Front row (left to right): Jennifer Algarin, Kelsey Kincaid, Cameron Volpe, Kathryne DeLeon, Louise Loyant, Holly Hurdle, Arabela Viana
Second row: Leticia Sanglard, Brittney Szafran, Lori Nichols, Erica Unz, Stephen Millar
Third row: Justin Krol, Katherine LeJeune, Leigh Townsend, Andres Gibbs, Dr. Russell Carr, Dr. Barbara Kaplan
Fourth row: Dr. Camilo Bulla, Dr. Attila Karsi, Dr. Jennifer Gambino, Dr. Erdogan Memili, Dr. Andrea Varela-Stokes
Not pictured: Michael Tucker

Single voxel 1H proton MR spectroscopy at 3 Tesla for the evaluation of companion animals with seizures
Jennifer L. Algarin-Morales*, Jacqueline Leigh Townsend, and Jennifer Gambino

Magnetic resonance (MR) imaging (MRI) is the gold standard method for evaluating patients with brain disease and seizure disorders. Advanced MR techniques such as MR spectroscopy (MRS) have clinical value in diagnosing brain disorders in humans by providing a biomolecular brain profile (MR spectra). Many disorders can cause seizures. Our focus, idiopathic epilepsy (IE), is characterized by recurrent seizures with no identifiable cause. It is seen in humans, dogs and less frequently cats. Any breed of dog or cat can be affected with genetic predispositions seen in German shepherds and Labrador and Golden retrievers. Seizures begin between 6 months to 6 years of age in dogs and 1 to 4 months of age in cats. Animals with seizures are often euthanized due to inadequate control, owner commitment, limited therapeutic options and/or complications. The diagnosis of IE is complicated and achieved only after other diseases have been ruled out. We hypothesize that MRS will aid in providing a more definitive diagnoses in veterinary patients, as it does in humans. MRI and single voxel MRS was performed between 2011 and 2014 in 79 animals for a prospective brain tumor study evaluating dogs and cats with seizures or neurologic dysfunction. Retrospectively, 8 dogs and 1 cat met the criteria for inclusion. Clinical history, examination, blood chemistry and cerebrospinal fluid analysis supported a tentative diagnosis of IE. The clinical utility of raw MR spectra and applied protocol was reviewed in these patients. MRS patterns were compared to classical human epileptic paradigms and to an unpublished database of normal dogs. Compiled data was added to MSU-CVM's MRS database. Future work is needed to evaluate the clinical utility of MRS in animals with

brain disease. Noninvasive biomolecular brain research may result in improved diagnosis, treatment and outcomes for animals and humans with seizure disorders with the potential for interspecies translational information.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Effects on Juvenile Rat Emotionality following Repeated Low Level Chlorpyrifos Exposure

K.A deLeon*, L. Loyant, and R. L. Carr,

Organophosphorus (OP) insecticides are the most common class of insecticides used in the United States, with chlorpyrifos (CPF) being the most frequent OP used for agricultural insecticides. There is concern that developmental exposure to CPF can cause negative impacts in children such as decreased cognitive function and increased signs of ADHD. Developmental CPF exposure also alters signaling in the endocannabinoid system, a system which plays an important role in brain development. The endocannabinoid system also plays a role in the regulation of emotionality. Our laboratory has reported that low level developmental exposure to CPF decreases the response of rats to a novel aversive environment which is considered an indicator of decreased emotionality. Other measures of emotionality include the open field and the elevated plus maze but the response of animals to low levels of CPF in these tests is not known. To test this, 10 day old rat pups were exposed daily for 7 days to either corn oil, 0.5 mg/kg CPF, 0.75 mg/kg CPF, or 1.0 mg/kg CPF by oral gavage. To determine if the changes in behavior observed were the result of CPF-induced effects on the endocannabinoid system, an additional treatment group was exposed to a specific inhibitor of the same endocannabinoid metabolizing enzyme that is inhibited by CPF. On day 23, rats were placed into an open field and monitored for 20 min. On day 29, rats were placed into an elevated plus maze and monitored for 5 min. For the open field, total locomotor activity and total distance traveled were recorded. The number of entrances into and time spent in the center of the field were determined as a measure of emotionality. In the elevated plus maze, the entrances into and percentage time spent in the open arms were determined as a measure of emotionality.

