

Student Abstracts 2012



Front (L to R): Dr. Henry Wan, Sally Lynch, Rachel Heintz, Sharon Yang, Janet Gomez, Katy Fuqua, Heather Troutman, Lydia Shafer, Samantha Lesniewski

2nd row: Dr. Attila Karsi, Dr. Lora Petrie-Hanson, Dr. Job Lopez, Tyler Gamble, Sagen Gunnoe, Ralph Tran, Jennifer Nichols, Jenna Dale, Joyce Follows, Becky Telle

3rd row: Dr. Camilo Bulla, Dr. Mark Lawrence, Dr. Jeff Eells, [Hiram Garcia-Crescioni](#)

Not pictured: Kriston Weaver

Development of Feline Infectious Peritonitis Database. Jenna Dale*, Cody Coyne

Feline Infectious Peritonitis (FIP) is a very common virus among cats, especially in environments with multiple cats. The FIP vaccines available don't effectively protect against infection or clinical disease. Generally it's not possible to detect cats with FIP until clinical signs become evident because plasma anti-viral IgG titers only detect viral exposure. There's no specific treatment for FIP which is a fatal disease in cats. Much remains unknown about FIP in cats and many questions need to be answer before advances can be made in the development of improved therapies and vaccines. A research proposal has been established to acquire information through a national survey database mechanism that addresses the incidence, mortality, treatment and vaccination practices associated with FIP. Establishing this database will provide an opportunity to discover variables associated with regional location, breed, treatment, and husbandry/veterinary practices that influence vulnerability and resistance to FIP infection. In this context, the database will serve as a valuable approach to discovering regions of the country that have a lower case incidence of FIP-infected cats and identify individual cats or possibly certain cat breeds that have greater resistance to FIP. The intent of this investigation is to utilize

the information acquired to identify cats that biological materials can be used to perform analyses that will improve our understanding about FIP. Anticipated results include the discovery of biologically active proteins and cellular functions that confer resistance. Experimental data of this nature will facilitate the conduction of future research devoted to developing vaccines that more effectively prevent disease and establish more potent forms of therapy.

Student support: American Humane Association & Mississippi State University College of Veterinary Medicine

Effects of Temperature and Duration of Storage on Gene Expression of Cytokines by Cyclosporine-Exposed Canine T-Cells. Joyce Follows*, C. Riggs, Todd Archer, C. Bulla, C. Fellman, and A. Mackin

Cyclosporine, a potent immunosuppressive agent, is used to treat a variety of canine inflammatory and immune-mediated conditions including atopic dermatitis, anal furunculosis, and immune-mediated hemolytic anemia, but ideal dosing protocols for this drug have yet to be established in the dog. Cyclosporine exerts its immunosuppressive effects by decreasing activated T-cell production of cytokines such as IL-2 and IFN- γ . Our laboratory is currently developing a quantitative reverse transcriptase PCR-based test measuring activated T-cell gene expression of these cytokines, with the goal of using this test to assess the degree of immunosuppression in canine clinical patients receiving cyclosporine and to adjust doses accordingly. To develop our PCR-based test for use on blood samples sent to our facility, we needed to determine how typical sample storage and handling conditions affected activated T-cell cytokine expression in cyclosporine-exposed samples. Samples of canine whole blood exposed to a typical post-treatment blood concentration for cyclosporine (500ng/ml) were stored for 0, 24, and 48 hours both at room temperature and at four degrees Celsius. T-cells within stored samples were then activated, followed by RNA extraction. Our quantitative reverse transcriptase PCR-based test was then used to measure gene expression of the cytokines IL2 and IFN- γ in activated T-cells. To date, we have obtained adequate RNA yields for all storage temperatures and durations. Sample storage for up to 48 hours at varying temperature with continuous T-cell exposure to cyclosporine did not appear to significantly alter the results compared to samples that were processed immediately after collection. Preliminary results of our current study suggest that our quantitative reverse transcriptase PCR-based test of activated T-cell gene expression of cytokines IL-2 and IFN- γ is robust enough to be feasible under standard veterinary practice handling and submission conditions.

