 1)Department of Pathophysiology and Diagnostic Investigation, 2)Department of Small Animal Clinical Sciences, 3)Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Traditionally tarsal pain has been attributed to osteoarthritis (OA) and osteochondritis dessicans (OCD), with less diagnosis of injury to soft tissue structures. With previous studies demonstrating that severity of lameness is unassociated with degree of radiographic change, there is an increasing interest in evaluating tarsal soft tissue pathology. Recent work detailing the radiographic attachments of soft tissue structures in the tarsus can help practitioners to identify enthesiophytes. The purpose of this study is to determine the prevalence of radiographic abnormalities consistent with tarsal soft tissue injuries and compare its inclusion in the medical record. Descriptive statistics were performed. Tarsal radiographs and medical records were reviewed from 326 horses admitted to the Michigan State University Veterinary Teaching Hospital between 5/2015 to 5/2020. The mean ± SD age was 8.3 ± 5.9 years, sex was 54.2% geldings, 32.6% mares, and 7.3% stallions. Breeds included Quarterhorses (17.2%), Thoroughbreds (13.6%), Standardbreds (10.3%), Warmbloods (9.2%), and other (49.7%). In this study, 95% of patients had mild to severe radiographic changes associated with soft tissue attachment sites. The most common structures affected were the: medial collateral ligaments (65%), lateral collateral ligaments (60%), suspensory ligament (57.9%), and long plantar ligament (52.9%). 33.3% of cases had a soft tissue lesion described in the medical record. During clinical assessment, diagnoses were: 30.6% soft tissue lesions, 50.2% OA and 16.5% OCD. Our results suggest that soft tissue injuries in the tarsus may be more prevalent than previously believed and could be under-diagnosed in practice.

ACTIVE WARMING AND SURGICAL DRAPING FOR PERIOPERATIVE THERMAL SUPPORT IN LABORATORY MICE

Kaitlyn T. Bailey[1], Sanket Jantre[2], Frank Lawrence[2], F. Claire Hankenson1, Jacquelyn M. Del Valle1 1)College of Veterinary Medicine, Campus Animal Resources;2)Department of Statistics

Surgical research procedures are commonly performed using mice but can have major effects on their core body temperature including development of hypothermia. The aim of this study was to evaluate perioperative warming options for rodent surgery to improve animal welfare and optimize research experiments. Mice (CD1 strain) were randomized into treatment groups (n=6; 8 mice per group); treatments included exposure to a small animal critical care incubator set to 38°C for 30 minutes either before surgery(Pre), after surgery (Post) or before and after (Both), using surgical draping with transparent sheeting (Control/Drape, Both/Drape). The control group received neither intervention (Control). Mice were anesthetized with ketamine/xylazine and a laparotomy performed, leaving the abdominal cavity exposed for 10 minutes. Post-operatively, mice were placed in a standard cage on a warm water blanket set to 38°C or were placed back into the incubator. Rectal temperatures were taken every minute during surgery; subcutaneous temperatures were collected at several points perioperatively from transponders placed at least 72 hours prior to surgery. Anesthetic recovery was documented as the return to righting reflex. Mice that were prewarmed in the incubator demonstrated significantly increased body temperatures after 30 min compared to non- prewarmed (p <0.0001). Mice after incubation (Pre, Post, Both, Both/Drape) had significantly longer anesthetic recovery times compared to mice that did not receive incubator warming (Control, Control/Drape; p=0.0001), but were subjectively more alert when return of righting reflex was noted. In summary, perioperative warming though incubation provides superior thermal support to laboratory mice enrolled in surgical procedures.

COVID-19 AND ITS EFFECTS ON THE HUMAN ANIMAL INTERACTION

Megan Allen[1], Jane Manfredi[2], and Kirk Munoz[3], 1) College of veterinary medicine summer research program, 2) Pathobiology and Diagnostic Investigation, 3) Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