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Effects of Pentoxifylline on IL-2 and IFN-γ Gene Expression in Canine Whole Blood

A. Gibbs*, E. Kummari, C. Riggs, C. Fellman, J. Stokes, J. Thomason, A. Mackin, and T. Archer

Pentoxifylline is a methylxanthine phosphodiesterase inhibitor that is used as a hemorrheologic and anti-inflammatory agent in veterinary and human medicine. Based on human studies, pentoxifylline has been shown to decrease T-cell production of cytokines such as IL-2 and IFN-γ. Our laboratory has developed a quantitative reverse transcriptase PCR-based assay to measure activated T-cell gene expression of IL-2 and IFN-γ in dogs. The goal of this current study was to use this assay to investigate the ability of pentoxifylline to decrease cytokine gene expression in canine whole blood. We hypothesized that pentoxifylline would inhibit canine IL-2 and IFN-γ cytokine gene expression. Our objectives were to identify one or more activators that would maximize cytokine gene expression and to then use these activators to determine the effects of pentoxifylline on gene expression *in vitro.* Activators investigated were phorbol myristate acetate (PMA) combined with ionomycin, phytohaemagglutinin, and lipopolysaccharide. PMA with ionomycin proved to be the only efficient activator, and was then used at standard concentrations (PMA 12.5ng/mL; ionomycin 0.8µM) and incubation times (5 hours) developed as part of our established laboratory protocol. Whole blood from 7 different dogs was then incubated with pentoxifylline for 1 hour at concentrations of 1ug/mL, 2ug/mL, 10 ug/mL, 50 ug/mL, and 200ug/mL (concentrations spanning known attainable serum concentrations in dogs receiving oral pentoxifylline) prior to activation with PMA with ionomycin. Cyclosporine at a concentration of 500ng/mL was used as a positive control. Analysis of activated whole blood by qRT-PCR revealed that suppression of IL-2 and IFN-γ gene expression was not reliably achieved at any concentration of pentoxifylline in samples from any dog, suggesting that pentoxifylline does not suppress T-cell cytokine production in dogs, and that the drug therefore may not have potential as an immunosuppressive agent.

Student Support: NIH 5T35OD010432

Effects of firocoxib, flunixin meglumine, and phenylbutazone on platelet function and thromboxane synthesis in healthy horses

Holly Hurdle*, Brenna Burkett, Robin Fontenot, John Thomason

Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandins, including thromboxane A_2, a potent agonist for platelet aggregation. There are two main COX isoforms, COX-1, the primary enzymatic pathway for

thromboxane synthesis, and COX-2, primarily involved in inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are classified as either selective or non-selective based on COX-1 or COX-2 inhibition. It has been proposed that non-selective NSAIDs have a greater effect on platelet function via the inhibition of thromboxane synthesis. There are no studies that evaluate the immediate effects of non-selective and selective NSAIDs on platelet function in horses. The aim of the study was to determine the effects of a COX-2 selective NSAID, firocoxib, and non-selective NSAIDs, flunixin meglumine and phenylbutazone, on platelet function. In a cross-over study design, six healthy horses were administered firocoxib (0.27 mg/kg loading dose and then 0.09 mg/kg SID), flunixin meglumine (1.1 mg/kg BID), or phenylbutazone (2.2 mg/kg BID) for 5 days with a 14-day washout period. Platelet function was evaluated prior to (Day 0) and during (Days 1 and 5) therapy using turbidimetric aggregometry (collagen agonist) and PFA-100® (collagen/epinephrine cartridge). Aggregometry was performed in quadruplicate and PFA-100® in duplicate, and results were averaged. Compared to baseline (Day 0), firocoxib initially decreased maximal amplitude on aggregometry by 0.4% and then increased by 7.5% on Days 1 and 5, respectively, phenylbutazone decreased maximal amplitude by 2.2% and 4.8% on Days 1 and 5, respectively, and flunixin meglumine decreased maximal amplitude by 15.7% and 17.2% on Days 1 and 5, respectively. For all drugs on Days 1 and 5, there was no change in PFA-100® closure times. The results of our study suggest that similar to non-selective NSAIDs, COX-2 selective NSAIDs have minimal effects on platelet function.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Bile salts do not introduce oxidative damage into *Listeria monocytogenes* in anaerobic or aerobic conditions
Kelsey Kincaid*, Jessica G. Wilson, and Janet R. Donaldson