Student support: Meriel Veterinary Scholars Program and Mississippi State University College of Veterinary Medicine

Transposon Mutagenesis and Identification of Mutated Genes Causing Growth Deficiency in *Edwardsiella ictaluri*. Kathryn Fuqua*, Jingjun Lu, Hossam Abdelhamed, Michelle Banes, Mark Lawrence, Attila Karsi

Enteric Septicemia of Catfish (ESC) is caused by the bacteria *Edwardsiella ictaluri*. To the catfish industry, ESC is a devastating disease that leads to millions of dollars lost due to production losses and treatment costs. Live attenuated vaccines have the potential as an alternative method for providing a high level of protection against ESC. However, more study is required about which virulence genes require alteration in order to become an effective vaccine. The purpose of this research was to generate growth deficient *E. ictaluri* mutants and to identify the functionally impaired genes that caused growth deficiency, which may be potential targets for vaccine development. To develop growth deficient *E. ictaluri* mutants, we utilized random transposon mutagenesis. Genomic DNA from these mutants was prepared and transposon ends were amplified using single primer PCR. Sequencing of the PCR products and subsequent database searches revealed the locations of 31 unique transposon insertion sites in the *E. ictaluri* genome. Blast2Go results indicated metabolic and cellular processes were highly represented. Also, most gene proteins were localized in the cytoplasm or cytoplasmic membrane. Microbial virulence database searches indicated 10 genes were associated with bacterial virulence, which makes them prime choices for vaccine development. Further research is needed to verify in vivo attenuation of these mutants.

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

Serological Survey of Normal Breeder Flocks within a Commercial Poultry Company. Tyler C. Gamble*, Philip A. Stayer, and Erin G. Riley

Nine billion broilers, valued at \$45 billion, are produced annually in the United States. Commercially reared birds are at risk for devastating viral diseases. For this reason, poultry companies routinely run serological assays to determine viral challenge, vaccine efficacy, and flock health. It is imperative to have reference values to rapidly evaluate serology samples collected. This study was conducted to abridge past serological data collected from breeder flocks, create a set of reference values to be used when gauging future serology reports, and compare results to reported industry serology. Over a four-year period, samples were routinely collected from 12,651 breeder flocks between the ages of 11-15 weeks (pullets), 22-33 weeks (young hens), and 49-60 weeks (old hens). The birds in this study were vaccinated once with killed antigens for Infectious Bursal Disease Virus (IBDV), Infectious Bronchitis Disease Virus (IBV), Newcastle Disease Virus (NDV), and Infectious Tenosynovitis Disease Virus (REO) at approximately 15 weeks of age. Standard industry practice is to vaccinate pullets twice with these killed antigens at 12 and 18 weeks of age. The 189,765 samples were analyzed via enzyme-linked immunosorbent assay (ELISA) for IBDV, IBV, NDV, REO, and CAV. The geometric mean and coefficient of variance of the titers was found for the 15 samples taken from each house. Two standard deviations, which is a 95% confidence interval, from the average geometric mean were used to create the upper and lower limits of the reference ranges. IBDV, IBV, NDV, REO, and CAV titer ranges were found to be: 2,510-20,000, 458-12,085, 854-7,846, 1,034-14,218, and 0.022-0.155, respectively. Seventeen of the annual average geometric means were noted to be outside their respective ranges, which led to further investigation. The means for the five antigens examined did not appear to be different between the company's single killed vaccination and the industry standard of vaccinating twice.