Pets are a vital part of people's lives, and the COVID-19 pandemic may have impacted how we interact with them. Our hypotheses are that due to COVID-19, non-medically trained (NMT) people would have increased awareness of interactions with their pets, there would be a greater impact on cat and dog owners than horse owners, and there would be a negative impact on those procuring veterinary care during stay-at-home orders (SAHO). An anonymous email survey, open from May to June 2020, was sent to staff, faculty and students of Michigan State University's College of Veterinary Medicine (CVM), active veterinary hospital clients within the last year, and a random number of non-CVM faculty and staff. Inclusion criteria was dependent on owning at least one dog, cat, or horse. Questions included areas such as handwashing, frequency of walks/rides, and access to veterinary care. Statistical analysis included ANOVA, chi square tests, and thematic assessment. Significance was set at p<0.05. There was a 15.3% survey response rate overall (19% medically trained (MT) and 81% NMT). Dog owners had the most marked differences in behaviors during the SAHO, primarily with not allowing their dog to have direct contact with other dogs and people outside their households (p<0.01). Many people postponed veterinary care (40.7% routine care and 7.4% care for a medical issue). Only 2.5% of people were unable to find veterinary care. Behaviors between MT and NMT did not differ as much as expected, however there were significant differences when comparing pre and during SAHO.

ASSESSMENT OF COMPANION ANIMALS FOR NOVEL CORONAVIRUS (SARS-COV-2) INFECTION

Olivia Child, Steve Bolin[1], Daniel Langlois[2], 1) Department of Pathobiology and Diagnostic Investigation, 2) Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University

The novel coronavirus (coronavirus disease 2019 [COVID-19] or severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) has rapidly led to a global pandemic after its original description in late 2019. Companion animals have become a point of interest amidst reports of natural infections in dogs, cats, and large felids following exposure to COVID-19 infected humans. The susceptibility of companion animals to COVID-19, and their role in disease transmission, remains unknown. The objectives of our study were to (1) identify COVID-19 infections in dogs and cats through RT-PCR testing of nasal and pharyngeal swabs and through serologic testing with a virus neutralization assay, (2) to characterize clinical signs associated with infection, and (3) to identify pet and owner related factors that are associated with infection. Samples were collected from pets undergoing veterinary evaluation, and owners were surveyed to obtain information related to both pet and owner health and exposure risk. Overall, 2 dogs and 1 cat were PCR positive for SARS-CoV2. None of the affected animals had respiratory signs or fever, nor were they exposed to known infected humans. All XX serum samples (xx feline and xx canine) had virus neutralization titers ≤ 16. The patient population was generally low risk as very few pets were exposed to known infected humans. These results highlight that the overall prevalence of SARS-CoV-2 infections in pets is low, but subclinical infections might be more common than previously speculated. Additional studies are needed to determine if pets are capable of transmitting infection to household members.

ANTIMICRIOBIAL RESISTANT NON-TYPHOIDAL *SALMONELLA ENTERICA* PREVALENCE AMONG POULTRY FARMS AND SLAUGHTERHOUSES IN CHITWAN, NEPAL

Peter D. Fowler[1], Sumit Sharma[2], Dhan Kumar Pant[3], Subir Singh[2], Melinda J. Wilkins[1]

1. Michigan State University, College of Veterinary Medicine,
2. Agriculture and Forestry University,
3. National Zoonoses and Food Hygiene Research Centre

Antibiotic resistant (AMR) infections are one of the leading threats to public health globally. Indiscriminate use of antibiotics in food animal production is an important driver of resistance, particularly among foodborne pathogens like Non-typhoidal *Salmonella enterica* (NTS). While there has been extensive research on AMR *S. enterica* globally, there have been few studies in countries in South Asia including Nepal. This descriptive study seeks to identify the prevalence and resistance patterns of poultry-associated NTS spp. in Chitwan, Nepal's leading poultry producing district. Samples were collected from 18 poultry farms and 20 slaughterhouses across 13 villages of Chitwan. Samples were cultured and biochemically tested for the presence of NTS. Positive isolates were serotyped and tested for antimicrobial resistance. Out of 708 samples (288 environmental and 420 biological), 103 (15%) tested positive for NTS: 26 (9%) environmental and 77 (18%) biological. Out of 103 positive *S. enterica* isolates, 48.5% were identified as *Salmonella Typhimurium*, 35% *Salmonella Enteritidis*, 7.8% *Salmonella Gallinarum* 4.9% *Salmonella Virchow* and 3.9% were *Salmonella Agona*. Of the 103 positive isolates, 80 (78%) were resistant to at least one antibiotic and 21 (20%) were multidrug resistant. Resistance was highest against cefotaxime (48%), followed by doxycycline (35%), nalidixic acid (19%), enrofloxacin (18%), ampicillin (13%), ciprofloxacin (5%) colistin (4%), chloramphenicol (3%), ceftazidime (3%) and gentamicin (2%). This baseline data has important implications for poultry production and consumption, providing the opportunity to update guidelines for improved therapeutic management practices. Data-driven management practices can help improve both human and animal health in the region.

