

2019 Summer Research Abstracts

Assessing diversity of antimicrobial resistance phenotypes and genotypes in bovine *Mannheimia haemolytica* isolates

Hannah F. Carter, Amelia R. Woolums, Robert W. Wills, Frank W. Austin, J. Dustin Loy, Brandi B. Karisch, William B. Epperson, John R. Blanton Jr.

Bovine respiratory disease (BRD) threatens stocker cattle operations; this may be exacerbated by increasing prevalence of antimicrobial resistance (AMR) in *Mannheimia haemolytica* (Mh), a leading cause of BRD. Research is ongoing to determine AMR causes and impacts. Characterization of AMR in Mh by culture and susceptibility testing is complicated by pathogen diversity in the host and uncertainty regarding AMR phenotypes (antibiograms) and genotypes in a sample. The objective of this study was to assess diversity of Mh phenotypes and genotypes on nasopharyngeal swabs (NPS) from stocker cattle. NPS were collected from 20 cattle. Phenotype was determined by measuring minimum inhibitory concentrations (MIC) by broth microdilution (Sensititre YBOPO7F). Genotype was indirectly determined by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) biomarkers via previously reported methods. NPS from 7 of 20 cattle yielded at least one Mh colony; average number (+ range) of colonies isolated per NPS was 45 (1-76). NPS from 3 cattle yielded one phenotype, 3 NPS had two phenotypes, and 1 NPS yielded three. NPS from all 7 cattle yielded one genotype. All phenotypic differences were due to one MIC dilution. The number of different phenotypes and genotypes was restricted in this population, suggesting that all Mh phenotypes and genotypes might often be identified by selection of relatively few colonies. These results will be used in a model to estimate the number of colonies that should be selected to identify all Mh phenotypes and genotypes on a bovine NPS with a desired level of confidence. The results will strengthen research to determine factors that drive AMR in stocker cattle.

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Identifying changes in canine urothelial cell membrane morphology in response to increased glucose concentrations

Morgan L. Currin, Victoria E. Fields, and Elizabeth A. Swanson

Bacterial biofilm populations located in the urinary bladder are a significant cause of chronic and recurrent urinary tract infections (UTIs) in medicine, both human and veterinary. This is due to lack of treatment strategies that can effectively target and degrade biofilm populations. Patients that produce high concentrations of glucose in their urine, such as diabetics, are reportedly at an increased risk for UTIs compared to those with normal urine. This study aimed to observe changes in urothelial cell membrane morphology when exposed to an environment with a high glucose concentration. Canine urothelial cells were isolated from combined free-catch urine samples from 4 dogs and incubated in cell culture plates in two environments: physiologic media conditions (control) and physiologic media with increased glucose concentrations (10%). In a 12 well culture plate, 6 wells of each group (control and increased glucose) were incubated on poly-L-lysine coated coverslips. In another 12 well culture plate, 6 wells of each group (control and increased glucose) were grown on porcine small intestinal submucosa (SIS) in an attempt to promote cellular adhesion and growth. After an incubation period of 5 days, a confluent cellular mat of viable cells was not obtained in either group. SEM and TEM imaging were then used to observe the cellular structure and membrane morphology. Cellular imaging and analysis is pending. Further studies are warranted to develop techniques for urothelial cell culture in high glucose concentrations.

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

Analysis of multi-drug resistant bacteria isolated from shelter and foster dogs and cats

Heather Daw, Peter McGinn, Kwang won Seo, Joo Youn Park, Sunghyun Yoon, Keun-Seok Seo, Cooper Brookshire

