



Front row (l to r): Emerald Barrett, Josh Pierce, Leah Myles, Lorraine Lopez-Soberal, Katrina Quinn  
 Second row: Dr. Jennifer Gambino, Taylor King, Alexis Parisi, Jessica Drakeford, Jennifer Lovvorn, Elizabeth Mitchell  
 Third row: Kimberly Brito, Pearce Sloan, Mary E. Andrews, Matthew Scott, Ashley Plover  
 Fourth row: Dr. Elizabeth Swanson, Yianelly Rodríguez Ruiz, Courtney Griffin  
 Fifth row: Kyle Johnson, Dr. Jeffrey Eells, Dr. Russell Carr, Dr. Andrea Varela-Stokes, Dr. Mark Lawrence

### **Developmental Chlorpyrifos Exposure Alters Endocannabinoid Metabolism in Brain Regions Linked to Emotionality**

Mary E. Andrews\*, Rachel L. Hybart, Carole A. Nail, Hannah R. Nelson, and Russell L. Carr

Low level developmental exposure to the organophosphate insecticide chlorpyrifos (CPF) results in inhibition of the endocannabinoid metabolizing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) in the absence of inhibition of its traditional target, cholinesterase (ChE). FAAH and MAGL degrade the endocannabinoids arachidonoyl ethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), respectively, and accumulation of these could disrupt signaling processes in a developing brain. In fact, this exposure results in altered emotional behavior in juvenile rats. The object of this study was to evaluate the level of enzyme inhibition in brain regions that are highly associated with emotionality, namely the hippocampus and amygdala. Rat pups were orally exposed daily from postnatal days 10-16 to either 0.5, 0.75 or 1.0 mg/kg CPF or 0.02 mg/kg PF-04457845, a specific FAAH inhibitor, and the activities of FAAH, MAGL, and ChE were determined 12 hours after the last administration. As expected, significant ChE inhibition only occurred at the high dosage of CPF in both the hippocampus (19%) and amygdala (25%). MAGL activity was significantly inhibited by the high dosage of CPF in the hippocampus only (13%) with no inhibition in the amygdala. In contrast, significant inhibition of FAAH was present with all dosages of CPF and with PF-0445785 in both regions. However, the level of inhibition in each dosage group was greater in the amygdala (30-45%) than in the hippocampus (15-

28%). The amygdala is more involved in regulating emotional behavior than the hippocampus. Our data suggest that the greater FAAH inhibition in the amygdala during exposure could contribute to the altered behavior observed at later ages.

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### **Normal canine brain single and multi-voxel <sup>1</sup>H MRS and conspicuity of varying doses of MR contrast at 3T**

Emerald D. Barrett\*, Jennifer M. Gambino, Alison M. Plumley, Michaela Beasley, Robert Wills, John E. Ball, and Judy James

Magnetic resonance (MR) imaging is the gold standard test in veterinary patients with brain disease. Regardless, diagnosis is limited to invasive biopsy or cerebrospinal fluid centesis, which can increase morbidity and mortality. In people, <sup>1</sup>H MR spectroscopy (MRS) is an adjunct test that increases conventional MR image interpretation accuracy by providing a biomolecular spectra of normal and abnormal states. MRS is an emerging area of interest in veterinary imaging. Specific study aims were to investigate two growing topics of interest in animal MR imaging, while maximizing the use of live pilot study dogs. First, the performance of single- and multi-voxel spectroscopy (SVS and MVS), were investigated. Little research exists determining statistically significant differences between SVS and MVS. Eight normal dogs underwent brain MR and both SVS and MVS. SVS and MVS biomolecular spectra and metabolite ratios were qualitatively and quantitatively compared. Second, little veterinary research exists establishing an ideal diagnostic MR contrast dose. Currently, animal doses are extrapolated from people. Thus, variable fractions of the standard dose of an intravenous MR contrast agent (gadodiamide) were given to evaluate the conspicuity of normal patterns of cranioccephalic enhancement to help establish an ideal dose. All (five) dose categories were lower than the anecdotal published dose (0.2 mmol/kg). Quantitative pixel intensity evaluation was performed. Preliminary results show that: 1) although performed with different techniques, SVS and MVS will yield similar results without statistically significant differences; and that 2) adequate T-1 shortening and contrast conspicuity is obtained with lower doses of MR contrast.