Listeriosis is a deadly disease caused by the consumption of food contaminated with *Listeria monocytogenes. L. monocytogenes* spreads through intracellular movement in the body but it is not understood how this organism responds to the stressful environment of the gastrointestinal (GI) tract prior to invading surrounding tissue. One of the stressors within the GI tract is bile, which induces oxidative damage to bacterial cell membranes through its bile salt component. *L. monocytogenes* is highly resistant to bile, though variations in resistance to bile salts is seen among different strains. This suggests another component in bile may protect *L. monocytogenes* against the oxidative damage of bile salts. Bilirubin, an additional component in bile, has been shown to possess antioxidant properties by scavenging reactive oxygen species. To determine if bile resistance is due to a protective nature of bilirubin, strains of *L. monocytogenes* that vary in capability to cause systemic infections were tested in combination with 1% bile salts and 2% bilirubin under both aerobic and anaerobic conditions. *L. monocytogenes* strains F2365, EGD-e, 10403S, and HCC23 were cultured in Tryptic Soy Broth to mid log, then exposed to porcine bile extract combined with bilirubin. As controls, cells were also treated with bile salts or tert-butyl hydroperoxide (TBHP) and esculetin, a known antioxidant. Cells were exposed to the conditions for up to 2h at 37C, serially diluted and plated on Brain Heart Infusion (BHI) agar. After 16 hours of incubation at 37°C, colonies were counted and analyzed for percent survivability. Though bile salts impeded the growth of all strains of *L. monocytogenes* tested, bilirubin did not protect cells against the bactericidal effect of bile salts. Esculetin provided protection against TBHP, which is known to induce lipid peroxides. Together, these results indicate the bactericidal effect of bile salts is not due to induction of oxidative damage in *L. monocytogenes.*

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A comparative study of *Edwardsiella ictaluri* isolated from zebrafish and channel catfish
Justin Krol*, Mark L. Lawrence, and Wes Baumgartner

Edwardsiella ictaluri is a gram negative pathogen of high economic importance affecting the channel catfish aquaculture industry. However, within the last year *E. ictaluri* causing edwardsiellosis epidemics in zebrafish colonies have been described (Hawke 2013). In the current study, a zebrafish isolate (*E. ictaluri* 11-100) was compared to a catfish isolate (E. ictaluri 93-146) in terms of virulence in zebrafish, method of detection, and mechanisms of attenuation. Virulence of strains 11-100 and 93-146 were compared in zebrafish. Plasmid pAKlux1 was transferred into strain 11-100 to allow bioluminescence imaging to track the pattern of infection in zebrafish at specific time points. Tissues were fixed and processed using a modified method of in situ hybridization developed in our lab to locate the isolate within zebrafish tissues through fluorescent imaging. A mutation in the gene encoding fumarate reductase (*frdA*), which caused attenuation in strain 93-146 in catfish, was transferred into strain 11-100. Zebrafish are an important biomedical animal research species, understanding zebrafish edwardsiellosis is important to not only improve the welfare of the zebrafish, but also to help prevent loss of valuable NIH resources invested in zebrafish research.