This study aimed to isolate and characterize extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* present in healthy shelter/foster dogs and cats. The presence of ESBL producing enterobacteriaceae poses a concern for both animal and human health. The Centers for Disease Control and Prevention has identified ESBLs as a serious threat to public health. Aural, oral, and fecal samples were collected from 83 dogs and 37 cats. ESBL producing bacteria were selected for using TSB enrichment broth and serial MacConkey broth incubations containing cephalothin and then ceftiofur. PCR was performed on isolated colonies phenotypically consistent with *E. coli* and *K. pneumoniae* for species identification. 50.6% of dogs and 32.4% of cats surveyed harbored at least one isolate of suspected ESBL producing *E. coli* or *K. pneumoniae*. Of the 68 *E. coli* and 19 *K. pneumoniae* isolates suspected to be ESBL producing, multiplex PCR identified at least one ESBL gene in 66 of the *E. coli* and 4 of the *K. pneumoniae*. Aural, oral, and fecal samples were also screened for *Staphylococcus* spp. 60.2% of dogs and 21.6% of cats surveyed harbored at least one isolate of *Staphylococcus* spp. Of the 122 samples that were phenotypically consistent with *Staphylococcus* spp., 70.1% were confirmed to be *S. intermedius* with PCR. These data demonstrate an alarming prevalence of ESBL producing enterobacteriaceae and *Staphylococcus intermedius* in shelter/foster dogs and cats and suggest the need for urgent development of One Health approaches to disease prevention and implementation of antibiotic stewardship strategies.

Student Support: Mississippi State University College of Veterinary Medicine

Investigating the effects of supplemental feeding on environmental fecal density and strongyle-type egg counts of white tailed deer.

Chris Deignan, Miranda Huang, Steve Demarais, Cooper Brookshire

Supplemental feeding of white-tailed deer is a common management practice aimed at improving herd health and thriftiness. However, provision of foodstuffs attracts a wide variety of wildlife and concentrates game in proximity to feeders. The purpose of this study was to determine if there is a relationship between feeder placement and increased fecal density and parasites, thereby increasing potential for disease transmission. Strongyle-type nematodes are very common in white-tailed deer and have a direct life cycle. The hypothesis of this study was that fecal density and fecal egg count load would be higher in a 25-meter radius around established feeders (fed sites) when compared to ecologically similar control sites without supplemental feeding (unfed sites). All visible fecal material was sampled at each 25-meter radius site. To analyze fecal density between fed and unfed sites, a paired-sample t-test was utilized. Fed sites had a significantly higher fecal density as measured by total number of unique fecal piles ($M=26.8$, $SD=25.5$) compared to unfed sites ($M=14.2$, $SD=20.5$); $t(23)=3.05$, $p=0.006$. Only a subset of fecal samples has been suitable for strongyle-type eggs per gram analysis. At the time this abstract was written, insufficient samples exist for a full statistical evaluation. However, currently available samples from unfed sites averaged 67.4 ($SD=116.3$) strongyle-type eggs per gram of feces while fed sites averaged 110 ($SD=162.8$) strongyle-type eggs per gram of feces, respectively; $t(93)=1.27$, $p=0.219$. These preliminary data suggest a possible association between supplemental wildlife feeding and environmental fecal density/white-tailed deer parasitism.

Student Support: Mississippi State University College of Veterinary Medicine and Mississippi Department of Wildlife, Fisheries and Parks

Use of trazodone and gabapentin to reduce fear, anxiety and stress during veterinary visits in client owned dogs.

Rachel Doenges, Christine Calder

Fear and anxiety associated with veterinary visits can lead to both adverse psychological and physiological effects, making patients more difficult to examine leading to delayed treatment, limited preventative care options, potential injuries, and overall poor patient welfare. In cats, studies have demonstrated the efficacy of trazodone and gabapentin separately in reducing the clinical signs of fear and anxiety, facilitating veterinary examinations and transportation to the

veterinary hospital However, the author could not find similar studies in dogs using these medications for veterinary visits in combination or as a single agent. We hypothesized that when administered prior to veterinary visits, a combination of trazodone and gabapentin would decrease anxiety related clinical signs associated with the veterinary visit and facilitate handling during a physical examination in dogs compared to placebo. This was a double blinded, placebo controlled, randomized crossover study. Prior to a scheduled veterinary visit and examination, each dog was randomly assigned to receive a trazodone and gabapentin medication combination or placebo PO. After a 1-week period, each dog received the opposite treatment and the protocol was repeated. At each visit, the dog was assigned a stress score, behavior summation score, and tractability score by the client and PI. No significant difference was found in any of the scores, for each category, when compared to placebo although the overall total score in each category was increased on placebo. The most common adverse event reported was sedation. Although the results did not support the hypothesis in this study, both trazodone and gabapentin appeared to be well tolerated by most dogs.