IMPACT OF EARLY LIFE STRESS ON THE ACTIVATION OF GASTROINTESTINAL MAST CELLS

Carmen-Maria Garcia, Neco Wilson, Kyan M Thelen, Mahsa Fardisi and Adam J Moeser.

Early weaning (EW) is a common practice in pig production, which causes high levels of stress during a critical period of gastrointestinal (GI) immune development. We previously showed that EW causes long-term deleterious changes in GI barrier and immune development and increased disease risk compared to piglets weaned at a later age (LW). How EW triggers long-term developmental alterations in GI function and disease is poorly understood. Our lab showed that in comparison to LW, EW piglets exhibit higher plasma levels of histamine, a major product of mast cells (MC), and increased intestinal histamine receptor expression. MC activation and histamine may play an important role in initiating intestinal inflammatory responses that can deleteriously impact GI development. The objective of this research is to understand why EW piglets exhibit higher plasma histamine levels and intestinal histamine receptor expression. We hypothesize that the EW piglets that have increased numbers of intestinal MCs which will correlate with higher plasma histamine levels. Ileum was harvested from 15d old (EW) and 26d old (LW) piglets at 0hr and 24hr post-weaning, and then stained with Toluidine blue to identify MC; they were then quantified and categorized. Learning how wean age influences early intestinal MC activation could lead to management, nutritional or therapeutic interventions to mitigate the long-term deleterious impacts of EW to improve animals' well-being, growth performance and disease resilience. Further, given the biological similarities between pigs and humans, this research could increase our understanding of the link between early life stress and susceptibility to chronic GI-related diseases, such as IBS, in humans.

THE *ALOUATTA PIGRA* DIET: THE INFLUENCE OF AGE ON DIETS

Shaun Goulet, Dalen Agnew, Jane Thomas. College of Veterinary Medicine, Michigan State University, East Lansing, Michigan (Goulet, Agnew); Department of Human Evolutionary Biology, Harvard University, Cambridge, Massachusetts (Thomas)

Zoo nutritionists must design diets that complement the needs of animals in all age groups. Different species require different nutrients to remain healthy; however, there is less knowledge on age-related variation in dietary needs within a species depending on age. Therefore, age-based studies that observe the dietary patterns of wild populations can help to better formulate diets for captive animals. This study aims to examine the dietary patterns of Yucatán/Guatemalan black howler monkeys in the region of Tabasco, Mexico, specifically, how age influences their diets. The null hypothesis is that there is no statistically significant difference between the eating patterns of adult versus juvenile *Alouatta pigra*. Data for this project was collected in 2015 through a series of focal studies/observations. Individuals fell into two age groups: juvenile (N=4) and adults (N=9). The time spent consuming each plant was recorded as a percentage of the total time the individual spent eating. Preliminary results indicated that age does not have an influence on *Alouatta pigra* diets, meaning there is no difference between the plant choice of adults versus juveniles. Multiple unpaired t-tests along with further analyses, indicate that there is no statistically significant difference in the consumption rates of plant species between the adult and juvenile *Alouatta pigra* in this study. These results may not be valid for other species, however, so further intraspecies dietary studies in other species are highly encouraged to improve the longevity and quality of life of animals cared for in captivity.