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

### **Behavioral changes in Nurr1 heterozygous mice with the administration of aspirin and bexarotene**

Kimberly C. Brito\*, Jeffrey B. Eells, Shirley Guo-Ross

Schizophrenia is a serious and debilitating neuropsychiatric disorder occurring in approximately 1% of the U.S. population. Despite its unknown etiology, research suggests a link between schizophrenia and the transcription factor Nurr1, which is responsible for the development and survival of dopamine neurons. The central hypothesis was that either inflammation or attenuated expression of dopamine neurotransmission genes, due the Nurr1 heterozygous genotype, alters behaviors in mice. To test this hypothesis in an animal model, Nurr1 heterozygous (+/-) mice and wild-type (+/+) mice were evaluated using a tail suspension test, activity in an open field, and prepulse inhibition (PPI) after administration of either aspirin or bexarotene, a retinoid X receptor (RXR) ligand with preference for activating RXR-Nurr1 heterodimers, for 14 days. There was a significant bexarotene treatment effect that caused an increase in PPI while aspirin treatment resulted in a decrease in PPI. There was a significant +/- genotype effect on the tail suspension (an increase in the number of immobile episodes and a decrease in latency for immobility) with treatment effect. In the open field test, the +/- mice were more active than +/+ mice which was absent after aspirin treatment. A similar trend on open field activity was also observed with bexarotone treatment. These data demonstrates that Nurr1 +/- genotype causes various behavioral changes while, administration of aspirin and bexarotene also caused behavioral changes independent of the genotype. This suggests that both inflammation and attenuated expression of dopamine neurotransmission may contribute to alterations in specific behaviors in Nurr1 +/- mice.

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## **Trypsin potentiates the effects of pneumolysin on airway epithelial cells**

Jessica E. Drakeford\* and Justin A. Thornton

*Streptococcus pneumoniae* (pneumococcus) is an important human pathogen causing diseases such as pneumonia, meningitis, and otitis, affecting children and adults worldwide. Pneumococcus produces a pore-forming cytotoxin, pneumolysin (PLY), which is one of its key virulence factors. The goal of this study is to characterize how the functionality of PLY is affected by protease cleavage. **We hypothesize that the cytotoxic effects of PLY are affected by protease cleavage, thereby impacting pathogenesis.** We used flow cytometry analysis to determine the sub-lytic concentrations of PLY on A549 human lung epithelial cells and Detroit-562 nasopharyngeal cells used throughout this study. Qubit analysis of DNA release and BCA protein assays were performed and demonstrated an increase in DNA and protein released from Detroit cells treated with PLY followed by trypsin versus cells treated with PLY alone. Western blot was performed to determine the extent of PLY cleavage. A lactate dehydrogenase (LDH) release assay was performed on A549 cells treated with PLY followed by trypsin and cells treated with only PLY to determine loss of membrane integrity. We have demonstrated that trypsin has an effect on the cytotoxicity of PLY. These results indicate that airway trypsin or other proteases could enhance the effect of PLY and potentially other pore-forming toxins.

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## **Evaluation of on-arrival vaccination and deworming on stocker cattle health and growth performance**