Student Support: NIH 5T35OD010432

Twist1 gene expression after "in vitro" cannabinoid challenge to canine osteosarcoma cells
Katherine LeJeune*, Erika Maria Terra, Letícia Abrahão Anaí, Sandra Curotto Bulla, Rodrigo Costa da Silva, Kari Lunsford, Camilo Bulla

Cannabinoid receptor agonists have been shown to reduce cancer growth and metastatic tendencies in animal models but the mechanisms are still unclear. Epithelial-mesenchymal transition (EMT) and its reversal mesenchymal-epithelial transition (MET) regulate invasiveness and aggressiveness in cancer through transcription factors such as Twist1. E-cadherin expression is common in static epithelial cells, while N-cadherin expression is common in motile, more invasive mesenchymal cells. By reducing N-cadherin expression, cells can differentiate and begin the MET-like process. This study tests the hypothesis that the EMT and MET pathways are involved in the anti-metastatic actions of cannabinoids. To do so, we analyzed Twist1 gene expression after treating canine osteosarcoma (OSA) cells with a cannabinoid receptor agonist, WINN 55-212,2. Canine OSA cells (OSA-8), generously provided by Dr. Jaime Modiano, were cultured in a 6-well plate (100,000 cells/well^{-1}) at 37°C, 5% CO_2, for 12 hours. The cells were then challenged with 1µM WINN 55-212,2 (3 wells, challenge group) or 0.1% DMSO (3 wells, control group) and incubated at the same conditions for 48 hours. The cells were then harvested and the mRNA extracted. Gene expression was measured through the use of Twist1 TaqMan gene expression assay (Cf02690938_gH), targeting 101bp of the canine chromosome 14, GoTaq® Probe 1-step RT-qPCR kit, and canine GAPDH primer and probe for normalization purposes. All reactions were run in a 7500 Fast Real-Time PCR thermocycler. The relative quantification of mRNA samples was analyzed by one-way ANOVA followed by the Student's t-test. The treated group presented a 27.93% reduction in Twist mRNA expression compared to the control group (p-value = 0.0125). These results support the proposed hypothesis, along with the importance of further research in this area. It would also suggests the presence of an increased E-cadherin and a decreased N-cadherin expression in these tumor cells.

Student Support: Merial Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Effect of Developmental Low Level Chlorpyrifos Exposure on Social Behavior in Adolescent Rats
Louise Loyant, Kathryne de Leon, and Russell Carr

There is a concern that chlorpyrifos (CPF), an organophosphorus insecticide, may cause developmental neurotoxicity in children leading to long term effects. Our laboratory has reported that low level developmental exposure to CPF alters emotionality in juvenile rats. Developmental CPF exposure also alters signaling in the endocannabinoid system of the brain, a system which plays a role in the regulation of emotionality and other emotion-related behaviors such as social interaction. Thus, it is possible that developmental CPF exposure could result in persistent effects on social behavior. To test this, 10 day old rat pups were exposed daily for 7 days to either corn oil, 0.5 mg/kg CPF, 0.75 mg/kg CPF, or 1.0 mg/kg CPF by oral gavage. To determine if the changes in behavior observed were the result of CPF-induced effects on the endocannabinoid system, an additional treatment group was exposed to a specific inhibitor of the same endocannabinoid metabolizing enzyme that is inhibited by CPF. On day 36, following a 24 hour isolation period, two rats of the same treatment, sex, age and size were placed together in an empty litter-filled cage. Social behaviors of the rats were observed for 10 min. The parameters recorded were: time to first interaction, grooming, chasing, body sniffing, anogenital sniffing, nape attack, crawling over/under, play fights, pinning, and time spent playing. In addition, the rats were sacrificed immediately following completion of testing to obtain the brain which was sliced and tissue punches containing the amygdala were obtained. Social play causes increased phosphorylation of the cannabinoid receptor 1 (CB1) in the amygdala and the levels of CB1 phosphorylation were determined in all treatment groups to correlate with behavioral changes.