SYSTEMATIC REVIEW INVESTIGATING THE COMPARATIVE EFFICACY OF REGENERATIVE THERAPIES IN HORSES WITH TENDONITIS AND DESMITIS

Kimberly E. Guzmán, Char Panek, Aimee Colbath, and Annette M. O'Connor, College of Veterinary Medicine, Large Animal Clinical Sciences, Michigan State University, East Lansing, MI

The aim of the systematic review will be to evaluate the literature for the comparative efficacy of MSC and PRP therapy for equine tendonitis and desmitis, focusing on return to performance and reinjury rate in studies that have at least a 6 month follow up period. Ligament (desmitis) and tendon (tendonitis) injury are common musculoskeletal injuries in sport horses. Unresolved ligament and tendon tears in horses can prevent return to athletic performance resulting in loss of use and potential economic loss. Intra-lesion injection of platelet rich plasma (PRP) and mesenchymal stem cells (MSC) have been assessed in experimental and clinical studies for treatment of equine musculoskeletal disorders. A systematic review evaluating MSCs vs. PRP vs. no treatment has not been conducted. Knowledge of the comparative efficacy would enable informed decisions about treatment options. It is also important to assess adverse events associated with these interventions, such as local or systemic inflammation. All current studies published in PubMed, CABI, and equine and orthopedic conference proceedings will be evaluated for relevance to the review. The eligible population is previously active horses diagnosed with tendinitis and desmitis who are expected to return to normal performance following treatment. Eligible studies include parallel and historical cohort studies and controlled trials. We will extract the effect size for days to return to function, reinjury risk and adverse event risk and use metaanalysis to estimate the pooled effect size and report the 95% confidence interval as a measure of our uncertainty about pooled effect size.

PROPENSITY OF SERUM AMYLOID A TO MISFOLD AND AGGREGATE IN ZOO, EXOTIC AND WILDLIFE ANIMALS

Hannah K. Hipkiss[1] and Jessica S. Fortin[1,2] ,1) College of Veterinary Medicine, 2) Department of Pathobiology and Diagnostic Investigation, Michigan State University

AA Amyloidosis is characterized by the deposition of fibrils in the liver, spleen, and kidneys. These deposits are comprised of fragmented serum AA (SAA), a protein that is synthesized by the liver during states of chronic inflammation. SAA is highly prone to misfold and aggregate due to high density of β-sheet. Fibrils are generated through a seeded-nucleation process, inducing neighboring healthy SAA peptides to elongate into mature fibrils and aggregate in the tissue. The accumulation of insoluble fibrils results in subsequent tissue damage and loss of function (organ failure). AA amyloidosis has been reported in humans, domestic and wild animals, with prevalence, clinical presentation, and pathogenesis of the disease varying between species. Transmission of AA amyloidosis is an investigative topic that has significance in zoos and captive management of exotic animals due to the interspecific transmissibility of AA amyloid fibrils. This project aims to evaluate the propensity of amyloid formation in zoo and wildlife animals by designing serum AA peptide fragments and in-silico aggregation profiling to determine species-specific risk of disease. Bioinformatic algorithms including TANGO and Aggrescan were used to calculate aggregation probability of the residue fragment (1-25 amino acids) and highlight regions of the peptide that were prone to misfolding. Protein sequence alignment of SAA was assembled via BioEdit to identify variance, and a phylogenetic analysis was performed to determine potential amyloidogenic lineages in MEGA. Future steps include a combination of biophysical assays of the designed peptide library to pinpoint region(s) or residues that contribute to SAA misfolding.

RETINOIC ACID AFFECTS BARRIER INTEGRITY IN BOVINE MAMMARY ENDOTHELIAL CELLS

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In veterinary medicine, there are numerous neurodegenerative conditions that cause blindness in canine by damaging the retina and or optic nerve head. These conditions include glaucoma, sudden acquired retinal degenerative syndrome (SARDS), progressive retinal atrophy (PRA), and retinal detachment. Various treatments that have been developed for the sole purpose of neuroprotection, and some already developed treatments have been found to provide neuroprotection as a secondary effect. However, no drug has passed clinical trials to earn approval from the FDA, both in the veterinary and human field, for neuroprotective purposes. Despite lack of FDA approval, some veterinarians prescribe treatments for neuroprotection as a last resort effort since many neurodegenerative conditions will, at some point, cause loss of vision. Thus, we developed a survey to gauge whether veterinary ophthalmologists on which treatments they use and the efficacy of the treatment in their experience. By evaluating these responses, our goal is to determine the efficacy of these treatments as neuroprotectants in order to stimulate future research and neuroprotective drug development for ophthalmic treatments.