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

Determination of optimal culture media for the growth and study of urinary pathogen *Enterococcus faecalis*

Victoria E. Fields, Morgan L. Currin, Elizabeth A. Swanson

Bacterial biofilms play an important role in urinary tract infections (UTIs), responsible for persistent infections leading to recurrences and relapses in both human and veterinary medicine. The precise mechanism of bacterial adhesion to abiotic surfaces such as synthetic material and minerals is well known; however, the host cell mechanisms that facilitate adhesion and subsequent biofilm formation in soft tissue are not well understood. In this study, we aimed to establish a reliable strain of gram-positive *Enterococcus faecalis* isolated from a clinical urine sample and to analyze the use of canine urine as a culture medium for growing uropathogenic biofilms that best mimics conditions naturally found in the urinary bladder. Microtiter plate biofilm assays were performed on eight clinical isolates of *E. faecalis* using different culture media. Seed cultures of each isolate were plated in 96-well plates in one of three media (RPMI, RPMI + urine (1:1), or urine) and then incubated at 37°C for 72 hours. Wells were washed with PBS and crystal violet staining was performed. Optical density (OD) was measured and results showed one strain (Strain 8) that consistently demonstrated denser biofilm formation. This strain can be utilized in future studies to identify the effect that host modulations have on biofilm formation. Furthermore, biofilm development was significantly denser in canine urine, confirming our hypothesis that bacteria will adhere and grow better in urine versus traditional cell and bacterial culture media. These results suggest urine can be utilized as a physiologic model culture medium to study biofilm formation of urinary pathogens.

Student Support: Mississippi State University College of Veterinary Medicine

Analysis of the thermoregulatory effect of applying socks to the feet of dogs undergoing general anesthesia for ovariohysterectomy

Flinchbaugh, Rebecca, Cooper Brookshire, Kimberly Woodruff

Hypothermia is a common perioperative complication that occurs in veterinary and human medicine. Maintaining a proper body temperature under anesthesia is an equilibrium between heat loss and heat production. Factors that can influence this balance include ambient temperature, weight, duration of surgical procedure, impairment of the thermoregulatory centers in the hypothalamus, amount of anesthesia given, passive or active heating mechanisms, etc. The aim of this study was to evaluate the thermoregulatory effect of applying socks to the feet of anesthetized dogs undergoing ovariohysterectomy (OHE). All animals received standard surgical warming support (heating pad, blanket, etc). At the time of surgery, patients were randomly assigned to control (no socks) or treatment (socks) groups. Socks were made in custom lengths using a double layer of surgical stocking to cover the entire foot up to the carpus/hock of each limb. A DataTherm2 digital thermometer was utilized to record rectal temperatures every two minutes until approximately 10 minutes after surgery was completed. The thermoregulatory effect was analyzed by comparing the loss of body heat in degrees Fahrenheit between the treatment groups utilizing an independent-samples t-test. Patients wearing socks lost significantly less body heat (M=2.6 degrees F, SD=1.2) compared to control patients without socks (M=3.9 degrees F, SD=1.83); $t(55)=3.087$, $p=0.003$. These data demonstrate significant thermoregulatory properties of socks when applied

to the feet of dogs anesthetized for OHE and suggest that routine application of socks during anesthesia may improve patient care in veterinary medicine.

Student Support: Mississippi State University College of Veterinary Medicine

Expression of immune-related genes in channel catfish following supplementation of trans-cinnamaldehyde

Emerald Ford, Reshma Ramachandran, Ozan Ozdemir, Hossam Abdelhamed

Pond-raised channel catfish (*Ictalurus punctatus*) is an economically important agricultural commodity in the United States. Bacterial pathogens including *Edwardsiella ictaluri*, a causative agent of enteric septicemia of catfish (ESC), threaten profitable catfish production through inventory losses and emergence of antimicrobial resistance. Due to selection pressure, resistant microbial strains are emerging, hastening the need for alternative therapies. Trans-cinnamaldehyde (TC) is a phytophenolic compound derived from cinnamon bark. Previous study revealed that TC inhibits the growth of *E. ictaluri in-vitro* and reduced mortality of catfish fingerlings following *E. ictaluri* infection. No information is available concerning effect of TC on immune related genes expression in catfish. The aim of this study was to investigate the protective effect of TC against *E. ictaluri* as well as to assess the impacts of TC on expression of immune-related genes. Ten days after the start of the feeding TC, fish were challenged with *E. ictaluri*, and liver, spleen, and anterior kidneys were collected at various timepoints. Subsequently, real-time PCR was employed to determine the expression levels of twelve immune-related genes. Results showed upregulation of MHC-I and MHC-II on day 7 and CD8- α , IgM, and TNF α on day 14 on TC administrated fish. Moreover, on day 28 genes including IgM, CD8- α , CD4-2, MHC-II, IL-1 β , TNF α , and IFN- γ were upregulated. This finding demonstrated that dietary TC supplementation can improve catfish immunity by initiation of innate and adaptive immune responses in catfish immunocompetent tissues. This research provides useful insights about use of TC as an antimicrobial alternative in aquaculture.