Griffin CM\*, Karisch B, Woolums AR, Blanton J, Kaplan RM, Epperson W, Smith DR

The effect of vaccinating and deworming stocker cattle at arrival is poorly documented and potentially detrimental. Our objective was to evaluate the effect of on-arrival vaccination and deworming on bovine respiratory disease (BRD) incidence, mortality, and growth of stocker calves. Calves (n=80) received from an order-buyer were stratified by d-3 weight and fecal egg count into 20 pens of 4 animals each. Pens were randomly assigned to treatments in a 2x2 factorial design to test d0 vaccination (modified-live BRD and clostridial vaccine or not) and deworming (oral fenbendazole and levamisole or not). Body weight and blood was collected days 0, 14, 28, 42, 56, 70 and 85. Fecal egg counts were measured days -3, 28, 56, and 85. Clinical signs of BRD (depression, anorexia, rapid respiratory rate, cough, nasal discharge, and rectal temperature of 104F) were monitored daily. Treatment effects on BRD incidence, mortality, and growth were tested using Poisson, logistic, or linear regression, respectively ( $\alpha \leq 0.05$ ). BRD incidence was greater for calves with d0 vaccination (RR=3.2), high ( $\geq 104F$ ) fever at day 0 (RR=6) and higher d-3 FEC (RR=1.2 per 100 epg). Mortality was greater for d0 vaccination (OR=8.3) and high fever (OR=41.6). Growth was lower for d0 vaccination (-10.3 lbs), moderate (103-103.9F) and high fever (-24.1 lbs and -16 lbs, respectively), and number of times treated for BRD (-17.5 lbs/treatment). Deworming at arrival was not found to be significantly associated with BRD morbidity, mortality, or weight gain. Health and growth performance of stocker calves may be adversely affected by vaccination, parasitism, and fever at arrival.

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## **The correlation between ground beef contamination with E. coli O157:H7 and human foodborne outbreaks in the US, 2001-2013**

Kyle Johnson\* and David Smith

*Escherichia coli* O157:H7 (STEC-O157) is a zoonotic foodborne pathogen that causes an estimated 96,000 illnesses and as many as 50 deaths annually in the US. The objective of this study was to test the relationship between the annual probability of ground beef contamination (GB) with STEC-O157 and outbreaks of human STEC-O157 disease attributed to various food vehicles (FV). Centers for Disease Control and Prevention (CDC) and Food Safety and Inspection Service (FSIS) data from 2001 through 2013 on the number of outbreaks (n = 300) of laboratory diagnosed human illness and the percentage of positive samples of contaminated GB (n = 350) were tested for correlation by Spearman's rank correlation. As the annual probability of GB contamination increased so too did the number outbreaks of disease attributed to GB ( $r=0.557$ ,  $P=0.047$ ), intact beef ( $r=0.544$ ,  $P=0.054$ ), and any beef products ( $r=0.635$ ,  $P=0.019$ ). The greatest correlation

was between annual probability for contamination of GB and the number of outbreaks attributed to beef products as well as outbreaks of unknown etiology ( $r=0.715$ ,  $P=0.006$ ). However, the annual probability for GB contamination was not correlated with the total number of STEC-O157 outbreaks ( $r=0.429$ ,  $P=0.143$ ). GB contamination is positively associated with outbreaks attributed to beef and unknown sources. However, GB contamination does not explain all outbreaks of STEC-O157 in humans.

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

### **Impact of pneumococcal polyamine transport deficiency on host response in a mouse model of pneumonia.**

Taylor King\*, Aswathy N. Rai, Leslie A. Shack, Edwin Swiatlo, Wes Baumgartner, and Bindu Nanduri

*Streptococcus pneumoniae* (pneumococcus) is the leading cause of community-acquired pneumonia worldwide and causes meningitis, otitis media, and septicemia. Polyamines are ubiquitous small cationic molecules. Their transport and synthesis have been shown to be involved in pneumococcal virulence. Deletion of polyamine transport operon, *potABCD*, in *S. pneumoniae* TIGR4 resulted in attenuation in a mouse model of pneumonia. Previous studies found a significantly higher number of *S. pneumoniae*  $\Delta$ *potABCD* in lung homogenates versus wild type (WT) at 4 hrs post-infection. In addition, our *in vitro* data indicated that there was >14-fold increase in the expression of biosynthetic genes, *speE* and *cadA*, in  $\Delta$ *potABCD* compared to the WT strain. One aim of this study was to use histopathology to compare early immune responses in the lung tissue of mice challenged with WT or  $\Delta$ *potABCD* at 4 and 12 hrs post-infection. Histological analysis of lungs at 4 hrs post-infection with  $\Delta$ *potABCD* showed mild neutrophilic inflammation, centered on distal bronchioles and associated alveoli compared to WT, which had no appreciable inflammation. At 12 hrs,  $\Delta$ *potABCD* infected mice exhibited mild acute to subacute inflammatory changes compared to WT, which had no appreciable inflammation. A second aim was to use qRT-PCR to quantify changes in gene expression in various strains. We quantified *potD* expression in  $\Delta$ *cadA*+ $\Delta$ *speE* and found a slight, statistically insignificant increase compared to WT. We also quantified the expression of 3 oxidative stress genes—*tpx*, *clpP*, and *ciaH* in  $\Delta$ *potABCD*,  $\Delta$ *cadA*+ $\Delta$ *speE*, and  $\Delta$ *potABCD*+ $\Delta$ *cadA*+ $\Delta$ *speE* compared to WT. We found a statistically and biologically significant increase in *tpx* and *ciaH* in  $\Delta$ *potABCD*+ $\Delta$ *cadA*+ $\Delta$ *speE*.

