Starting at bottom front row (l to r): Elizabeth Moyer, Kayla Alexander, Nicole Rowbotham, Sarah Middlebrooks, Olivia Mann. Second row: Audrey Tucker, Anna Walker, Alexis Thompson, Emily Bucak, Scott McMullin. Third row: Keli Weigle, Matty May, Christa Gilfeather, Julie Holdridge, Ashley Varley. Fourth row: Dr. Jun Liao, Robert Stenger, Madeleine Hendrix, Wil Moorhead, Peyton Williams, Josh Bennett. Fifth row: Dr. Jeffery Eells, Nancy Gavron, Tommy Ware, Dr. Russell Carr, Dr. Andrea Varela-Stokes. Sixth row: Dr. Barbara Kaplan, Dr. Trey Howell, Dr. Mark Lawrence, Dr. Matt Griffin. Seventh row: Dr. Jean M. N. Feugang, Dr. Andrew Mackin, Dr. John Thomason. Not pictured: Jenna Scott, Sydney Hayter, Courtney Williams.

Flow Cytometric Evaluation of Canine Lymphocytes after Oral Mycophenolate Mofetil

Kayla Alexander*, Charlee Mulligan, Barbara Kaplan, and Andrew Mackin

Mycophenolate mofetil (MMF) is an immunosuppressive agent that has been used in human medicine for organ transplant recipients. The low cost of MMF has led to an increase in use in dogs for the treatment of autoimmune disease. MMF modulates immune responses by inhibiting inosine-5′-monophosphate dehydrogenase (IMPDH), an enzyme needed for the synthesis of purines. Two isotypes of IMPDH exist: IMPDH1 and IMPDH2. IMPDH2 is used exclusively by lymphocytes during proliferation, whereas other cells are able to utilize a salvage pathway for the synthesis of purine nucleotides [3]. Inhibition of IMPDH2 selectively inhibits lymphocyte mitosis, leaving cells unable to replicate. Researchers hypothesize that the

immunomodulatory effects of MMF are mostly due to an inability of lymphocytes to proliferate [1]. Other studies suggest that MMF also induces lymphocyte apoptosis [4].
Ongoing studies in our laboratory have documented that MMF, after two weeks of administration, inhibits lymphocyte proliferation. This current study was designed to use flow cytometry to determine whether MMF in dogs, at maximally tolerated doses for two weeks, also causes lymphocyte death and apoptosis. In addition, flow cytometry was used to determine the effects of MMF on canine CD4+ and CD8+ lymphocyte populations. Our results indicated that, compared to pretreatment values, oral MMF for two weeks had minimal effect on lymphocyte apoptosis (early or late), cell death, or populations of CD4+ and CD8+ cells. Our findings support the theory that MMF inhibits lymphocyte proliferation primarily by causing cell cycle arrest, rather than cell apoptosis or death.

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

Acute toxicity evaluation in rats of potential chemical warfare agent antidotes

Joshua P. Bennett*, Edward C. Meek, Shane W. Bennett, Ronald B. Pringle, Howard W. Chambers, and Janice E. Chambers

Novel substituted phenoxyalkyl pyridinium oximes (US patent 9,227,937 B2), previously shown to reactivate rat brain cholinesterase inhibited by nerve agent surrogates and enhance survival and decrease time to cessation of seizure-like behavior following lethal dosages of nerve agent surrogates, were tested for potential acute toxicities in rats. Rat cohorts were treated intramuscularly with 292 μmol/kg of oxime (2x the human equivalent dosage) or vehicle (Multisol) control and were weighed daily after administration. There was no statistical difference detected in brain cholinesterase activity between treatment groups at times of 1 hour, 24 hours, and 2 weeks post-dosing. *In vitro* testing of these oximes at 2 different concentrations using rat brain homogenate revealed a range of 2-18% inhibition of cholinesterase activity. In addition, there were statistical differences detected in weight gain among Oximes 20 and 55 at 2 weeks post-dosing, but no difference among treatments was found after 24 hours. Also in the 2-week cohort, a statistical difference was observed among the kidney weights and indices of Oximes 1, 15, and 20. Post-mortem evaluation of the 2-week cohort's organs showed changes in kidney size and color, in some, of the rats treated with Oximes 1, 15, and 20. No other gross pathological changes were noticed among treatment groups, except for irritation at the injection site. Previous studies performed in our lab have shown that these oximes have exceptional promise as a potential therapy to prevent nerve agent-induced lethality and attenuation of seizure-like behavior; therefore, further preclinical studies on safety and efficacy are needed.