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Species differences in the susceptibility of juvenile rats and mice to chlorpyrifos: Role of hepatic esterases

Lauren B. Glenn, Juliet E. Ryan, Katelyn N. Sette, Caitlin N. Seay, Shirley X. Guo-Ross, Russell L. Carr

High levels exposure to the organophosphorus insecticide chlorpyrifos (CPF) induces toxicity via the inhibition of brain cholinesterase (ChE) resulting in overstimulation of the nervous system. Recent evidence suggests that low level CPF exposure is also important as it is linked to developmental delays in children. Based on preliminary work, juvenile mice and juvenile rats differ in their susceptibility to low level CPF exposure. The basis for this difference is unclear but may be related to species differences in the levels of alternative binding sites throughout the body. These binding sites are commonly enzymes such as ChE and carboxylesterase (CES) in non-nervous tissues. These can bind the active CPF metabolite and remove it from circulation before it can interfere with the nervous system. To investigate this, post-natal day 10 (PND10) rats and mice were exposed daily for 7 days to either corn oil or a range of dosages of CPF by oral gavage. On PND16 (4 hrs post-exposure), brain, serum, and liver were collected. CPF treatment resulted in greater inhibition of brain ChE in rats than mice. In the liver, rats had slightly higher levels of CES than mice, but the level of inhibition of CES was higher in rats than mice. In the serum, mice had 8-fold higher levels of CES, and a dosage that almost eliminated CES activity in rats only resulted in 24% inhibition in mice. For ChE, greater inhibition was observed in both tissues in rats. Our data suggest that the blood CES provides a major CPF detoxication mechanism in mice that is great than that of rats. The lower level of rat serum CES suggests more rapid saturation of the protective enzymes than occurs in mice resulting in more CPF reaching the brain to inhibit brain ChE.

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Innate Effectors of Neutrophil Homing in Peripheral Lung in Pasture-Associated Severe Equine Asthma

Lauren E. Ingraham, Cyprianna E. Swiderski, Christa Frodella, Soma Mukherjee, Jackie E. Bowser, Cathleen Mochal, Alison Eddy, Andrew K. Claude.

Severe equine asthma (SEA) is characterized by reversible airway obstruction, non-specific airway hyper-responsiveness and chronic neutrophilic airway inflammation. Two forms of SEA are described. One is elicited by barn dust in association with indoor housing in continental climates. The second is associated with grazing grass pasture during hot humid conditions. Though neutrophilic airway inflammation that characterizes SEA segregates with severe and corticosteroid refractory forms of human asthma, the molecular mechanisms responsible for neutrophilic airway inflammation are not clearly defined. Anti-IL-17 therapy, directed at TH17-mediated adaptive responses that mediate neutrophilic inflammation, has not been successful in the treatment of human asthma. Accordingly, we hypothesize that innate immune responses provide key signals for early and continued neutrophil recruitment. Our modeling of differentially expressed genes from serial lung biopsies of pasture-associated SEA horses during asthma exacerbation versus remission (N=6) identified significant increases in innate immune signaling pathways that mediate neutrophilic airway inflammation ($p < 10^{-5}$). We therefore examined expression of one of these signaling molecules, TREM1, in peripheral lung from horses with pasture-associated SEA (N=4) and non-diseased control horses (N=2) using semi-quantitative RT-PCR to confirm this finding. As diseased and control horses shared the same environment, differences in this innate immune signaling pathways known to home neutrophils to the lung will substantiate the relevance of this pathway in defining the mechanistic basis of neutrophilic inflammatory responses that characterize severe asthma.