Student Support: Merial Veterinary Scholars Program Europe

Endemic Foci of *Borrelia turicatae* and Tick-Borne Relapsing Fever in Texas
Stephen Millar*, H.K. Wilder, W.K. Boyle, J.E. Harris, R.A. Hargrove, J.E. Lopez

Tick-borne relapsing fever (TBRF) is an endemic, zoonotic disease caused by many *Borrelia* spp. spirochetes throughout the globe and is characterized by recurring episodes of fever, aches, pain, and headaches. Historically, little has been done to study *Borrelia turicatae* distribution since their identification in the early 20th century. This gap is exacerbated by the non-specific symptoms, which suggests the disease is likely misdiagnosed and underreported.
Recent molecular advances allow the ability to identify regions of endemicity for *B. turicatae* using the recombinant antigens GlpQ (Glycerophosphodiester phosphodiesterase) and BipA (*Borrelia* Immunogenic Protein A). GlpQ is a highly conserved and broad antigen to determine exposure to species of relapsing fever *Borrelia,* while evidence indicates that BipA is a species specific antigen. In this project, Texas murine samples were collected and serologically surveyed using enzyme-

linked immunosorbent assay (ELISA) with *B. turicatae* recombinant GlpQ (rGlpQ) and immunoblotting assays with *B. turicatae* recombinant BipA (rBipA). These assays found seropositivity indicating endemic foci of *B. turicatae* exposure in Edwards County, TX and *Borrelia* relapsing fever exposure in Edwards, Cottle, Hemphill, and La Salle Counties.

Student Support: NIH 5T35OD010432

Determine parameters necessary to measure DOPA levels within Toxoplasma gondii cell cultures by HPLC
Lori Nichols* and Jeffery Eells

Toxoplasma gondii affects up to 30% of the world's population and has been linked to multiple neuropsychiatric disorders including schizophrenia, depression, and suicide. One mechanism proposed through which *T. gondii* contributes to these diseases is via increasing dopamine levels in the host's central nervous system. 3,4-dihydroxyphenylalanine (DOPA) is a precursor to dopamine (DA), and within neurons, the enzyme tyrosine hydroxylase (TH) almost immediately converts DOPA to DA. The rapid conversion makes DOPA levels challenging to measure. The main theory of this research project hypothesizes that *T. gondii* requires DOPA to survive in addition to DA. Before the hypothesis can be tested, however, the optimal parameters need to be determined for extracting DOPA from tissue culture media and measuring it using high pressure liquid chromatography (HPLC). Since catecholamines bind to alumina at basic pH but not at acidic pH, several different parameters were tested including the amount of alumina and extraction solvent. Perchloric acid gave the best recovery of DOPA relative to citric acid. Less than 20 mg of alumina gave poor results, but 40 mg produced adequate extraction of DOPA from 1 mL of tissue culture media. The pH of the mobile phase buffer affects the time the catecholamines stay on the column, so the pH of the mobile phase necessary to measure DOPA was also tested. At a pH of 3.88, the DOPA peak comes off around 4.5 minutes, which was frequently obscured by the solvent peak. A buffer with pH 3.01 was found to shift the DOPA peak to approximately 6 minutes, giving a clear reading away from the solvent peak. Finally, these conditions were found to be able measure a DOPA concentration down to 10 pg/□l. Therefore, this study concludes that using 0.1 M perchloric acid, 40 mg alumina and a mobile phase buffer with pH 3.01 can measure a DOPA concentration of 10 pg/□l in tissue culture media when measuring by HPLC.

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Invasion capacities of novel *Listeria monocytogenes* mutants in human intestinal epithelial cells
Leticia Sanglard*, Hossam Ahdelhamed, Mark Lawrence, and Attila Karsi