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### **Utility precision cut lung slices for ex vivo investigation of airway hyper-responsiveness in horses**

Lorraine Lopez-Soberal\*, Jacquelyn Bowser, Santosh Kumar TK, Jim Cooley, and Cyprianna Swiderski.

Airway hyper-responsiveness (AHR) is a hallmark of Recurrent Airway Obstruction and Inflammatory Airway Disease, which account for the majority of equine respiratory disease. Despite the clear importance of airway homeostasis to equine health and athletic potential, the molecular mechanisms by which inhaled stimuli result in AHR and bronchoconstriction remain poorly characterized in horses, limiting both diagnosis and therapeutic management of these conditions. We have identified differences in gene product expression in lung that segregate with AHR in disease. To determine the functional significance of these gene products, an *ex vivo* experimental system that can isolate the effects of these gene products on airway function is needed. To address this need, we hypothesized that airways in fresh and cryopreserved equine precision-cut lung slices (PCLS) would demonstrate dose dependent constriction and dilation to cholinergic and  $\beta_2$ -adrenoceptor agonists, respectively. Our initial experiments confirmed that 350-500 mls of 1-1.5% low melting point agarose infused into the caudal dorsal nondependent lung via a BAL catheter (placed with the aid of an endoscope) provides superior lung expansion relative to cannulation of lung tissue obtained at necropsy. Airways in 1mm thick lung slices derived from a non-diseased horse using a mouse brain slicer demonstrated viability with trypan blue exclusion, and dose dependent constriction to carbachol that was also identified following 24 hour incubation in DMEM with 10% FBS at 37°C in 5% CO<sub>2</sub>. Freezing equine PCLS was limited by difficulty attaining thin sections necessary for cryoprotectant penetration ( $\leq 250$   $\mu$ m), with the EMS-4000, despite its published utility for this purpose.

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## **The effects of clopidogrel and omeprazole on platelet function in normal dogs**

Jennifer Lovvorn\*, Brittany Thames, Todd Archer, Andrew Mackin, and John Thomason

Clopidogrel is prescribed in humans as an inhibitor of platelet aggregation, but is associated with gastrointestinal bleeding. Omeprazole, a proton pump inhibitor, is used to reduce GI side effects. However, when these two drugs are combined in people, the anti-platelet effects of clopidogrel are reduced. In dogs, these drugs are also commonly administered together, but it is unknown if omeprazole minimizes the anti-platelet effects of clopidogrel as it does in humans. The goal of our study was to determine if the concurrent use of clopidogrel and omeprazole in dogs reduces the anti-platelet effects of clopidogrel. We hypothesized that dogs receiving clopidogrel alone would have greater inhibition of platelet function compared to dogs receiving both drugs. In a three-way randomized crossover study, 8 dogs were given clopidogrel (1mg/kg, SID, PO), omeprazole (1mg/kg, SID, PO), or both for 5 days with a washout period before switching groups. Blood was collected and analyzed on Day 0, Day 3, and Day 5 of treatment. Platelet function was determined via turbidimetric aggregometry (ADP and collagen) and the PFA-100® (Collagen/ADP) platelet function analyzer. When both drugs were administered, there was an increase of 17% and 68% in platelet aggregation using ADP as an agonist on Days 3 and 5 respectively, compared to clopidogrel alone. With the PFA-100, when compared to clopidogrel alone, there was a 15.7% and 9.5% decrease in closure time when both drugs were administered. Our study suggests that concurrent omeprazole reduces the anti-platelet effects of clopidogrel in dogs, thereby decreasing the effectiveness of clopidogrel. More research must be conducted to better understand this drug interaction.