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

Analysis of nosocomial bacteria in a veterinary teaching hospital

Peter N. McGinn, Kwang Won Seo, Cooper Brookshire, Heather Daw, Joo Youn Park, Sunghyun Yoon, Frank Austin, Keun Seok Seo

Antimicrobial resistance (AMR) is a serious threat to public health. Emergence of hospital acquired methicillin resistant *Staphylococcus* spp. and extended spectrum beta-lactamase (ESBL) infections demand the implementation of infection control programs in medical systems. However, very little effort has been made in veterinary medicine. In this study, we collected environmental samples from 22 different locations at the Mississippi State University veterinary teaching hospital over 12 weeks and phenotypically and genotypically characterized Gram positive (GP) and negative (GN) isolates. We found recurring GP bacterial populations with five distinct phenotypes. White colonies with single-zone hemolysis (WSH) were most common and accounted for 27.63% (79/286) of isolates followed by white double-zone hemolysis (WDH) at 23.07% (66/286) and white gamma-zone hemolysis at 23.07% (66/286). Yellow colonies with no hemolysis (YNH) accounted for 18.5% (53/286) of isolates while yellow double-zone colonies accounted for 4.54% (13/286). As a pilot study, species was determined in one isolate from each phenotype by 16s rRNA sequencing. Results showed that *Aerococcus urinaeequi* was identified from the WGH isolate. Interestingly, three *Staphylococcus* species were identified from the WSH (*S. epidermidis*), WDH (*S. pseudointermedius*), and YDH (*S. waneri*) isolates. These results suggest that *Staphylococcus* species are a major nosocomial bacteria at the veterinary hospital. We will further determine AMR patterns of these isolates and additional isolated GN bacteria. Results of this research will be useful for establishing an infection control program at the veterinary hospital.

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Modeling the Effects of Antimicrobial Use Policies on Profitability of Post-Weaning Beef Production Systems

Tyler B. McMurray; Robert W. Wills, David R. Smith

The objective of this study was to understand how profitability of post-weaning beef production systems in the US is affected by health and production factors, including antimicrobial use policies, such as prohibiting the use of mass medication or applying price incentives for cattle not treated with antimicrobials. Vensim Personal Learning Edition software was used to create causal loop and stock and flow supply-chain models describing the post-weaning beef

production system. A spreadsheet was used to calculate the breakeven purchase price of the systems modeled. A causal loop diagram was created to describe the factors important to profitability in post weaning beef production systems, including body weight, purchase price, compensatory gains, risk and cost of bovine respiratory disease (BRD), tolerance for BRD, metaphylaxis, and social concerns regarding antimicrobial use. Converting the causal loop to a stock and flow model revealed that cattle flow through six basic systems with the primary factor being whether the calves are considered high or low risk for BRD. Those systems are: high risk calf-fed feedlot, high risk backgrounder, high risk yearling feedlot, low risk calf-fed feedlot, low risk backgrounder, and low risk yearling feedlot. High risk calf-fed systems with metaphylactic use of antimicrobials have higher breakeven purchase prices (higher relative profit) compared to low risk calf-fed systems without metaphylaxis. Changes in antimicrobial-use policy affect which systems are the most profitable. This model creates a platform to evaluate different antimicrobial use policies and to understand the implications of those policies on the beef cattle industry.

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Life Cycle Elucidation & Exploration of Diplostomid Trematode Diversity in Pond-Raised Catfish Aquaculture

Meisha N. Mychajlonka, Ethan T. Woodyard, D. Tommy King, Wes A. Baumgartner, Matt J. Griffin, David J. Wise, and T. Graham Rosser