Student Support: Mississippi State University College of Veterinary Medicine and National Institutes of Health U01 NS083430

Sperm Characteristics Associated with Bull Fertility

Emily Bucak*, E. Menezes, F, Santos, S. Baird, S. Lu, F. Austin, A. Kaya, A. Moura, E. Memili

Bull fertility is crucial for sustainable and efficient reproduction of cattle. Despite producing large amounts of sperm with normal motility and morphology, some bulls have low fertility data. Molecular, cellular and physiological mechanisms causing low fertility are elusive. The objectives of this study were to determine the cellular parameters of cryopreserved sperm from 10 commercial Holstein bulls with different fertility data and ascertain bacterial presence in whole semen samples. Sperm parameters were characterized using Computer

Assisted Sperm Analysis (CASA), light microscopy, and flow cytometry. Sperm viability, morphology, velocity parameters, as well as cell membrane, DNA, and acrosome integrity were analyzed. Bacterial presence was evaluated using biochemical tests and Polymerase Chain Reaction (PCR). CASA analysis showed a statistically significant difference between high (HF) and low fertility (LF) bull samples in average pathway velocity, amplitude of lateral head displacement, and straightness. Both light microscopy and flow cytometry analyses did not show any statistical difference in sperm parameters between high and low fertility bulls. Results of the biochemical tests showed that only two bull samples have bacterial presence; a HF bull with *Pseudomonas aeruginosa/putida* (100%), and a LF bull with *Comamonas testosteroni* (75.48%), *Pseudomonas alcaligenes* (11.45%), and *Pseudomonas flourescens* (8.67%). PCR analysis of 16S ribosomal subunit DNA and gel electrophoresis showed that all semen samples had detectable bacterial DNA. These findings are significant because they help advancement in fundamental animal science and biotechnology.

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

Refining approaches to assess microbiota and low-profile pathogens in the tick vector, *Amblyomma maculatum*

Nancy A. Gavron*, John V. Stokes, Si Hong Park, Steven C. Ricke, Jung Keun Lee, Kaitlin J. Graham, Sharon Cannaliato, Andrea S. Varela-Stokes

Amblyomma maculatum, Gulf Coast ticks (GCT), harbor species of bacteria and protozoa, including emerging zoonotic pathogens, *Rickettsia parkeri* and Panola Mountain *Ehrlichia* (PME), as well as the canine protozoal pathogen *Hepatozoon americanum*. While infection rates of *R. parkeri* in GCT populations have been recently documented, little is known about infection rates of PME and *H. americanum*. Further, recent studies on tick microbiota are limited; with reports analyzing extracts not enriched for prokaryotic DNA, and protozoal contributions not considered. Our objectives were to explore the bacterial microbiome of individual adult GCT, while evaluating infection rates of *H. americanum* and PME in archived GCT. We hypothesized that the microbial community of our GCT would include bacterial species previously not detected, while PME would be rarer than *H. americanum*. We collected questing adult GCT and extracted DNA using a protocol that selects for prokaryotic DNA; extracts were submitted for sequencing using an Illumina MiSeq platform, with results pending. To evaluate infection rates in archived GCT, we first addressed challenges in detection assays. The rarity of PME prompted production of a plasmid control to be used in a TaqMan quantitative (q)PCR. Initial assays for *H. americanum* used published 18S rRNA primers in a nested PCR. We could successfully detect *H. americanum* in blood but not in ticks due to significant similarities in the primer sequences to GCT sequences. New primers were designed and utilized in a SYBR green qPCR. The optimization of these protocols will be used to better understand the infection rate of these pathogens in GCT and evaluate the influence of microbial diversity on pathogen transmission.