Student Support: Mississippi State University College of Veterinary Medicine

## ***Rickettsia parkeri* and “*Candidatus Rickettsia andeanae*” in unfed and fed *Amblyomma maculatum* ticks**

Elizabeth Mitchell\*, Jung Keun (Kevin) Lee, Gail Moraru, Kaitlin Graham, Andrea Varela Stokes

*Rickettsia parkeri* is a tick-borne spotted fever agent that uses *Amblyomma maculatum* (Gulf Coast Tick; GCT) as its principle vector. “*Candidatus Rickettsia andeanae*,” a suspected non-pathogen, may also be found in GCTs, and rickettsial co-infections have been reported. This study evaluated *R. parkeri* and “*Ca. R. andeanae*” infection rates in field-collected unfed GCTs and in salivary glands (SG) and legs of experimentally infected GCTs at 0, 6, and 12 days after host feeding, using a multiplex QPCR targeting the rickettsial *ompB* gene. Infection rates in 102 GCTs collected from Mississippi in 2015 were 73% (75/102) with *R. parkeri*, 20% (21/102) with “*Ca. R. andeanae*” and 3% (3/102) with both rickettsiae. In *R. parkeri*-infected GCTs, SG and leg infection rates decreased over time, with up to 10% infected with *R. parkeri* on day 0 to 0% on day 12. In the “*Ca. R. andeanae*” group, infection rates were low but more consistent over time, with up to 20% positive on day 0, and up to 13% positive on day 12. In experimentally co-infected GCTs, *R. parkeri* was detected in 10% of SGs on day 0 and 0% by day 12, and infection rates in legs were 40% on day 0 and 0% by day 12. In co-infected ticks, “*Ca. R. andeanae*” had a higher infection rate on day 0 (70% in SG and 50% in legs), decreasing to below 10% by day 12. Within this group, 20% were co-infected on day 0. These results demonstrate higher infection rates for both rickettsiae from Mississippi GCTs compared to previous years, and apparent loss of rickettsiae in SG and legs from experimentally infected ticks during blood feeding. Future studies on potential interactions between these rickettsiae will allow for a better understanding of disease risk to humans.

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## **New strategy to incorporate nanoparticles within mammalian spermatozoa**

Leah T Myles\*, Peter L Ryan, Scott T Willard, Jean Feugang

Full comprehension of the pathway of mammalian spermatozoa through the utero-tubal lumen is imperative to develop methods of increasing fertility and pregnancy rates. At present, not much is known about the passage of spermatozoa through the female genital tract and pioneer studies have investigated the labeling of mammalian spermatozoa with quantum dot nanoparticles (QD) for non-invasive bioluminescence imaging. However, further incorporation of QD within spermatozoa may still be possible through induced-capacitation. Here we suspended mature boar spermatozoa in the

modified Tris-Buffered Medium containing BSA fraction-V (mTBM-BSA), supplemented or not with QD, QD+Caffeine, or QD+Heparin (n=4 replicates). After 30 and 60 minutes incubation at 37-38°C, samples were analyzed for sperm motility to evaluate the impact of induced-capacitation (Caffeine and Heparin), assessed for bioluminescence signal emission, and imaged with a Transmission Electron Microscope (TEM) to localize the QD within spermatozoa. The presence of caffeine significantly increased the sperm motility parameters (motile and progressive spermatozoa) after 30 minutes and velocity characteristics (Straightness, Linearity, and Straight-line velocity) after 30 and 60 minutes. All QD-labeled spermatozoa showed strong bioluminescence signals that were not observed in the control group (mTBM-BSA). While waiting for the TEM imaging, the current results confirm the non-toxicity effect of QD on sperm motility and revealed beneficial effects of caffeine known to induce sperm hyper-activation and capacitation. We expect the TEM imaging to confirm whether the induced-capacitation allows further incorporation of QD within the spermatozoa.