Pond-raised catfish are the most extensively grown freshwater food fish in the United States. Losses due to piscivorous birds and the trematode parasites they introduce represent significant threats to the sustainability and profitability of this economically important industry. Management of these infections depends on the accurate identification of all hosts involved in these complex life cycles. As part of a larger survey of trematodes in piscivorous birds, American white pelicans *Pelecanus erythrorhynchos* ($n=12$) were necropsied and their live trematodes collected. Two novel adult diplostomid species in the genera *Bursatintinnabulus* and *Bursacetabulus* were morphologically identified from the large intestinal and cecal contents of these birds. Each species was molecularly characterized by sequencing ribosomal and mitochondrial gene regions for supplemental identification and life cycle elucidation. Simultaneously, the potential intermediate snail host *Biomphalaria havanensis* was collected from catfish production ponds and examined for patent trematode infections. Cercarial stages shed by snails were molecularly identified and used in experimental infection trials with channel catfish *Ictalurus punctatus*. Histopathological analysis of catfish tissues at select time points was used to document potential pathological changes associated with developing metacercariae. Evaluation of mitochondrial cytochrome *c* oxidase subunit 1 sequence data from all stages indicated conspecificity and the hosts involved in the life cycles of both novel species include: *P. erythrorhynchos*, *B. havanensis*, and *I. punctatus*. Through the elucidation of these life cycles, integrated pest management plans can be implemented for prevention.

Student Support: Mississippi State University College of Veterinary Medicine

Hepatozoon americanum in cell culture and field-collected Gulf Coast ticks.

Natalie D. Parkins, John V. Stokes, Andrea S. Varela-Stokes.

Hepatozoon americanum is a protozoan parasite and the causative agent of the debilitating disease, American Canine Hepatozoonosis (ACH). Canids are infected through ingestion of the vector and definitive host, *Amblyomma maculatum* (Gulf Coast tick), or via consumption of paratenic hosts. Determining prevalence of *H. americanum* in vector populations would reveal areas of transmission risk to canids. Further, isolating the protozoan in culture would allow for better characterization of the organism and expansion of diagnostic assays. We addressed these needs by first using a multiplex TaqMan qPCR assay for detection of *H. americanum* in wild-caught adult *A. maculatum* using the 18S rRNA gene target. Of 102 Gulf Coast ticks collected in Oktibbeha and Lowndes counties in Mississippi, we did not detect *H. americanum*; however, results from ticks collected in Huntsville, Alabama, where ACH was recently diagnosed, are pending. DH82 (canine monocytic cell line) and ISE6 (*Ixodes scapularis* embryonic tick cells) cultures inoculated with either *H. americanum*-positive tick tissues or canine buffy coat cells, were evaluated for cultivation of *H. americanum*. Suspect organisms in ISE6 cells were visualized in cytopspins from culture samples that yielded CT values by TaqMan qPCR. Samples from selected cultures were archived for continued passage and additional analyses. The lack of detectable *H. americanum* in Gulf Coast ticks from this season, coupled with no to low infection rates in past years continues to suggest

that adult ticks may not be a primary route of transmission to dogs. Early evidence of *H. americanum* in cell culture samples implies that co-culture in tick or mammalian cells may support cultivation if conditions are optimized.

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Safety profile study on novel oxime acetylcholinesterase reactivators versus 2-PAM

Reid Pearlman, Janice Chambers, Edward Meek

Organophosphate (OP) poisoning is a frequent occurrence worldwide, both accidentally and intentionally. The mechanism of the acute poisoning produced by OP's occurs through the inhibition of nervous system acetylcholinesterase which leads to an accumulation of acetylcholine and subsequently excessive stimulation of peripheral muscarinic /nicotinic (PNS) receptors and central nervous system (CNS) receptors. In general, following OP exposure salivation, lacrimation, urination, defecation, gastric signs and emesis (SLUDGE) occur rapidly, often within minutes. Additionally, signs may be chronic following exposure, often arising after periods where no symptoms occurred. These compounds are highly likely to affect the central nervous system adversely because of the prolonged and persistent seizures induced by OP poisoning. The CNS is protected by a blood brain barrier which serves as an additional layer of protection from any substance which is harmful to the brain present in the peripheral circulation, including an antidote for the OP. The currently available oxime reactivator is 2-PAM (pralidoxime). Pralidoxime's drawback is that it cannot effectively cross the blood-brain barrier effectively due to its positive charge, and its therapeutic action is restricted to the peripheral nervous system. Several novel oxime compounds have shown efficacy in preventing the long term damage to brain architecture caused by OPs.