Student support: Sanderson Farms and Mississippi State University College of Veterinary Medicine

Cannabinoid Suppression of VEGF Expression in Canine Osteosarcoma Cell Line. Hiram J. García-Crescioni*, M.K. Ross, A.S. Figueiredo, C. McIntosh, S.C. Bulla, K. Lunsford, Camilo Bulla

Vascular endothelial growth factor (VEGF) is a pro-angiogenic signaling molecule, frequently over expressed in malignant tumors. In order to grow and in response to stimulus such as hypoxic/low-nutrient microenvironment, neoplastic cells release pro-angiogenic factors such as VEGF. These factors promote new blood vessel formation from the normal tissue vascular network. Cannabinoids, molecules that bind to CB1 and CB2 receptors, have been shown to inhibit angiogenesis in different types of cancer. Recently, in studies involving human and mouse cancer cells, cannabinoids were found to decrease VEGF expression. There is no information on the effects of cannabinoids on canine cancer. The aim of this work was to assess VEGF expression in the canine osteosarcoma cell line 8 (OSA-8) after incubation with the synthetic cannabinoid WIN-55,212-2. OSA-8 cells were incubated with 1 μ M WIN-55,212-2 for 48 hrs, on three independent experiments. After total RNA extraction, RNA quality and concentration was spectrophotometrically evaluated. Reverse transcriptase quantitative PCR was performed by one-step reactions with primer and labeled-probe specific for canine VEGF (TaqMan® Gene Expression Assay, Invitrogen). A canine GAPDH labeled-probe was designed for normalization purposes and all samples were run in triplicate. Amplification efficiencies were obtained with the slope of each calibration curve dilution versus cycle resulting in 96% for VEGF and 99% for GAPDH. The comparative Cq method was employed for relative quantification. WIN-55,212-2 decreased by 35% \pm 10% (n=8) the amount of VEGF mRNA expression on average (p<0.0001) compared to the control (100% \pm 23%, n=6). In conclusion, WIN-55,212-2 decreases VEGF mRNA expression in the canine osteosarcoma cell line OSA-8.

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

Antigenic and Genetic Characterization of Swine Influenza Viruses from Ohio County Fairs. Janet Gomez*, Andrew Bowman, Jianqiang Ye, Sarah Nelson, Brigitte Martin, Sherry Blackmon, Kun Jia, Laura Bailey, Hailiang Sun, Guohua Yang, Kyoung-Jin Yoon, Richard Slemmons, and Xiu-Feng Wan

In the past decades, swine origin influenza viruses have caused sporadic human infections and human origin influenza viruses have also been reported to infect swine. The recent emergence of H3N2 variant viruses (H3N2v) has posed a potential threat to public health by causing 13 cases of swine origin influenza virus infection in mostly children across six states in the United States since August 2011. A few human infections were also detected in county fairs in years past. In this study, we aimed to assess the risk of county fairs as a potential interface for influenza interspecies transmission between human and swine by characterizing the genetic and antigenic diversity and dynamics of H3N2 swine influenza viruses (SIV) in county fairs. We collected 9 H3N2 SIVs from Ohio county fairs from 2009 to 2011. These SIVs will also be compared with other H3 subtypes of avian, canine, and swine origin as well as human seasonal H3 viruses and human H3v viruses. Antigenic characterization demonstrated that Ohio county fair H3N2 viruses can be separated into two antigenic subgroups: the 2009 fair isolates as one, and the 2010/2011 fair isolates with human H3N2v as the other. Genomic analyses demonstrated that the HA genes of these H3N2 SIVs and H3N2v belong to the same genetic lineage (IV) which has been predominant in North American swine populations since 2005. Furthermore, the M genes of the 2009/2010 fair isolates were from "classical" SIVs (North American lineage), but those of the 2011 fair isolates were from the 2009 pandemic H1N1 virus (Eurasian lineage). Our results suggested that H3N2 SIVs continued to evolve antigenically and genetically after they were introduced from human to swine. Our observations also suggest that agricultural fairs can serve as a potential animal-human interface for influenza interspecies transmission; therefore, surveillance shall be continued to monitor influenza viruses in both swine and humans at agricultural fairs.