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### **An observational study of the prevalence of heartworm disease in Mississippi shelter dogs and test efficacy**

Alexis M. Parisi\*, Uri B. Donnett, Christina M. Loftin, Min Wang, David R. Smith and Kimberly A. Woodruff.

Heartworm disease is a progressively fatal parasitic disease endemic in Mississippi (MS) dogs. Costs of tests vary but the most efficacious test for shelter dogs is unknown. The study objectives were to determine the prevalence of heartworm disease in MS shelter dogs and compare agreement between six heartworm detection tests. A cross-sectional study was conducted of dogs > six months of age in three MS shelters. Six tests including a direct blood smear, Modified Knott's test, commercial Heartworm antigen test and antigen batch test with and without heat treated sera were performed. The kappa statistic determined test agreement and test performance compared to a commercial antigen detection ELISA ( $p \leq 0.05$ ). Twenty-three of the fifty-seven dogs tested had positive antigen detected. Multivariable modeling showed dogs 5 years of age or older (odds ratio= 8.842,  $p=0.0408$ ) and every pound in weight added (odds ratio=1.041,  $p=0.0303$ ) increased the odds of having a positive heartworm test result. All commercial tests were found to have high agreement with a commercial ELISA (sensitivities >90%, specificities=100%, and kappa >0.90). Sensitivity was 60.87 and 80% and specificity was 97.06 and 57.14% for blood smear and Modified Knott's test respectively with kappa's of 0.6138 and 0.3514. The prevalence of heartworm disease in this sample population (40%; 95% CI 29%, 53%) was higher than data reported from reference laboratories for MS. Older and larger dogs were at increased odds of heartworm disease likely due to increased time of exposure and body surface. Heartworms appear to be endemic to shelter dogs and less expensive heartworm detection methods were less likely to detect dogs ELISA-positive for heartworms.

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

### **Pilot Study for the Prevalence of Biofilms in Chronic Wounds in Dogs**

Joshua Pierce\* and Elizabeth Swanson

Biofilms are a common complication of diabetic pressure wounds in humans. Biofilms have also been diagnosed in chronic limb wounds in horses. A recent case study identified wound biofilm in a dog, but the prevalence of biofilms in dog wounds has not been determined. This pilot study aimed to evaluate whether and how frequently biofilms can be detected in canine wounds of different durations. We planned to collect tissues from 5 wounds <1 day old and 15 wounds >1 day old. Samples were evaluated for biofilm by scanning electron microscopy and light microscopy. Biofilms were further characterized by standard bacterial culture and molecular bacterial identification. Wounds from two dogs were sampled during the study period. One was a 5-day-old, slowly healing wound. Scanning electron microscopic evaluation revealed planktonic bacteria as well as bacteria within a biofilm on the surface of the wound. Bacterial culture identified *Staphylococcus schleiferi*, and a gram positive cocci. The other wound was an acute bite wound that was less than 24 hours old. Scanning electron microscopic evaluation of the bite wound revealed only planktonic bacteria. Bacterial culture identified *Moraxella sp.*, *Brevundimonas sp.*, and *Arcanobacterium pyogenes*. Histology and molecular bacterial identification are still pending for these samples. These preliminary data support our hypothesis that biofilms occur in canine wounds and can be identified as early as 5 days after injury. This pilot study will continue until a sufficient amount of samples have been analyzed to determine the initial prevalence of biofilms in canines.