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Cannabidiol Use and Its Potential Immune Effects in Modulating EAE

C.L. Gilfeather*, G.Yray, J. Nichols, E. Kummari, S. Dhital, and B.L.F. Kaplan

Multiple sclerosis (MS) is an autoimmune disease in which the immune system attacks cells in the central nervous system (CNS) that provide neuronal myelination. A plant-derived cannabinoid, cannabidiol (CBD), is currently being investigated to help reduce spasticity in MS patients.With its potential for increased use, it is important to understand the mechanism by which CBD is effective in MS. We used a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), to study disease following a 5-day oral treatment with

CBD. EAE was induced by injecting mice with a self-peptide, myelin oligodendrocyte glycoprotein (MOG_{35-55}), which is found in myelin. We hypothesized that CBD would suppress T cells in EAE. Clinically, CBD delayed initiation of disease by 2 days, although mice at end stage disease exhibited similar clinical signs to EAE mice. Upon necropsy, splenocytes (SPLC) and draining lymph node (LN) cells were stimulated with MOG or MOG + anti-CD3/28 beads for 48 hrs. No CBD effect was observed on extracellular production of IFN-γ or IL-17A in either lymphoid organ as assessed by ELISA. There was a slight decrease by CBD in intracellular IFN-γ in both CD4+ and CD8+ T cells in LN as compared to EAE mice as assessed by flow cytometry. Similarly, CBD decreased intracellular IFN-γ in CD4+ T cells in SPLC. Direct analysis of CD25 and CD69 activation markers on CD4 or CD8 cells revealed a slight increase in CD69 in LN cells with disease but no further alteration by CBD. In conclusion, while CBD delayed disease there was little effect on immune function in SPLC and LN at end-stage disease. The results suggest a longer CBD exposure might be effective for clinical signs and produce immune suppression in EAE.

Student Support: Mississippi State University College of Veterinary Medicine

Identifying antimicrobial resistance patterns in *Salmonella enterica* serotype Typhimurium from human and cattle sources

Sydney R. Hayter*, David R. Smith

Antimicrobial resistance is an issue important to the health of humans, companion and food-producing animals. These concerns affect public health policy. The National Antimicrobial Resistance Monitoring System (NARMS) is a collaborative effort between the CDC, USDA, and FDA to collect and report data regarding antimicrobial resistance in foodborne pathogens isolated from human clinical cases, food-producing animals, and retail meat samples. The objective of this study was to determine the pattern of antimicrobial resistance among *Salmonella enterica* serotype Typhimurium among human clinical samples and beef carcass samples from the NARMS datasets. Fifteen years of data (1998-2013) were analyzed to determine the effects of source (human or beef carcass) and year on the probability of reporting *S.* Typhimurium resistant to tetracycline, ceftiofur, gentamicin, ciprofloxacin, multidrug resistance (MDR) as defined by CDC, or an MDR pattern known as ACSSuT. Data were analyzed for linear trend over time using the logistic regression and the Chi square test for trend. Significant interactions between source and time existed for MDR ($p<0.0001$), tetracycline ($p<0.0001$), and ceftiofur ($p=0.0014$). Tetracycline (OR = 0.92, $p<0.0001$) and MDR (OR = 0.9, $p<0.0001$) resistance from human samples decreased over time. ACSSuT resistance significantly decreased over time (OR = 0.92, $p=0.0071$). The probability to recover ceftiofur-resistant samples from cattle increased (OR = 1.1, $p<0.0001$) over time. No significant relationships were detected for gentamicin or ciprofloxacin resistance. The probability of isolating resistant *S.* Typhimurium was dependent on the time and source of sampling, and differed among drugs of public health importance.

Student Support: Mississippi State University College of Veterinary Medicine and Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction

Identification and characterization of *Yersinia ruckeri* from farm-raised catfish in Mississippi

Madeleine V. Hendrix*, Stephen R. Reichley, Lester H. Khoo, Patricia S. Gaunt, David J. Wise, and Matt J. Griffin