Student support: NIH Grant #9T35OD010432 and RC1AI086830

The Role of Canine Professional Antigen Presenting Cells in the Generation of Regulatory T cells in Dogs Exposed to Babesia canis. Sagen Gunnoe*, Kriston Weaver, John Stokes, Joyce Follows, Lydia Shafer, John Thomason, Andrew Mackin, Joo Youn Park, Todd Archer, Lesya Pinchuk

Regulatory T cells (Tregs) are now recognized as an absolute requirement for the mammalian immune system. Abnormalities of Treg cell number or function have been implicated in multiple autoimmune, infectious, and allergic diseases, but the clinical relevance of canine Tregs is not yet known. Recent studies show that antigen presenting cells (APCs) such as dendritic cells and monocytes/macrophages are capable of priming naïve T cells which initiates the generation of Tregs. Liposomal clodronate (LC) has been used extensively to deplete APCs in small mammals by inducing apoptosis, thus providing an opportunity to investigate the role of these professional APCs in the generation of Tregs in multiple diseases. The objective of this study was to evaluate the role of APCs in the generation of FOXP3-expressing Tregs in dogs with a history of exposure to *Babesia canis* but without productive *Babesia* infection in their red blood cells (RBCs) by flow cytometry (FC) and blood smear analyses. Four healthy greyhounds known to be exposed to *Babesia canis* based on history, serology and past positive PCR results were given intravenous LC at low (0.5 ml/kg) and medium (1ml/kg) doses. RBCs were obtained from whole blood and stained with hydroethidine (HE) for the presence of *Babesia* infection. HE-incorporated RBCs were analyzed by one color FC with single histogram statistics. CD4+CD25+FOXP3+Tregs were separated from lymphocyte populations and analyzed by complex three-color FC with quadrant and single histogram statistics. FC analyses were performed before and after LC treatment. Our data indicated that dogs exposed to LC had moderately enhanced susceptibility to *Babesia* by slightly increasing the RBC HE incorporation. However, the population of FOXP3-expressing Tregs increased dramatically in all the dogs exposed to LC. These findings suggest that Tregs may provide protection against *Babesia canis* by controlling the APC depletion-dependent productive parasite infection in greyhounds.

Student support: NIH 9T35OD010432

Invasion and Adhesion of O157:H7 and Non-O157:H7 Shiga-toxin Producing Escherichia coli in Macrophages. Rachel M. Heintz*, Andrea Rodgers, Justin A. Thornton, and Janet R. Donaldson

Shiga-toxin producing *Escherichia coli* (STEC) cause disease by releasing shiga-toxins and adhering to and invading host gut epithelium. Bacteria are translocated across special gut epithelial cells, M cells, into a region densely populated by macrophages that phagocytize the pathogens. Research has looked at *E. coli* O157 survival in macrophages, but little work has assessed differences among non-O157 serogroups. The purpose of

this study was to determine whether *E. coli* O157, along with various non-O157 STEC serogroups, had differing levels of adherence and invasion in J774 murine macrophages, which could potentially correlate to differences in host responses or colonization patterns. Macrophages were infected with the following five serotypes: O157:H7, O103:K:H8, O145:H28, O26:H11, or O111:H8. An avirulent form of O157:H7 not possessing the *stx1* and *stx2* genes was also analyzed. Adhesion assays were conducted for three hours, and intracellular survival was analyzed at 1, 3, and 5 h post infection. Results indicate the serotype O103:K:H8 had the highest adherence level of all the serotypes, while the O157:H7 Δ *stx1 stx2* serotype had the lowest levels of adhesion. *E. coli* O157:H7 Δ *stx1 stx2* was also significantly less invasive than the other serotypes which showed similar invasion levels. Thus shiga-toxins may play an important role in the ability of *E. coli* to adhere to and survive in macrophages, which will need to be explored in further detail. Further research is currently being conducted on the adhesion and invasion properties of the serotypes in colorectal epithelial cells.