Listeria monocytogenes is a food borne pathogen causing listeriosis via consumption of contaminated food. Due to risks related to *L. monocytogenes* infection, it is essential to understand the molecular basis of the pathogen's virulence mechanisms. Previously, we identified and in-frame deleted six *Listeria* genes (*IspG*, f2365_0522, *f2365_2763*, *tkt*, *argf-1*, and *f2365_0661*) specific to high-risk strain 4b 2365. In this study, we investigated invasion characteristics of these mutants in the Caco-2 cell line. First, functional copies of the in-frame deleted genes were amplified and cloned into pPL2 using *Escherichia coli* strain Dh5-α. Then, the plasmid carrying the functional gene was transformed into specific *Listeria* mutant to obtain complemented strain. Finally, an invasion assay was conducted in Caco-2 cells to assess roles of the deleted genes in cell invasion. Results indicated that cell invasion capabilities of the *Lm*Δ*IspG*, *Lm*Δ*f2365_0522*, *Lm*Δ*f2365_2763*, *Lm*Δ*tkt*, and *Lm*Δ*argf-1* mutants were decreased, whereas *Lm*Δ*f2365_0661* mutant did not show any difference compared with the wild type *L. monocytogenes*. The complemented *Listeria* mutants showed higher cell invasion capacity than that of mutants. Further studies are underway to elucidate the role of these genes.

Student Support: NIH 5T35OD010432

A Comparison of Brain, Liver and Spleen Serine Hydrolase Activity in a Mouse Inflammation Model
Brittany Szafran, Sam Borazjani, Jung Hwa Lee, John Stokes, Matthew Ross, and Barbara Kaplan

Inflammation is an important part of the innate immune response and is involved in the healing of many disease processes; however, chronic inflammation is a harmful component of many diseases such as heart disease or atherosclerosis. Not all the regulatory mechanisms of inflammation have been completely understood. One such possible regulatory mechanism is the endocannabinoid system. Endocannabinoids such as 2-arachidonoylglycerol (2-AG) and anandamide have been shown to increase or reduce inflammation in a tissue-dependent manner; therefore, preventing the degradation of endocannabinoids by specific serine hydrolases such as fatty acid amide hydrolase, monoacylglycerol lipase, and carboxylesterases (CES). We hypothesized that unique inflammatory cell types would

increase while specific serine hydrolase activity would decrease with a subsequent increase in endogenous endocannabinoids in the presence of inflammation. Mice were injected with lipopolysaccharide (LPS) at 6-hour and 24-hour time points, and inflammation and serine hydrolase activity were assessed. Flow cytometry showed there was no significant difference in γδ T cell populations and their cytokines, interleukin (IL)-17 & interferon (IFN)- γ, in the spleen's response to LPS, suggesting these unique inflammatory populations might not be altered early after LPS administration. Activity-based protein profiling of serine hydrolases showed no significant difference in various serine hydrolase protein activities in the brain, liver, and spleen post-LPS exposure. However, high-pressure liquid chromotography showed a significant decrease in 2-AG hydrolase activity 6-hours post-LPS injection when compared to saline controls in the spleen. A modest inhibition of CES2g in the spleen could contribute to the decreased 2-AG hydrolase activity. In conclusion, although the γδ T cell cytokines were not altered in the spleen, 2-AG hydrolase activity difference suggested LPS alters endocannabinoid metabolism.

Student Support: Mississippi State University College of Veterinary Medicine

Computed tomographic (CT) anatomic reference for the endangered Kemp's ridley *(Lepidochelys kempii)* sea turtle

Jacqueline Leigh Townsend*, Jennifer L. Algarin-Morales, and Jennifer M. Gambino.

Concern for wildlife and the Gulf of Mexico ecosystem has prompted veterinary health providers to explore new diagnostic options in conservation medicine to improve the quality of care. Natural disasters; deleterious industrial, commercial, and recreational human activities can negatively impact the ecosystem. Five of seven species of sea turtles inhabit the Gulf. All species (Green, Kemp's, Loggerhead, Hawksbill, and Leatherback) are threatened by the oil and fishing industries. Since the Deepwater Horizon Oil spill in 2010, the number of stranded turtles admitted to IMMS for rehabilitation has greatly increased from 1-3 to greater than 200 annually, with the endangered Kemp's Ridley sea turtle being the most commonly stranded. Accurate, noninvasive diagnostic modalities, like CT, are critical to health assessment and improve diagnosis and treatment, which aid in successful rehabilitation and release. CT is also fast and easy to perform. However, image interpretation is complicated by the bony shell, anatomic variety among the sea turtles, lack of established species specific tomographic atlas and unfamiliarity of veterinary/wildlife care providers with CT sectional imaging. Our study retrospectively reviews medical records and non-contrast multidetector CT studies of stranded Kemp's Ridley sea turtles performed between 2010 and 2013. Studies were evaluated for diagnostic value. Protocol and scan quality were assessed to determine optimal protocols for future use. Normal and abnormal findings were described. Elegant CT post processing techniques were implemented to create 2D and 3D multiplanar (dorsal and sagittal) straight and curved, volume rendered and maximum intensity projection reconstructions for each dataset. CT is excellent for evaluating the species. The study resulted in creation of an anatomic reference for the Kemp's ridley sea turtle, which will serve as a valuable adjunct anatomic diagnostic clinical reference.