These novel oxime antidotes being studied do penetrate the CNS better than the currently approved antidotes and this summer's research objective is to investigate their safety profiles needed for further drug development. Limited data collected earlier suggested that toxicity to the kidney might be the main toxic effect of these novel oximes. Through dosing rats with the novel oxime antidotes, and then comparing their data to those dosed with 2-PAM and to a vehicle control, the objective is to demonstrate that the novel oxime antidotes can be safely administered. Data have indicated that 2-PAM is more acutely toxic than the novel oximes and that kidney damage is the most prominent adverse effect of the novel oximes

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The Effect of Cannabinoids on Pro-inflammatory Cytokines in Canine PBMCs and Granulocytes

Mimi Pelanne, Jaylan Sears, Matthew Ross, Todd Archer and Barbara L. F. Kaplan

Pet owner use of cannabinoids has increased in the past few years, however; the health effects and/or putative therapeutic benefits of cannabinoid treatment in veterinary medicine are not known. Owners are giving their animals cannabinoids without consultation of a veterinarian, with no knowledge of dosing, effective administration routes or adverse effects. This purpose of this project is to examine if cannabinoids affect gene expression of the proinflammatory cytokines, interleukin-17 (*IL17*) and tumor necrosis factor alpha (*TNF α*), in canine peripheral blood mononuclear cells (PBMCs) and granulocytes. The hypothesis is that the plant-derived cannabinoids, cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (THC) suppress pro-inflammatory gene expression. The results showed that *TNF α* and *IL17* react similarly when stimulated with various stimulants and treated with CBD or THC. After analyzing gene expression of both cytokines via qPCR, it was observed that expression is enhanced by CBD and THC in PBMCs stimulated with lipopolysaccharide (LPS), phorbol ester plus calcium ionophore (P/I) and Concanavalin-A (ConA). In contrast, cytokine expression was robustly inhibited by CBD and THC in granulocytes. We also quantified the intracellular amount of CBD following delivery to culture using LC-MS/MS to precisely define cannabinoid effects in vitro. Overall these results suggest that cannabinoids affect canine immune cell function in a cell-type specific manner.

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Effects of Swainsonine on Invitro Hepatocytes in Relation to Malaria Invasion Mechanisms

Audrey Pugh, Aumbriel Schwirian, Jonas G. King

The impact of the deadly vector-borne disease, Malaria, is a relevant concern to many doctors and scientist alike. Malaria affects nearly half the world's population, killing many each year and causing economic strife throughout endemic countries. Malaria is acquired by humans through transmission of infective *Plasmodium spp.* sporozoites from an infected mosquito vector following a blood meal. Once inside the human body, sporozoites travel through vasculature to the liver, where they invade hepatocytes and continue to mature. The hepatocyte invasion mechanism is the target of the current vaccine, whose antigenic target is the circumsporozoite-Heparan Sulfate Proteoglycan (CSP-HSPG). While it is known that CSP-HSPG is very important to the invasion mechanism, the vaccine is still only partially effective. The results lead scientist to believe that other hepatocyte epithelial state factors are involved in the invasion mechanism. Prior work within the King Lab involved determining these other epithelial state markers important to *Plasmodium* invasion. Hepatocellular carcinoma (HCC) lines have been found to have lower rates of *Plasmodium* sporozoite invasion, so a comparison between the HCC epithelial state and the primary hepatocyte epithelial state was conducted. In another effort, cell lines were treated with swainsonine, the toxin made within the locoweed plant, which is an inhibitor of Golgi alpha-mannosidase II. Swainsonine changes the glycocalyx of the treated cells. Treated cells and control cells were digested and then sent for RNA sequencing. Data from the RNAseq shows differences in expression of certain genes that might reveal other epithelial state markers than CSP-HSPG that are important in the invasion of *Plasmodium* sporozoites.

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Partitioning in red blood cells after continuous rate infusion and repeated intramuscular administration of dexmedetomidine in horses