Student support: NIH 9T35OD010432

Development of a Proximity Ligation Assay to Quantify Regional Tyrosine Hydroxylase Protein Levels. Samantha Lesniewski* and Jeffrey Eells

Although quite a few protein detection methods have been developed, many of the current methods are becoming outdated, too time-consuming, or fall short when it comes to protein quantification. The goal of the current research was to develop a protein detection method based on proximity ligation assay that uses laser capture microdissection (LCM) and quantitative PCR (qPCR) to measure protein levels in discrete areas of the brain. The protein of interest was tyrosine hydroxylase, the rate-limiting enzyme essential for dopamine synthesis. Focus was placed on dopaminergic target regions containing the highest amounts of tyrosine hydroxylase which includes the nucleus accumbens, the prefrontal cortex, and the striatum. A modified version of the proximity ligation assay, termed immunohisto-qPCR, was performed on forebrain tissue, collected via LCM, and analyzed using qPCR. Tyrosine hydroxylase protein levels were also measured using quantitative immunohistochemistry for comparison. The somatic sensory cortex was used as a region with very low level of tyrosine hydroxylase expression. Using immunohisto-qPCR, tyrosine hydroxylase levels were highest in the striatum (17.66 fold above the somatic sensory cortex) followed by the nucleus accumbens (7.09 fold higher) and prefrontal cortex (5.33 fold higher). The somatic sensory cortex was indistinguishable from the no primary antibody control. A similar order of results were obtained using quantitative IHC, however there was a larger range using the immunohisto-qPCR. These experiments demonstrate that protein levels of tyrosine hydroxylase can be measured in tissue using LCM and the immunohisto-qPCR technique. The advantage of this technique is that it provides a very sensitive measure of protein levels with high anatomical resolution and expands the proximity ligation assay to antibodies that function in immunohistochemistry.

Student support: NIH Grant #9T35OD010432

A Serological Survey of Wild Rodents for Evidence of Endemic *B. turicatae* in Texas. Sara A. Lynch*, Hannah K. Wilder, William H. Boyle, Amy Gilbert, Ceara M. Nicholson, Reid A. Hargrove, and Job E. Lopez

Tick-borne relapsing fever (TBRF) is a globally neglected zoonotic illness. The disease, caused by *Borrelia turicatae*, and transmitted by Argasid ticks, results in episodic fever, nausea, body aches, malaise, and a 5-30% mortality rate in humans. Though endemic in the United States, the ecology of TBRF in relation to its vectors and wildlife hosts is poorly understood, emphasizing the need for improved disease detection and prevention. Consequently, a serological survey of wild rodents was conducted in Texas in an effort to investigate mice and rats as potential carriers of TBRF spirochetes. Serum samples from captured rodents were analyzed by immunoblotting using a recently identified diagnostic antigen. Of forty-seven rodents captured, one *Peromyscus* was found to be positive for *B. turicatae* antibodies. While rodents are implicated in the maintenance of different species of relapsing fever spirochetes, this study is the first implicating the importance of *Peromyscus* in the tick-mammalian cycle of *B. turicatae*.

Student support: NIH Grant #9T35OD010432

Effects of Dietary Zearalenone Exposure on the Reproductive Performance of Mares. Jennifer Nichols*, Heath King, Susan Bowers, N. Schappell, David Christiansen, Kevin Walters, Richard Hopper and Peter Ryan