Student Support: Morris Animal Foundation Veterinary Student Scholars Program and Mississippi State University College of Veterinary Medicine

Prevalence of failure of passive transfer and association of serum total protein in beef calves in southwest Nebraska

Michael A. Tucker*, Suzanne G. Genova, and David R. Smith

Colostral immunity through passive transfer is the first line of defense against infectious pathogens for ruminants and other mammals. Failure of passive transfer (FPT) in beef calves is known to predispose to an increased risk of neonatal morbidity and mortality. The objective of this study is to estimate the prevalence of beef calves with failure of passive transfer in the herd in Nebraska. Blood samples were collected from 392 calves ranging from 1 day to 7 days of age. The serum IgG concentration was assessed using radial immunodiffusion as well as the serum total protein was measured with a Misco digital refractometer. Calves with serum IgG concentrations ≤800 mg/dL are classified as failure of passive transfer. Partial failure of passive transfer are those calves with IgG concentrations >800 mg/dL but ≤1600 mg/dl. Calves with serum IgG concentrations >1600 mg/dl are categorized as having adequate passive transfer. The recorded data revealed a 4% failure of passive transfer with an additional 5% partial passive transfer amongst the calves. The prevalence of FPT was lower in the cow herd compared to the the heifer population. The outcome of having a prevalence of 4% of the cattle herd was significantly lower than expected value due to the previous studies results reporting prevalence. The lower occurrence of FPT could be directly related to the herd management as well as the calving interval time from April to June.

Student Support: Boyd and Anne Epperson Summer Research Award for Food Animal Medicine

Infection rates of rickettsiae in the Gulf Coast tick*, Amblyomma maculatum*, from Mississippi
Erica R. Unz*, Jung Keun (Kevin) Lee, Gail M. Moraru, Andrea S. Varela-Stokes

Amblyomma maculatum is the primary vector for *Rickettsia parkeri*, an emerging human pathogen and one of the agents of spotted fever rickettsioses in the United States. Known as the Gulf Coast tick (GCT), this vector also carries a second rickettsia of unknown pathogenicity, "*Candidatus* Rickettsia andeanae*"*. Given that GCTs are known to bite humans and *R. parkeri* infections have been reported in Mississippi, continued monitoring of local GCTs for *R. parkeri* and *"Ca.* R. andeanae" is essential for evaluating human risk of a known and potential pathogen. In this study, we collected a total of 139 adult GCTs from three sites where these ticks are known to be established in Oktibbeha County, Mississippi. DNA was extracted from individual ticks and then initially tested to confirm presence of extracted DNA using a PCR assay targeting the tick mitochondrial 16S rRNA gene. We subsequently tested extracts for rickettsial DNA by multiplex quantitative PCR (qPCR) targeting the rickettsial outer membrane protein B gene. This multiplex qPCR used one primer set to amplify both rickettsiae and combined HEX-labeled and FAM-labeled Taqman probes specific for *R. parkeri* and *"Ca.* R. andeanae", respectively. Samples positive by qPCR are being bidirectionally sequenced to confirm identity. Thus far, we detected *R. parkeri* in 15.8% of GCTs by qPCR, which is within the range previously reported in our geographical location. Using qPCR, we also detected "*Ca.* R. andeanae" in 10.8% of GCTs and both rickettsiae co-infecting 1.4% of GCTs. The calculated index of co-infection was -2.4. Results from this study highlight the presence of *R. parkeri* in Mississippi, and demonstrate a higher infection rate of "*Ca.* R. andeanae" in GCTs and a lower index of co-infection than previously reported. This additional data highlights the continued need for monitoring in an endemic area, particularly since the significance of "*Ca.* R. andeanae" in singly and co-infected GCTs is still unknown.