Yersinia ruckeri is the causative agent of enteric redmouth disease in salmonid fish. Although most commonly isolated from trout, there have been limited documented cases in farm-raised catfish. In spring of 2016, *Y. ruckeri* was isolated from diseased channel (*Ictalurus punctatus*) x blue (*I. furcatus*) hybrid catfish from multiple ponds on a single operation. It is unknown if this isolation indicates a potentially emerging pathogen or

a fluke occurrence. Farm-raised catfish is the largest food fish aquaculture industry in the United States. Given the economic importance to Mississippi and its neighboring states, it is critical the susceptibility of catfish to this pathogen be evaluated. To this end, channel and hybrid catfish were challenged with *Y. ruckeri* by intraperitoneal injection as well as bath immersion to fulfill Koch's postulates and determine the relative virulence of *Y. ruckeri* in catfish. Results suggest hybrids may be more susceptible to *Y. ruckeri.* Interestingly, under the conditions used in this study the bacterium did not cause disease by immersion, even under experimentally induced oxygen stress, suggesting the pathogenicity in catfish may be limited. Additional studies at lower temperature are warranted. Furthermore, *Y. ruckeri* isolates from both catfish and trout were subjected to phenotypic and genetic analyses using commercial phenotypic test kits (BBL, Biolog, API20E), motility and TSI slants, estimations of G+C content, *gyrB* sequencing, plasmid characterization, and repetitive extragenic palindromic PCR (rep-PCR). These analyses indicate genotypic and phenotypic variations exist between isolates from catfish and trout, although the biological implications of these differences are presently unknown.

Student Support: Mississippi State University College of Veterinary Medicine

Alterations in hepatic glucose metabolism following direct exposure to organochlorine pesticide metabolites

Julie Holdridge*, Sandeep Kondakala, Margaret Davis, and George Howell III

Diabetes mellitus is an increasingly prevalent disease in both in the United States and worldwide with type II diabetes mellitus, characterized by hyperglycemia driven by insulin resistance, accounting for 90-95% of cases. Although a number of risk factors for type II diabetes are known, there is evidence that exposure to certain organochlorine pesticides are associated with the development of type II diabetes as well. Previous studies have demonstrated exposure to p,p'-dichlorodiphenyldichloroethylene (DDE), one of the most prevalent organochlorine compounds, promotes pathological alterations in glucose homeostasis. However, the underlying cellular mechanisms governing these effects remains poorly understood. The goal of the present study was to determine if direct exposure to the organochlorine compounds DDE and *trans*-nonachlor alters glucose homeostasis in the liver utilizing rat primary hepatocytes as an "*ex vivo*" model of hepatic function. To assess effects on glucose homeostasis, glucose production, glucose uptake, and glycogen content in the hepatocyte were examined. After 16 hours of exposure, DDE and *trans*-nonachlor significantly decreased glucose production in the primary hepatocytes in a concentration-dependent manner. While both DDE and *trans*-nonachlor decreased hepatocyte glucose production, glucose uptake and glycogen content were not significantly altered. These organochlorine effects on glucose production were observed at non-cytotoxic concentrations of both DDE and *trans*-nonachlor. In summary, our current data indicate direct exposure to select organochlorine pesticides negatively impacts glucose metabolism by decreasing hepatocyte glucose production which may promote fasting hypoglycemia.

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Characterization of N-linked glycan profiles in bat gastrointestinal tissues specific to influenza A viruses

Olivia Mann*, Henry Wan, Chun-Kai Yang

The influenza A viruses have caused and continue to cause threats to public health due to its large range of animal reservoirs and hosts. Bats are a potential reservoir, and it has recently been discovered that bats carry a newly-classified influenza-like virus. It has been demonstrated that this virus does not bind canonical human or avian receptors. The central hypothesis is that bats harbor unique glycans to which this new influenza-like virus binds, and that bat-origin influenza A virus has unique receptor glycans distinct from human-origin influenza A

viruses. To identify glycans in bat tissue, sections of bat small intestines were harvested, processed, and subjected to hydrophilic interaction liquid chromatography before being permethylated and identified via mass spectrometry. In addition, influenza-specific glycans were harvested using Nickel-ionized HisTrap columns and purified recombinant H1 and H17 proteins. Glycan profiles of two different bat species, *Myotis austroriparius* (My au) and *Eptesicus fuscus* (Ep fu), revealed that the majority of glycans resides in the duodenum, with the former species having more total glycans than the latter. H1-specific glycans of the two species showed that among the three portions of small intestine, the ileum contains more H1-specific glycan content, and most H1-specific sialyation are alpha 2,6-linked. In addition, the H1-specific glycans from bat tissues are commonly fucosylated. Interestingly, My au had far more H1-specific glycans than did Ep fu by a ratio of approximately 4 to 1. This data suggests that some bat species may be more prone to infection by influenza viruses than others, and that bat glycans that bind these viruses may not be sialyated like human glycans.