Zearalenone is a phytoestrogenic mycotoxin produced by the fungus family *Fusarium* that is often a contaminant of common animal feedstuffs. It is responsible for causing estrogenic effects in swine and reproductive disorders in cattle, but the effects of the mycotoxin in equids is relatively unknown due to limited data. Thus, the objective of this study was to assess the effects of zearalenone on reproductive efficiency in healthy mares at two different concentrations and to determine an acceptable tolerance level of zearalenone contamination of feedstuffs for horses. To this end, 21 mature, healthy, and reproductively sound mares (2 -16 yr) were age-matched and assigned to one of three treatment groups (0, 2 or 8 mg zearalenone/d). Mares were fed (08:00 h/d, 0.5 kg horse pellets) using nose-bags that included a horse-treat previously soaked with zearalenone in ethanol. Treatments commenced on day of ovulation (d 0) and continued for three consecutive estrous cycles. Reproductive activity was monitored every other day by ultrasound (ovary, reproductive tract) and stallion-teasing. Serum was collected on d 0, 2, 4, 8, and 16 of each cycle for progesterone and estrogen. Upon detection of a 32 mm follicle and uterine edema, serum was collected and reproductive activity examined daily until ovulation was confirmed. Preliminary results at the current dietary exposure levels show no recorded differences in pre-ovulatory follicle size in treated mares compared to control mares. However, low dose mares do appear to have a higher incidence of developing hemorrhagic follicles and trabeculated corpus lutea. Additionally, estrous cycle length was slightly longer (19d and 20.67d) for high and low dose mares compared to control mares (18.75d). While this study is ongoing, these data suggest that zearalenone may affect equine reproductive activity. Endocrine analysis may provide a clearer insight as to the effects of dietary zearalenone on reproductive performance.

Student support: NIH Grant #9T35OD010432

Comparison of Multiplate and Chrono-log Platelet Aggregometers for Assessment of Aspirin Resistance in Dogs. Lydia Shafer*, Jillian Haines, Andrew Mackin, Camilo Bulla, Kari Lunsford, and John Thomason

Low dose aspirin is the most commonly administered anti-platelet therapy in both humans and dogs. Unlike high doses of aspirin, low doses have minimal side effects, but may not consistently inhibit platelet function, a phenomenon termed 'aspirin resistance' in humans. Recently, aspirin resistance has also been identified in dogs. The gold standard for assessing platelet function and aspirin resistance in people, turbidimetric platelet aggregometry, is time consuming and impractical for use in clinic patients receiving aspirin. Several alternative techniques that assess platelet function may be more feasible for practice use. In humans, these techniques have been compared to turbidimetric aggregometry to determine efficacy at detecting aspirin resistance. Unfortunately, in dogs receiving low dose aspirin, there are no studies that compare these techniques. Our study objective was to compare different techniques that analyze platelet function in dogs receiving low dose aspirin. Our hypothesis was that techniques such as impedance aggregometry and point-of-care analysis (PFA-100) could provide results comparable to turbidimetric aggregometry in dogs on low dose aspirin. Blood was collected from 9 healthy dogs prior to low dose aspirin administration, platelet function was analyzed using turbidimetric aggregation, impedance aggregation (Chrono-log and Multiplate analysers), and PFA-100® analysis, and results were compared. The mean percentage of maximal aggregation for turbidimetric aggregometry was 97.3 (range, 54.2–153). The mean platelet aggregation for the Chrono-log® was 41 U (31–58) and the Multiplate® was 167 AUC (86–249). The mean PFA-100® closure time was 165 seconds (68–300). Our preliminary results suggest that impedance aggregometry and PFA-100® analysis compare favorably with turbidimetric aggregometry. Our study has established several different techniques that are feasible for clinical use for evaluating aspirin resistance in dogs.

Student support: Dr. Hugh G. Ward Endowed Chair Discretionary Funds and Mississippi State University College of Veterinary Medicine

Evaluation of *Edwardsiella ictaluri* In-Frame Deletion Mutants for Virulence and Vaccine Efficacy. M. Rebecca Telle*, Hossam Abdelhamed, Neeti Dahal, and Mark L. Lawrence