Andrea Rodriguez, Sarah Shane, Patricia Ellrich, Claudio Correa Natalini

Dexmedetomidine is an α_2 -adrenoreceptor agonist commonly used in equine anesthetic protocols. Currently, it is unknown whether dexmedetomidine undergoes partitioning within erythrocytes as is commonly seen with romifidine. Furthermore, there have not been any compartmental pharmacokinetic studies performed to investigate if this partitioning effect is occurring with dexmedetomidine. Once hemoglobin and dexmedetomidine concentrations are determined, it will be possible to normalize dexmedetomidine contents per erythrocytes. Hence, the aim of this study is to determine the concentration of dexmedetomidine (ng) per gram of hemoglobin. Eight adult, female horses were administered dexmedetomidine intravenously (Treatment A) and intramuscularly (Treatment B); blood samples were then collected at baseline and up to 3 hours. Whole blood was centrifuged, lysed, suspended with phosphate buffer solution (PBS) and homogenized. Hemoglobin concentration (g/dl) was determined at each timepoint for each sample. An exclusion filter (10KDa) was then used to separate hemoglobin from the remaining dexmedetomidine. The resulting homogenate was assayed using liquid chromatography-tandem mass spectrometry. Our results showed significance ($P < 0.0001$) between the concentration of dexmedetomidine in erythrocytes for both routes of administration at all time points except 120 minutes. Dexmedetomidine is undergoing partitioning in erythrocytes but is found at lower concentrations within the muscle at all time points. Understanding the pharmacokinetics of dexmedetomidine will allow practitioners to properly implement anesthetic protocols in equine patients and monitor for the drug's adverse side effects even after the patient has been recovered.

Student Support: Mississippi State University College of Veterinary Medicine

A collagen elastin-like-polypeptide hydrogel for delivery of doxycycline to treat osteomyelitis

Victoria Scites, Anna Rourke, Amol Janorkar, Lauren Priddy

Osteomyelitis is an infection of bone and/or bone marrow. While mild cases are treated with systemic antibiotics, severe cases may require surgical debridement of infected bone and soft tissue. As an alternative to systemic antibiotics, delivery vehicles that provide a localized repository of therapeutics directly to the site of infection are needed. Collagen and elastin are structural proteins found abundantly in connective tissue and the extracellular matrix and are used for guided bone

regeneration. When brought to 37 C, solutions of both elastin-like polypeptide (ELP) and collagen form gels. In this ongoing pilot study, a new collagen-ELP hydrogel was used to deliver a low concentration of bone morphogenetic protein-2 and a high concentration of doxycycline antibiotic to the site of osteomyelitis for treatment of infection induced with *Staphylococcus aureus* in rats. Our hypothesis is that the bacterial burden at the end of the study will be lower in treated rats than in control rats. For all *in vitro* work, a dose of doxycycline in gel above the known solubility limit was compared to the dose below the solubility limit used in previous studies. A release kinetics study demonstrated a peak release within the first 24 hours. The high dose gel released more doxycycline, but with a more sustained rate. Kirby Bauer assays showed a dose-dependent response of bacterial inhibition with increasing concentrations of doxycycline. Osteomyelitis was induced surgically in rats using a contaminated screw procedure. The rats received either doxycycline-loaded gel or a blank control gel. The *in vivo* study is ongoing. Bacterial counts will be performed on bone and soft tissue at four weeks post-treatment to quantify bacterial burden.

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

Effects of an environmentally relevant mixture of organochlorine pesticides on macrophages and Kupffer cells

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Activated Kupffer cells are the resident macrophages of the liver and serve a crucial role in liver repair and disease; however, altered function can lead to chronic inflammation and disease. Various persistent organic pollutants (POPs) are known to have differing effects on macrophage function. However, the effects of exposure to these POPs on Kupffer cell viability and function is not well elucidated. Thus, the goal of this study is to examine the effects of exposure to an environmentally relevant mixture of organochlorine pesticides (OCPs) on macrophage and Kupffer cell viability and function. Rat primary Kupffer cells and J774A.1 (J77) macrophages were treated for 24 hours with a mixture of DDE, trans-nonachlor, and oxychlorodane to recapitulate the approximate body burden ratio. Cell viability was measured using an MTT assay, and a caspase activity assay was used to measure apoptosis. Kupffer cells treated with the OCP mixture displayed significantly increased cell viability compared to controls, while J77 macrophages showed no significant difference. Furthermore, J77 macrophages treated with the OCP mixture displayed a significantly increased apoptotic drive, as indicated by increased caspase 3 activity, versus the control. However, the Kupffer cells showed no significant difference between the treated cells and control. Additionally, exposure to the OCP mixture significantly increased J77 phagocytosis and reactive oxygen species production in a concentration dependent manner. These findings demonstrate that the mixture of OCPs may cause increased viability and potentially increased proliferation of Kupffer cells which may alter the localized inflammatory tone of the liver and alter hepatic function.

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