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Antigen presenting cells promote protective immunity in catfish exposed to live *Edwardsiella ictaluri* vaccines

Matty May*, Adef Kordon, Hossam Abdelhamed, Hamada Ahmed, Joo Youn Park, Attila Karsi, and Lesya Pinchuk

We have identified the presence of Langerhans-like cells (LCs) containing Birbeck granules in the gill of uninfected catfish by immunohistochemistry. Also, we showed that catfish peritoneal macrophages are capable of actively phagocytosing and killing *E. ictaluri* opsonized with sera from catfish vaccinated with live attenuated vaccine (LAV) candidates compared to control fish. These results lay a foundation for our present study that is to determine the vaccine-induced production of innate and adaptive immune responses through catfish antigen presenting cells, such as dendritic cells, macrophages, and B cells. In this study, we demonstrate that *in vivo E. ictaluri* infection dramatically changes the numbers of LCs from visually undetectable in uninfected fish to highly abundant numbers of LCs in the infected catfish gill. Furthermore using a qPCR approach, we found several principle differences in the CD8α T cell co-receptor gene expression levels in both anterior kidney and spleen between the vaccinated and non-vaccinated groups suggesting the development of T cell-mediated immunity. Our *in vivo* infection study also showed a significant correlation in colonization of vaccine strains, active antigen uptake, and bacteria killing properties of anterior kidney mononuclear cells. We expect that these findings will be important in understanding the mechanisms of innate and adaptive immune responses against the most effective vaccines.

Student Support: National Institutes of Health T35OD010432

Biomechanical Properties of the Porcine Optic Nerve

Scott McMullin*, Sammira Rais-Rohani, Bryn Brazile, and Jun Liao

In space, astronauts experience several physical abnormalities due to microgravity. Some of these include intraorbital and intracranial pressure changes similar to the effects of idiopathic intracranial hypertension. When exposed to microgravity, many astronauts experience an enlarging of the optic nerve, resulting in the buckling of the optic nerve sheath. These abnormalities may result in blurred vision, which can ultimately lead to permanent blindness. It is believed that the optic nerve may be an integral part of both the cause and the solution to preventing or treating this affliction. Although there have been several studies concerning the correlation between intracranial pressure and the optic nerve, to the best of our knowledge there is no study that investigates the biomechanical properties of the optical nerve itself. Porcine heads were obtained from a local abattoir, and the optic nerves were dissected. The optic nerves were trimmed of all excess material leaving the

sheath intact and placed in phosphate buffered saline (PBS). Compression tests were performed on the optic nerves by mounting the nerves onto a Test Resource compression machine and wetted with PBS solution to obtain the tissue buckling behavior. The compression test shows that the optic nerve shows an increasing linear relationship between strain and stress until the stress reaches ~15Pa, at which point it plateaus from strains roughly 0.22 to 0.26. The stress then shows a sharp exponential increase after this until the end of the test. This study established a strong basis for optical nerve biomechanics. Future work will include histology, bending tests, and computational simulation of the effects of abnormal pressures.