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

## **Development of bioluminescent 12-140A *Edwardsiella ictaluri* and evaluation of pathogenesis in *Danio rerio***

Ashley N. Plover\*, Wes Baumgartner, and Mark Lawrence

*Edwardsiella ictaluri* is a facultative intracellular bacterium that is the causative agent of enteric septicemia of catfish (*Ictalurus punctatus*). Recently, *E. ictaluri* has become a primary pathogen in laboratory zebrafish colonies throughout the USA. The strain of *E. ictaluri* (12-140A) in zebrafish *Danio rerio* is markedly different, being much more virulent in the zebrafish than catfish. We sought to produce a bioluminescent strain of 12-140A to be used to investigate the etiology of this disease. To accomplish this, the pAKlux1 plasmid was conjugated into 12-140A as follows. The plasmid contains the luxCDABE operon from *Photobacterium luminescens* ligated into the pBBR2MCS4 broad host range plasmid, forming a plasmid with luxCDABE oriented downstream from the lacZ promoter. Plasmid pAKlux1 from *Escherichia coli* donor strain SM10  $\lambda$ pir into 12-140A by conjugation on 0.45 micron filter papers. Following incubation bacteria were washed and selected on BHI plates with ampicillin and colistin, resulting in bioluminescent 12-140A colonies (pAKlux1 12-140A). Trans Wild-type (RAG +/-) *Danio rerio* were challenged with pAKlux1 12-140A; thirteen fish via immersion at doses of  $3 \times 10^7$  and  $3 \times 10^9$  colony-forming units per milliliter (CFU/mL) and twenty three fish via intraperitoneal (IP) injection (twenty five fish) at doses ranging from  $1 \times 10^1$  to  $1 \times 10^5$  CFU/ $\mu$ L. Additionally, seven wild-type *Danio rerio* were immersed with the wild-type 12-140A *E. ictaluri* at a dose of  $3 \times 10^9$  CFU/mL. Infection assays failed to cause mortalities, suggesting prior exposure to *E. ictaluri*. Histologic examination of experimental zebrafish found no significant pathology. Bacterial cultures from the abdomens of IP infected fish, 9 days post-infection, had no growth.

Student Support: Mississippi State University College of Veterinary Medicine

## **An observational study of the probability of coccidia oocyst presence in shelter-housed cats**

Katrina A Quinn\*, Uri B Donnett, Min Wang, Christina M Loftin, David R Smith, Kimberly A Woodruff

Coccidia are protozoal parasites that easily establish infection in the gastrointestinal tracts of cats. The infection often results in diarrhea and death, particularly in immunocompromised and young animals. The objective of this study was to determine the probability of coccidia oocyst shedding in cats in animal shelters and foster care in Mississippi. Fecal floats were performed for intestinal parasite detection, identification, and quantification. Of the 53 pens of cats examined, 6 pens had coccidia oocysts detected with 5 pens having high concentrations of more than 30 oocysts per slide. The 11% probability (95% CI: 5-23%) of coccidia oocysts detected in this sample population is similar to that previously reported in other studies. Logistic regression was used to determine that the number of animals in the enclosure (n=1-7), concurrent detection of additional intestinal parasites (hook or roundworms), or the fecal score measured were not significant contributors to coccidia oocyst detection. Animals with a high number of coccidia oocysts detected were randomly assigned to one of three treatment groups either receiving no treatment (control), toltrazuril, or ponazuril. Fecal floats were repeated on Days 3 or 4 and 6 or 7. Both the toltrazuril pens (n=2) and the ponazuril pens (n=2) had no coccidia oocysts detected on follow-up fecal exams while the control pen (n=1) had shedding detected on the final follow-up fecal exam. Due to the low number of pens containing cats actively shedding detectable levels of oocysts, the difference in efficacy of the two treatment could not be determined. Future studies are needed to determine if there is a significant difference in the efficacy of ponazuril and toltrazuril.

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

### **Characterization of immune responses to staphylococcal enterotoxin C1 in mouse T cells**

Yianelly Rodríguez Ruiz\*, Nogi Park, Eun-Ju Yang, Jeffrey Eells, Keun-Seok Seo, and Barbara L. F. Kaplan.