Edwardsiella ictaluri is the causative agent of Enteric Septicemia of Catfish (ESC), one of the most detrimental bacterial diseases of channel catfish (*Ictalurus punctatus*). Every year, catfish farmers suffer tremendous

economic losses because of ESC, and the currently approved methods of prevention and treatment are not sufficient to aid in the problem. To improve catfish health, an efficient live attenuated vaccine is needed to combat ESC. The TCA cycle is central to bacterial energy metabolism, and it is also important for virulence. Previously, five single and four double *E. ictaluri* mutant strains (*Ei*Δ*sdhC*, *Ei*Δ*frdA*, *Ei*Δ*mdh*, *Ei*Δ*glyA*, *Ei*Δ*gcvP*, *Ei*Δ*frdA-sdhC*, *Ei*Δ*mdh-sdhC*, *Ei*Δ*gcvP-glyA*, *Ei*Δ*sdhC-gcvP*) were constructed by using allelic exchange to result in in-frame deletions. The virulence of each mutant was evaluated by an immersion challenge of 16-day old channel catfish fry. After 21-days post-vaccination, all fry groups were re-challenged with wild type *E. ictaluri* to determine the efficacy of the mutant strains as a live attenuated vaccine candidate. In addition, a complementation experiment was conducted for each of the five single deletion mutants. Each gene was amplified and cloned into the pBBR1MCS4 plasmid. The resulting recombinant plasmids were transferred into the mutant strains by conjugation. The newly constructed complemented *E. ictaluri* mutant strains will be used to verify that the attenuated phenotype is indeed the result of the individual gene deletions.

Student support: NIH Grant #9T35OD010432

Leukocyte Profiles Change with Age in *Nothobranchius furzeri*, an Emerging Model for Age Associated Disease. Huyanh Ralph Tran*, Claudia Hohn, Russell Carr, and Lora Petrie-Hanson

Nothobranchius furzeri is a South African fish that inhabits small ponds that dry seasonally. While different populations of *N. furzeri* show large variations in longevity, the laboratory strain, "Gona-re-Zhou" (GRZ) has a life span of three months when raised at 28C. It has been suggested that within that time period, the physiological processes of these fish progress through the natural changes associated with aging. This senescence would make this fish a critical laboratory model for investigations involving the aging immune and neuro-endocrine systems. Additionally, their unique habitat and reproductive requirements are extremely difficult to mimic in a laboratory setting. We have successfully spawned and reared these fish under different temperature regimes. Differential counts of peripheral blood leukocytes revealed younger fish had higher lymphocyte counts compared to older fish, 68% to 16%, respectively. The neutrophil was the predominate phagocyte. Flow cytometric analyses of kidney hematopoietic tissue additionally demonstrated a decreasing lymphocyte population and increasing phagocyte population with age. Anatomical development and lymphoid tissue architecture were also evaluated.

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

Pressure Induced Osteoblast-Like De-Differentiation of Porcine Aortic Valve Interstitial Cells is Regulated Via the MAP Kinase Pathway. Heather M Troutman*, Abdolsamad Borazjani, Valtresa Myles, James Warnock

Calcific aortic valve disease (CAVD) results in the osteogenic de-differentiation of valve interstitial cells. The cellular transformation can be initiated by elevated pressure and is thought to be related to the mitogen-activated protein kinase (MAPK) signaling pathway. However, the role of the MAPK pathway in development of CAVD is not known yet. We hypothesize that blocking the signaling proteins could prevent osteoblast-like de-differentiation of the cells. To this aim, porcine aortic valve leaflets were incubated in a bioreactor and exposed to a pressure of 120mmHg, 80mmHg or 0mmHg. Half of the leaflets were incubated with 300nM Angiotensin II prior to being placed in the bioreactor. Activation of the MAPK pathway was detected by western blotting. Valve leaflets were then incubated with inhibitors of JNK, p38, or ERK1/2 for 1 hour and then exposed to 120mmHg in the bioreactor for one hour. mRNA expression of the osteoblast markers RUNX2, OPN, and ALP was measured using quantitative real time PCR. Our results indicate that osteogenic de-differentiation is increased when leaflets are exposed to 120mmHg as compared to the paired controls. Our results indicate clearly that the MAPK pathway may be instrumental in osteogenic de-differentiation of porcine aortic valve interstitial cells under conditions of elevated pressure.