Student Support: NIH 5T35OD010432

Sperm Cryopreservation and Regenerative Medicine Improved by Biotechnology
Arabela G. Viana*, Kelli Matheny, Rodrigo Oliveira, Arlindo Moura, and Erdoğan Memili

The cryopreservation of semen and isolation of adult stem cells are essential for reproductive biotechnology and regenerative medicine, respectively. The viability of cryopreserved stallion sperm and epigenetic stability of adipose derived stem cells are low and the underlying reasons are poorly understood. These gaps in the knowledge base are important because lack of such knowledge in these areas is preventing improvement of horse reproduction and regenerative medicine, respectively. The objectives of this study were to improve post-thaw viability of stallion sperm (Study 1), and ascertain epigenetic stability of porcine adipose derived stem cells, pASC (Study 2). The plans for the methods for the first study were cryopreservation of sperm from four stallions in an extender supplemented with antioxidant resveratrol (0, 1, and 10 mM) followed by analyses of motility by computer assisted sperm analyses (CASA) and membrane permeability using hypo osmotic swelling test (HOST). The plans for the methods for the second study were 1) isolation and culture of pASCs in a basal medium (control) and in a chondrogenic differentiation medium followed by the analyses of stemness, differentiation to chondrogenic lineage, and epigenetic stability using immunohistochemistry, staining, and flow cytometry, and 2) analyses of epigenetic networks of genes controlling stemness using Metacore®. The results obtained thus far from the first study using the HOST showed an increase sperm membrane damage in extenders supplemented with resveratrol than the control, 1.17 and 1.223 times, when added 1mM and 10mM, respectively ($P<0.005$). The results thus far obtained from the second study demonstrated that stem cells can be isolated from adipose tissue of pigs and cultured *in vitro*. The findings are important because they help us better understand the fundamental biology of sperm and the stem cells. The results also enhance biotechnology of mammalian reproduction and regenerative medicine.

Student Support: Boyd and Anne Epperson Summer Research Award for Food Animal Medicine

Thermal imaging assessments of body temperature in the equine eye, muzzle, and coronary band
Cameron Volpe*, Susan Bowers, Lauren Hodges and Scott Willard

Measuring vital signs such as body temperature in an efficient manner is crucial to monitoring the health of both humans and animals. Digital infrared thermal imaging (DITI) is a fast non-invasive method of measuring body surface temperature gradients by converting a heat signature into a color picture. This study utilized a DITI camera manufactured by Flir to evaluate the temperatures of the equine eye, muzzle, and coronary band and investigated their correlation with internal body temperature. Mares and foals (n=45) were imaged in a covered barn at a distance of one meter to determine any correlation between DITI temperatures and rectal temperatures (RT) measured with a digital thermometer. Individual images were captured, including a lateral view of each eye, a dorsal and lateral view of the muzzle, and a dorsal view of each coronary band of the hoof. Individuals were imaged multiple times to increase numbers to 85 image sessions. DITI temperatures were compared to RT using Pearson correlation coefficients, regression analysis, and paired/unpaired

comparisons where appropriate (StatView). RT was positively correlated with eye DITI (0.44 to 0.48; P< 0.001) and muzzle DITI (0.393 to 0.436; P<0.001) at a moderate level. Front and rear coronary DITI differed (P<0.02) by 0.27 to 0.29°C and were positively correlated with RT at a moderate level (0.289 to 0.367; P<0.0074). In conclusion, DITI measures were positively correlated, albeit at a moderate level, with RT. Additional research is needed to determine if this is sufficient as a non-invasive measure of body temperature in the equine species.

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