IMPACT AND PREVALENCE OF CRANIAL CRUCIATE LIGAMENT DISEASE IN FIELD TRIAL LABRADOR RETRIEVERS

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Cranial cruciate ligament disease (CCLD) is the most prevalent orthopedic problem in canines, affecting 2.6-4.9% of dogs, causing stifle instability, mobility dysfunction and pain. The objective of the study was to evaluate the field trial community's knowledge of CCLD and estimate its prevalence in this population. A survey instrument was created, validated, and emailed to a population of retriever field trial participants to collect information on perceptions and experience with CCLD, and experience with field trials, as well as dog specific data. Statistical analyses included: descriptive statistics, Chi square tests and thematic analyses with the significance set at P<0.05.

The majority of respondents appropriately identified the connection between genetics and CCLD (68.7%). However, there was under-recognition (22.8%) of the degenerative nature of the disease, with 60.7% inappropriately identifying trauma as the main cause. Respondents also indicated that diagnosis of a dog's sibling or offspring affected their breeding decisions less than diagnosis of their parents, indicating a misunderstanding of genetics. More than half of respondents indicated prior experiences with CCLD. Finally, the prevalence of CCLD was found to be 11.8% in field trial Labrador Retrievers (72/610) which is higher than the 5.8% prevalence in all Labradors indicated by previous studies. These results indicate a lack of knowledge in the field trial community regarding the causes of CCLD, which could result in perpetuation of this disease. The increased prevalence of CCLD in this population demonstrates the potential impact of the disease on this community.

COMPARING THE EFFECTIVENESS OF MANUAL TOOLS TO PERFORM FEMORAL HEAD AND NECK EXCISION

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This study compared femoral head and neck excisions using manual tools against ideal computer-generated ostectomies. Three canine and three feline cadavers had bilateral surgery performed using Gigli Wire on one leg and an osteotome contralaterally. Three-dimensional models were created from Computed Tomography images, and an ideal ostectomy was made on pre-surgical models and compared with post-surgical models evaluating ostectomy angles, bone volumes, and part comparisons. Surgery duration and time to perform ostectomies were recorded. Statistical analyses included Student's t-tests and Fisher's exact tests with significance set at p<0.05. Surgery, but not ostectomy, duration was longer for osteotome procedures (p<0.05). Mean ±standard deviation differences for ostectomy angle and femur volume were 12.90 ±8.49° (Gigli Wire) and 18.59 ±9.46° (osteotome), and 205.30 ±116.45mm^3 (Gigli Wire) and 205.17 ±169.08mm^3 (osteotome), respectively. All femurs had residual bone (242.65 ±172.99mm^3 Gigli Wire; 230.30 ±190.37mm^3 osteotome), often at the caudal edge of the femoral neck. Seven femurs (3/6 Gigli Wire, 4/6 osteotome) had excess bone removal (19.15 ±41.66mm^3 Gigli Wire; 92.51 ±188.11mm^3 osteotome). Part comparison analyses allowed more accurate identification of residual bone regions. Both tools performed equally and may be used to perform femoral head and neck excision, but it is recommended to check for residual bone at the caudal edge of the neck and avoid excessive bone removal.

SARS-COV-2 PHYLOGENETIC EVOLUTION S GENE IN HUMAN AND ANIMAL ISOLATES USING GSAID

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SARS-CoV-2, the virus causing COVID-19, which was first reported in Wuhan, Hubei Province, China in late December 2019. Studies have proposed that the origins of the virus may stem from bat colonies, where betacoronaviruses possibly serve as a reservoir. Currently, SARS-CoV-2 vaccine efforts target the spike glycoprotein, important mediators in host cell receptor binding and cell membrane fusion. However, little information is available concerning the relationship between the closest SARSCoV-2 viral relatives spreading through animal populations. Our project goal is to phylogenetically map the evolution of the SARSCoV-2 spike protein, of human and animals infected with COVID19. Understanding relatedness of COVID-19 circulating animal strains of coronaviruses will assist studies of viral function, contributing to vaccine development. GISAID global database interrogated and full genome sequences of 90 human SARSCoV2 strains and 22 animal strains spanning January 2020 to June 2020, were downloaded. Sequences were aligned to the S gene and trimmed using MEGA X. A phylogenetic tree will be constructed using clade specific human reference strains from Wuhan, China (original outbreak 11/01/2019-01/31/2020) and cross referenced against all available animal strains. BEAUti & BEAST software will account for time of divergence of animal SARS-CoV-2 and identification of amino acid changes that resulted in human host specificity. We provide evidence of human SARS-CoV-2 strains have proximal origins with horseshoe bat coronaviruses (RaTG13) and Malaysian pangolins. Additionally, mink, canine, and feline SARS-CoV-2 strains appear to originate from human SARS-CoV-2. Understanding relationships of circulating coronaviruses in animal populations help inform future studies of viral function.