Student support: National Institutes of Health T35OD010432

T. Gondii Infection and Nurr1-null Heterozygous Genotype Affect Spatial Learning

Sarah Middlebrooks*, Jeffrey Eells, Shirley Guo-Ross, Ciarra Smith

Schizophrenia is a poorly understood disease that alters the CNS by eliciting positive symptoms and cognitive symptoms; however, most of the etiopathology is unknown resulting in inadequate management strategies. *Toxoplasma gondii* is an intracellular protozoan that infects one third of the population worldwide. Many studies have shown that *T. gondii* is an important risk factor for schizophrenia via its ability to encyst in the brain and alter neurotransmitter levels. Another risk factor is the mutation of the Nurr1 nuclear receptor that is needed for dopamine synthesis and dopamine neuron survival. To investigate the mechanisms through which a genetic mutation and infection with T. gondii can contribute to risk for schizophrenia and cognitive deficits, we determined the behavioral effects of *T. gondii* infection in adult mice. Furthermore, we incorporated the Nurr1-null heterozygous (+/-) mice into this study to observe any cumulative effects that could contribute to cognitive deficits. Our hypothesis was that the +/- mice will be more sensitive to *T. gondii* infection which will elicit more severe cognitive deficits. Adult mice were tested for open field activity and spatial learning using a Barnes maze. Both genotype and treatment effects were observed in which Nurr1-null +/- and *T. gondii* infected mice exhibited longer total escape latency times in the Barnes maze. In an open field test, Nurr1-null +/- mice were more active. Another important effect was that both T. gondii treated groups experienced weight loss. By combining these factors into one model, we were able to observe cognitive deficits through quantitatively scoring our mice models based on their behavior.

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Gender differences in protein detection of swine muscle and fat tissue

Wil A Moorhead*, Casey L Durfey, Gustavo D A Gastal, Peter L Ryan, Scott T Willard, Jean M Feugang

Two commonly assessed proteins in the swine industry, myoglobin (MYO) and fatty acid synthase (FAS), are considered biochemical indicators of meat quality. MYO carries oxygen within tissues and gives meat its red color. FAS assembles the various fatty acids that have differential effects on meat quality. The purpose of this study was to determine if these proteins are expressed in muscle and fat tissues at different levels between genders.
Gilts maintained in our experimental farm were inseminated with extended boar semen and farrowing occurred at approximately 114 days post-insemination. At weaning, twenty piglets (10 female and 10 male) were randomly selected and allowed to grow to market weight. Longissimus muscle and subcutaneous back fat samples were collected and subsets were subjected to western immunoblotting (WIB) to confirm specificity of protein targets using MYO and FAS antibodies. Remaining samples were prepared for immunofluorescence (IF) detection. Standard protocols were used for both WIB and IF. Images were captured (EVOS microscope) and intensities were quantified (ImageJ). Data were analyzed (ANOVA/Wilcoxon) to compare protein fluorescence intensities within tissues across gender.

Results revealed high levels of MYO protein in male muscle and female fat. Females showed higher muscular FAS content, while no significant difference was found in gender detection of FAS in fat tissues.
These biochemical differences have implications on meat quality as differential FAS content could affect intramuscular fat accumulation or marbling, a subjective indicator of palatability to consumers. The higher MYO content of male muscle will produce a darker red meat, which is typically associated with greater tenderness.

Student Support: Mississippi State University College of Veterinary Medicine

Evaluation of intradermal testing in five horses

Moyer L*, Tucker A, Gunter J, Bowser J, Cooley JA, Swiderski CE.

Intradermal skin testing (IDT) is commonly applied in the diagnosis and management of allergic diseases in humans and dogs. Allergen panels, developed and refined for human allergy, have been successfully adapted for the dog, a species that cohabitates with man. Due to inconsistencies with testing outcomes, IDT has been widely criticized and considered of limited utility in horses. However, the environmental allergens of horses are radically different from humans and dogs. Insect bite hypersensitivity, an IgE-mediated skin disease of horses, substantiates horses develop IgE sensitizing antibodies that arm skin mast cells. Accordingly, we hypothesize that limited utility of IDT in horses is influenced by a bias towards antigens of significance in human allergy, and an absence of replicates in IDT protocols from which to make valid assessments. To test this hypothesis, we performed intradermal skin on 3 with pasture-associated asthma, and two non-diseased controls, employing antigens of relevance to the environmental exposure of the horse. We performed replicate injections for each antigen. Wheal size (mm) and induration (0-4) were evaluated at 20 minutes and 6 hours (reactions considered primarily IgE- mediated) and also at 24 and 48 hours. Reactions were considered positive for an antigen when wheal and induration were identified at all replicate sites. Consistent reactions to grass and mold antigens were identified within individual horses. IgE-mediated reactions were documented by response to replicate injections of Johnson Grass, *Bipolaris spp*, Meadow Fescue, and Grass Smut at 20 minutes and 6 hours. We conclude that modifications of testing protocols for horses are likely to improve the diagnostic utility of IDT.