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with unknown etiology. It affects over 2 million people worldwide causing variable clinical signs and symptoms depending on the affected area in the CNS, which may include motor, sensory, autonomic as well as cognitive disabilities. Experimental autoimmune encephalomyelitis (EAE) is the most common animal model used to study MS and causes paralysis and spinal cord inflammation. Regulatory cells have been shown to control EAE and MS, including CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Tregs), as well as CD8<sup>+</sup> T suppressor cells (Ts). One potential therapeutic strategy to limit the severity of the disease is to increase Tregs. Staphylococcal enterotoxin C1 (SEC1) is a pyrogenic toxin produced by *Staphylococcus aureus*, and is a superantigen (SAg), which can induce Tregs at low concentrations. The goal of this work was to characterize the degree to which Tregs and Ts could be induced in response to SEC1 in mouse splenocytes. First, we evaluated cellular proliferation in mouse splenocytes in response to SEC1 and found SEC1 induced proliferation at high concentration. Next we performed flow cytometry for CD25 and FoxP3 expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells using various SEC1 concentrations and time points. SEC1 induced modest CD25 and FoxP3 expression in CD4<sup>+</sup> cells but was more effective at lower concentrations. Although there was little FoxP3 expression induced in CD8<sup>+</sup> cells, the CD25 expression was high in response to SEC1. The results suggest that treatment of splenocytes with SEC1 could provide a source of Tregs and Ts that could help control EAE.

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### **Identification of dendritic cells in channel catfish**

Matthew A. Scott\*, Adef Kordon, Wes Baumgartner, Lesya M. Pinchuk

Multiple dendritic cell (DC) populations, that strikingly resemble the most powerful professional antigen presenting cells (APCs), DCs in mammals, have been identified and characterized morphologically and functionally in teleost fish models. The presence of DCs with remarkable similarities to human Langerhans cells (LCs) in the spleen and head kidney (HK) of salmonids has been reported. In this study, we assessed DC-like cells with light microscopy in the immunocompetent tissues of channel catfish. In addition, the presence of LC-like cells in catfish lymphoid organs were studied with flow cytometry and immunohistochemistry, using commercially available purified and directly conjugated antibody against human Langerin/CD207. Cells with mammalian DC-like dendritic morphology have been identified in channel catfish spleen, HK and peripheral blood samples. In addition, flow cytometric analysis revealed the presence of LC-like Langerin/CD207<sup>+</sup> cells in the spleen and HK, but not in peripheral blood of catfish. The supporting evidence of the presence of LC-like cells in the immunologically related organs of catfish was obtained by applying immunohistochemistry approach with human Langerin/CD207-specific purified and fluorescence-labeled antibodies. Our preliminary data suggest that multiple cell populations with DC-like morphology in the lymphoid organs of catfish may share morphological and functional properties with previously reported DCs in teleost fish and mammals. This data lays a foundation for further studies aimed at the enrichment of DC-like cells from catfish peripheral blood, HK and spleen as was previously demonstrated in the rainbow trout model and their further morphological and functional characterization.

Student Support: Mississippi State University College of Veterinary Medicine

### **Tensile properties of cannon bones in horses with hereditary equine regional dermal asthenia**

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Hereditary Equine Regional Dermal Asthenia (HERDA) is a debilitating connective tissue disease found in American Quarter horses and related breeds. It is caused by a c.115G>A missense mutation in peptidyl-prolyl cis-trans isomerase B, the gene encoding cyclophilin B, a protein responsible for post-translational modification of type I and III fibrillar collagens. We have previously demonstrated that HERDA alters tensile properties of skin, tendons, and vessels, but a bone phenotype has not been identified. Because bone is almost exclusively mineralized type I collagen, we hypothesized that tensile properties of HERDA bone could differ from control horses. To test this, a four-point bending test was conducted on frozen canon bones from 12 HERDA affected and 11 control horses. Young's modulus ( $E$ ), Modulus of Toughness (MT), Failure Stress ( $\sigma$ ), and Failure Strain ( $\epsilon$ ) were calculated. Linear regression was executed using SAS 9.2

PROC GLM program (SAS Institute, 2009). Multivariate analysis of age, sex, and time frozen were not associated with any of the four values. HERDA status was significantly associated with  $E$  ( $p=0.0005$ ),  $\sigma$  ( $p=0.0027$ ) and  $\epsilon$  ( $p=0.0065$ ). These results indicate that bone from horses with HERDA has significantly increased stiffness, requires significantly more force to break, and shows significantly less deformation before failure than the control bone, and documents the presence of a subclinical bone phenotype in association with the HERDA c.115G>A missense mutation in peptidyl-prolyl cis-trans isomerase.

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