GENETIC MECHANISMS OF RESISTANCE TO BTK INHIBITION IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is a common, aggressive cancer affecting both humans and dogs, and effective therapies without toxicities are lacking. In a previous study, our laboratory performed a clinical trial to investigate the efficacy of BTK inhibition in canine DLBCL using the BTK inhibitor acalabrutinib. The objective response rate was 25%; thus, most patients demonstrated primary resistance. In the current study, we performed whole exome sequencing of canine lymphoma cells before and after acalabrutinib therapy to discover mutations in major drivers of B-cell cancers that could propagate cell survival and proliferation despite proximal BCR inhibition with acalabrutinib. We identified 242 unique variants affecting 202 different genes, of which 39 were high impact mutations and 203 were moderate impact. On average, there were 21.7 variants (median=21.5 variants). We identified frequent mutations in the genes *P53, DDX3X, MYC, MAP3K14, POT1, TRAF3, FBXW7, SETD2, TM9SF3, AMBRA1, CSMD1, FRAS1, PAK2, RAB2A, RAB38, ENSCAFG00000014207, ENSCAFG00000029236, ENSCAFG00000029996, ENSCAFG00000032078*, and *ENSCAFG00000030258*. Additionally, we identified a compelling increase in mutation frequency of *SETD2*, though this difference lacked statistical significance. Further investigations must be performed to verify trends that lacked statistical significance. These data will guide future investigations to determine how specific mutations and genes alter molecular pathways driving lymphoma cell survival and drug resistance.

SURVEY OF AMYLOIDOSIS CASES AMONG DIFFERENT WILDLIFE AND ZOO SPECIES

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Amyloidosis regroups a range of diseases (i.e. protein-folding disorders) characterized by a buildup of amyloid deposits in one or multiple organs. The pathogenesis and pathologic findings of amyloidosis can vary widely due to the nature of the precursor protein. In veterinary medicine, there are ten proteins known to form amyloid deposits in various organs. This review aims to compare amyloidosis cases among different wildlife and zoo animals focusing on the determination of the 1) species particularly susceptible to amyloid formation, 2) prone-to-aggregate protein commonly involved, and 3) associated condition(s) that might trigger fibril formation. This review discusses about transmission of AA amyloidosis which is relevant in institutions such as zoos, housing multiple individuals and species in relatively close proximity. Additionally, this review includes guidelines for reporting single or multiple cases of amyloidosis. Insights into the diversity, transmission, and pathogenesis of known amyloidogenic proteins and species prevalently affected may help to establish preventive intervention and stimulate the discovery of new therapeutic strategies.

SPONTANEOUS BACKGROUND ARRYTHMIA INCIDENCE IN CANINE, SWINE AND NON-HUMAN PRIMATES

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New compounds used to pursue advancements in drug therapy can be accompanied by life threatening side effects. Drug induced arrhythmia is a serious concern. An important process in the development of new medications is the use of electrocardiogram (ECG) telemetry analysis in preclinical studies to evaluate drug safety. However, some arrhythmias occur naturally and can be influenced by diurnal effects, interindividual differences, and species-specific differences which confound these studies. To address this issue, we studied the incidence of spontaneous background arrhythmias in healthy canine, swine, and cynomolgus monkeys. Our goal is to improve the accuracy of pharmacologic and toxicologic drug studies by expanding our ability to discern background arrhythmias from drug induced arrhythmias. To accomplish this, retrospective analysis of continuous 24 h telemetry data from male canine (n=12), swine (n=12) and cynomolgus monkeys (n=12) were analyzed for arrhythmias using Rhythm Express software (VivaQuant, LLC). Arrhythmia type, incidence, and time of day were identified and interindividual comparisons were made within and between species. We determined variability of ventricular arrhythmias, atrial arrhythmias, and premature junctional complexes for all three species and found indications of diurnal effects on arrhythmia incidence distinct to each species and found differences between individuals of the same species. Time of day and interindividual differences may play a key role in the future design of predictive preclinical drug studies. By understanding diurnal effects and normalizing background arrhythmia incidence in pre-treatment individuals we can better evaluate risk.