Student Support: Mississippi State University College of Veterinary Medicine

Involvement of increased CB1R activation in altered social play induced by developmental chlorpyrifos exposure

Nicole E. Rowbotham*, Carole A. Nail, Jenna A. Mosier, Aubrey M. Lewis, Russell L. Carr.

Childhood exposure to chlorpyrifos (CPF), an organophosphorus insecticide, results in negative long-term neurologic effects. We have previously reported that low-level developmental exposure of rats to CPF disrupts endocannabinoid (EC) degradation through fatty acid amide hydrolase (FAAH) inhibition and leads to increased social play behavior once adolescence is reached. Increased activity in the EC system may be responsible for the observed altered behavior. Phosphorylation of the cannabinoid receptor (CB1R) is an indicator of EC activation. This study compared the phosphorylation of the CB1R in brain regions of control and treated rats immediately following behavioral testing. On postnatal day (PND) 10, rats were exposed orally to either corn oil, 0.5 mg/kg CPF, 0.75 mg/kg CPF, 1.0 mg/kg CPF or 0.02 mg/kg PF-04457845 (a specific FAAH inhibitor) daily for 7 days. On PND 36, social behavior was monitored and all treated groups spent more time playing than did controls. Western blotting was used to quantify the amount of CB1R and phosphorylated CB1R proteins in the hippocampus, amygdala, prelimbic cortex, agranular insular area, and nucleus accumbens. There were no significant effects of treatment on the amount of CB1R protein in any of the five brain regions. Increased CB1R phosphorylation occurred in all brain regions following social play, but no effects of CPF

treatment were observed. This suggests that increased EC activation is not a causative factor in the increased social play observed in CPF treated rats. It remains unclear whether developmental CPF exposure induces an alteration in EC tone or an alteration in other neurotransmitter systems that could explain the observed changes in social behavior.

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Effects of deworming and vaccination on seroresponse to BVDV1 and BHV1 in stocker calves

Jenna A. Scott*, Brandi Karisch, Amelia R. Woolums, John Blanton, Ray M. Kaplan, William Epperson, and David R. Smith

Recently weaned calves moving through market channels into growing or feeding systems may not be immunocompetent at arrival. The objective of this study was to evaluate the effects of deworming, time of vaccination, and health characteristics on arrival on seroresponse to BHV1 and BVDV1. Calves (n=80) were blocked by d-3 fecal egg count (FECd-3) and weight (BWd-3) and assigned to 20 pens (4 calves/pen). Pens were randomly assigned to treatments in a 2 x 2 factorial design of deworming (oral dewormer at d0 (DWM) or not (NoDWM)) and vaccination (5-way MLV respiratory and 7-way clostridial vaccines at d0 and 56 (VAC) vs. d56 only (VAC56)). Serum was collected at d0, 28, 56 and 85; log titers and change in log titers from d0-28, 28-56 and 56-85 were analyzed using linear regression. Main effects were vaccination and deworming with random effect of pen and covariates of sex, FECd-3, BWd-3 and fever at d0. Calves with higher FECd-3 values had lower BVDV1 titers at d0 ($P = 0.05$). From d0 to 28 VAC calves had, on average, a 5.3 ± 0.8 greater rise in BVDV1 titers compared to VAC56 calves ($P < 0.0001$). From d28 to 56 VAC cattle had a 0.9 ± 0.4 greater increase in BVDV1 titers than VAC56 cattle ($P < 0.0322$). From d56 to 85 VAC56 calves had a 4.4 ± 0.8 greater increase in BVDV1 titers compared to VAC calves ($P < 0.0001$). There were no factors which explained arrival BHV1 titers. From d0 to 28 VAC calves had a 2.2 ± 0.5 greater change in BHV1 titers than VAC56 calves ($P < 0.0001$). BHV1 titer change from d28 to 56 and d56 to 85 did not differ by any factor. These results indicate that stocker cattle do respond to vaccines given at arrival. Seroresponse to vaccines differ by pathogen, and deworming does not appear to affect seroresponse.