Student support: NIH Grant #9T35OD010432

CD14+ Monocytes and FOXP3-Expressing Regulatory T Cells in the Peripheral Blood of Babesia-Exposed Greyhounds. Kriston Weaver*, Sagen Gunnoe, John Stokes, Joyce Follows, Lydia Schafer, John Thomason, Todd Archer, Andrew Mackin, Lesya Pinchuk

In the US, greyhounds are often infected with *Babesia canis*, a protozoal parasite of red blood cells (RBCs). Although babesiosis is usually subclinical in greyhounds, it can cause serious illness in dogs inadvertently transfused with infected blood. Identification of donors with occult *Babesia* infection is therefore essential for transfusion safety. Liposomal clodronate (LC) has been shown to deplete macrophages via induction of apoptosis. Since macrophages are needed to remove *Babesia*-infected RBCs from the circulation, it has been proposed that LC may 'unveil' hidden babesiosis in infected greyhounds. The main objective of our study was to determine if LC would unveil the hidden organism in greyhounds with a history of exposure to *Babesia canis*. Since monocytes/macrophages are capable of priming naïve T cells and initiating the adaptive immune response, our study concurrently evaluated the effects of LC on CD14+ monocyte and FOXP3-expressing regulatory T cell levels in peripheral blood. Four greyhounds known to be exposed to *Babesia canis* were given IV LC at low (0.5 ml/kg) and medium (1ml/kg) doses. Serial flow cytometric analyses were performed before and after each dose of LC. RBCs were collected and stained with hydroethidine (HE) for the presence of *Babesia* infection via flow cytometry. CD14+ monocytes separately gated from the peripheral blood mononuclear cell population based on size and granularity were analyzed by one color flow cytometry analysis with single histogram statistics. CD4+CD25+FOXP3+ regulatory T cells were separated from canine lymphocyte populations and analyzed by complex three-color flow cytometry analysis with quadrant and single histogram statistics. Six normal greyhound dogs were used as controls. According to data, throughout the course of exposure to LC levels of CD14+ remained variable with no significant changes. Tregs showed a slight increase just following administration of LC then remained high not returning to normal levels. Although, slight increases were seen in the amount of HE incorporated by RBCs, levels remained variable throughout exposure to LC and do not indicate changes in susceptibility to *Babesia canis*.

Student support: Dr. Hugh G. Ward Endowed Chair Discretionary Funds and Mississippi State University College of Veterinary Medicine

Identification of Leucocytozoon spp. in Captive Birds Using Conventional and Novel Methods. Sharon Yang*, Kelli Jones, Whitney Smith, John Stokes, and Andrea Varela-Stokes

Leucocytozoon, one of three hemosporidian genera parasitic in avian species, is transmitted by black flies (*Simulium* spp.) and may cause mild to severe disease in birds. A black fly outbreak associated with avian morbidity/mortality occurred in 2009 in Mississippi; no additional outbreaks have been reported. Although black flies may cause disease directly, detection of Leucocytozoon in blood smears of affected birds in 2009 prompted further investigations of this protozoan as a cause of disease. Our study objective was to identify Leucocytozoon spp. in naturally infected birds in Mississippi using microscopy, PCR assay, and a novel technique, flow cytometry. We collected 84 blood samples from captive wild and domestic birds at seven sites in Mississippi. Among all samples, 25% were positive by blood smear and 19% were positive by PCR. However, blood smear and PCR results did not always agree, possibly due to co-infections with other hemosporidians. Results were also inconsistent with empirical identification of gametocytes by flow cytometry. This was possibly due to low parasitemias in non-clinical birds, or interspecies differences in Leucocytozoon morphology. Using flow cytometry, 3.5% of samples had evidence of Leucocytozoon gametocytes in blood. We also observed a decreasing number of detectable gametocytes by flow cytometry in a positive sample over time. Therefore, only fresh blood samples should be analyzed. While our results suggest flow cytometry shows promise in identifying Leucocytozoon, future studies exploring its utility will require systematic analysis of infected and non-infected birds in order to optimize the technique for various Leucocytozoon and avian species.

Student support: NIH Grant #9T35OD010432.