EFFECT OF CHEMOTHERAPY AGENTS ON FECAL MICROBIOME OF CANINE LYMPHOMA PATIENTS

Eric Spilker, Hinako Terauchi and Linda Mansfield

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 vincristine would decrease the diversity of the canine fecal microbiome and create dysbiosis of 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 in the microbiome. From each dog, 16s sequencing data from ten longitudinal samples were analyzed using QIME2 and bacterial taxa assignments made using the SILVA database. During intervals of vincristine treatment, changes in the relative abundance of bacterial taxa were observed using heatmaps and percentage of change phylogeny plots. These plots provided evidence that vincristine administration resulted in microbiota shifts within the canine gastrointestinal tract. Shannon diversity plots created exhibited distinct changes in diversity corresponding to CHOP chemotherapeutic administration. The specific bacterial taxa affected suggested that commensal bacteria decreased, and opportunistic pathogens increased. Further analysis will focus on determining if a significant association exists between the GI microbiota changes observed and clinical signs of CIGD.

ASSESSMENT OF PLASMA 25-HYDROXYVITAMIN D AND IONIZED CALCIUM CONCENTRATIONS IN TWO COLLECTIONS OF CAPTIVE MAGELLANIC PENGUINS

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Vitamin D is essential for the maintenance of calcium and bone metabolism in vertebrates. Deficiency of vitamin D can contribute to a variety of forms of metabolic bone disease (MBD). MBD has been documented in various species of birds. The objective of this study was to evaluate whether plasma 25-hydroxyvitamin D and ionized calcium (iCa) concentrations differ between indoor housed Magellanic penguins without access to UVB lights and outdoor housed penguins exposed to natural sunlight. Frozen banked plasma samples from 16 healthy, captive Magellanic penguins (n=10 outdoor housed, n=6 indoor housed) with similar husbandry practices were evaluated. Mean +/- standard deviation plasma 25-hydroxyvitamin D concentrations did not differ significantly (P=0.372) between the penguins housed indoors (48.9 +/- 16.7 nmol/L) and those housed outdoors (50.9 +/- 26.5 nmol/L), however plasma iCa concentrations differed significantly (P=0.02) between indoor (1.04 +/- 0.09 mmol/L, n= 5) and outdoor (1.21 +/- 0.02 mmol/L, n=3) penguins. The results suggest that indoor housed penguins without access to UVB have comparable levels of vitamin D to outdoor housed penguins. To evaluate if plasma could be used to analyze vitamin D and iCa in penguins, tandem serum and plasma samples were submitted from one zoo population. iCa concentrations in plasma (1.21 +/-0.02 mmol/L, n=3) and serum (1.29 +/- 0.34 mmol/L, n=6) differed significantly (P= 0.01), however the obtained 25-hydroxyvitamin D values were not significantly different (P= 0.429, n=6). These results suggest that banked plasma samples may provide an accurate representation of vitamin D levels in Magellanic penguins.

M. KANSASII: EVOLUTION, DIVERSITY, AND DIAGNOSTICS

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Mycobacterium kansasii is a pulmonary mycobacterial infection that mimics *M. tuberculosis*, *M. bovis* and the *M. avium* complex pathologically and histologically. Distinguishing these infections is extremely difficult with current diagnostic techniques in cattle. *M. kansasii* has been split into its own complex further complicating differentiation. Our lab has whole genome sequenced and assembled a *M. kansasii* isolate from a bovine granuloma. I annotated the genome, and developed a phylogenetic tree to define its relatedness to other *M. kansasii* lineages. I used the RAST server to annotate our genome, selected genes and differentiating sequences were pulled from our *M. kansasii* genome as well as from reference isolates. These were concatenated, and aligned using ClustalW via MEGA X. The alignment was used for phylogenetic analysis as well as for highlighting regions of difference between species. Selected unique regions of difference will be examined and confirmed by BLAST and confirmed by PCR. A set of potential diagnostic targets will be proposed from this pool.

DOWN UNDER IN THE LAND DOWN UNDER: MONOTREME REPRODUCTION

Zachary Woloszyk and Dalen Agnew

Monotremes (Monotremata) are one of the oldest living taxon of the class Mammalia that branched off from the lineage two hundred million years ago. These mammals exist today as one of five extant species. This includes four distinct species of echidnas (Tachyglossidae), three of which are long-beaked (Zaglossus) and one of which is short-beaked (Tachyglossus); and the famous amphibious duck-billed platypus (Ornithorhynchidae). All five of these species are native to "the land down under", Australia. Australia as a continent has a very diverse system of mega-habitats, ranging from tropical forests and deserts, to grasslands and tundra. The survival, conservation, and reproduction of these species in this varied environment begs the question of why these mammals lay eggs and does this provide some evolutionary benefit in a highly varied continent like Australia? One of the main reasons for their survival is credited to the complex reproductive tract that both male and females utilize. The reproductive tracts in monotremes are seen to bridge aspects of reptilian, avian, and even mammalian reproduction. The focus of this review article is to dissect the intricacies of reproductive anatomy of the two subgroups of monotremes, both male and female. This information might provide insight as to what conservation endeavors can be implemented to better understand the impact these organisms have on the natural world and how we can support the populations and acknowledge these iconic species.

EQUINE HERPES-VIRUS 1 AND 9 INFECTION IN ADDRA GAZELLE (*NANGER DAMA RUFICOLLIS*)

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In domestic and wild equids equine herpes-virus (EHV) infection can range from asymptomatic carriers to clinical disease resulting in respiratory signs, abortions, and myeloencephalopathy. Cases of EHV at zoos have been associated with zebra as the reservoir responsible for causing fatal myeloencephalopathy in a variety of non-equid spp. including; giraffes (*Giraffa camp*), Thomson's gazelles (*Eudorcas thomsonii*), and polar bears (*Ursus maritimus*).Binder Park Zoo had an outbreak of EHV-1 in 2020 that affected 50% of the addra gazelle (*Nanger dama ruficollis*) herd at the zoo (n=4). A 9 mo. male presented with dehydration, head tilt, OD eye lid paresis, circling, and exertional myopathy. Animal responded to supportive treatment, however condition declined after 7 days from worsening vision deficits from EHV associated retinitis resulting in euthanasia. 72 hours later this animal's dam presented recumbent with convulsions and died. Animals diagnosed with multifocal histiocytic to lymphoplasmacytic meningoencephalitis. PCR on brain tissue was negative for the male and positive for the female; zebra associated EHV-1 strain T-616 and isolate B6 404 identified with bidirectional sequencing. Immunohistochemistry of male's retinal tissue positive for EHV-1. Banked serum samples from the surviving herd members and a subset of the zebra pop. evaluated for titers to EHV-1 via serum virus neutralization. Another case of death in addra gazelle due to EHV-9 identified at another zoological institution. These cases reinforce the risks of mixed species exhibits. Investigation into the effects of EHV vaccination on viral shedding in zebra is warranted to decrease risk to susceptible species.

EXAMINING DIFFERENCES IN EQUINE SERUM AND WHOLE BLOOD SELENIUM REFERENCE INTERVALS

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Reference intervals (RIs) are critical for clinical data interpretation as well as clinical decision-making and treatment plan development for veterinary professionals. RIs, however, are not necessarily consistent between laboratories, equipment, or the type of sample submitted. In this study, we compared existing RIs for equine whole blood and serum selenium concentrations from the MSU Veterinary Diagnostic Laboratory (VDL) with 3 other regional veterinary diagnostic laboratories and further examined data collected by the MSU VDL during the past 5 years for consistency with stated RIs and any observed differences in age, breed, and sex. We found unique differences in both RIs supplied by each regional veterinary diagnostic laboratory and how intervals were generated. When examining the MSU VDL dataset, we compared 61 breeds of horses, sexes/reproductive statuses, and age groups. Of these, differences were only observed by separating horses by age. Using several statistical approaches including Hoffman, Bhattacharya, and Maximum Likelihood Estimation, we observed differences between the stated reference intervals and those identified within the dataset. This *a posteriori* evaluation of equine selenium concentrations may have implications for the generation of new RIs and future clinical decision-making by veterinarians.