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

Helminths in mountain gorillas: conservation through intestinal parasite monitoring

Robert J. Stenger*, David R. Smith, Gladys Kalema-Zikusoka, and Stephen Rubanga

The mountain gorilla (*Gorilla beringei beringei*) brought to international attention by Dian Fosey's *Gorillas in the Mist* is critically endangered. Through great effort from local peoples, governments, and conservation NGOs the mountain gorilla population is rising. Yet with rapidly growing local human populations and greater tourist visitation there is an ever increasing risk of zoonotic disease transmission. Using fecal samples collected in a longitudinal monitoring program we looked for variation in intestinal parasite prevalence, hypothesizing that prevalence would differ by factors which were recorded at sample collection (gorilla age, gorilla group, month of collection, etc.). Parasite prevalence was determined using fecal flotation (Anoplocephala, Strongyle, Ancylostoma, and Taenia eggs identified). Our objective was to quantify intestinal parasite prevalence and test if prevalence varied by the different factors of interest. We looked at the mountain gorilla populations in Bwindi and the Virungas and at three different time periods (2005, '06, & '09). Results of our multivariable logistic regression indicate that there is statistically significant variation in the prevalence of intestinal parasites within mountain gorillas ($\alpha = 0.10$). Parasite prevalence varied by the individual gorilla (Virungas, '09) and month of collection (Bwindi and Virungas, '05 & '09). The next step is to determine if this variation is due to natural

differences in ecology or human induced factors. These data are currently used to monitor gorilla health. However, a better understanding of the epidemiology of intestinal parasitism in the mountain gorilla will allow for more informed management decisions.

Student Support: National Institute of Health T35OD010432

Evaluation of refractometry to detect failed transfer of passive immunity in pre-weaned beef calves
Alexis Thompson*, Liesel Schneider, Min Wang, David R. Smith

Failed transfer of passive immunity (FTPI) is associated with increased morbidity and mortality in pre-weaned calves. The gold standard for detecting passive transfer is radial immunodiffusion (RID). An alternative is refractometry which indirectly measures immunoglobulins by estimating the concentration of serum total protein (STP). The objective of this study was to measure the agreement and establish a cut-off point for FTPI between STP and RID and evaluate other refractometry scales. Serum IgG concentration from 818 calves from 4 farms was measured by RID and refractometry using scales for Brix%, specific gravity (SG), and STP. The correlation coefficient between the refractometry scales and the RID IgG concentration were calculated. Sensitivity and specificity of STP was calculated for each 0.1 g/dL increase in STP to determine an optimal cut-off using an RID value of 1,000 mg/dL IgG to define FTPI. Based on RID, the prevalence of FTPI in beef calves in our study population was 4.8% (95%CI=4.0%, 6.3%). The association between Brix% and RID, SG and RID, and STP and RID were significant ($P<0.01$, $r=0.86$, 0.85, 0.84, respectively). Based on ROC curve, an optimal cut-off for STP of 5.9 g/dL to classify calves to FTPI status was identified. At this cut-off the test performance of STP measured by refractometry was 93% specific (95%CI=91%, 95%) and 95% sensitive (95%CI=83%, 99%). This level of test performance makes refractometry diagnostically useful to identify calves with FTPI in populations with a high prevalence of calves with FTPI (e.g. 40%). However, as a screening tool in low prevalence populations STP refractometry the test has a low positive predictive value and will over-estimate prevalence of FPTI.

Student Support: Mississippi State University College of Veterinary Medicine

Survey of Grass- Associated Fungi to Identify Aeroallergens Relevant to Summer Pasture- Associated Recurrent Airway Obstruction (SPA-RAO).
Audrey Tucker*, L Moyer, M Tomaso- Peterson, R Lemus, C Wenzel, V Maddox, J Bowser, CE Swiderski

(SPA-RAO) is an asthma-like disease that affects horses living on pasture during conditions of high heat and humidity. The disease is prevalent in the southeastern United States, but reported less frequently in other states and the United Kingdom. Affected horses demonstrate rapid clinical improvement when removed from pasture, implicating pasture-associated aeroallergens as etiologic agents. Associations between disease exacerbation and seasonal increase in molds and grass pollens are documented. Fungal aeroallergens, including those of the phylum basidiomycota, are recognized as important etiologic agents of asthma in southeastern states and are also associated temporally with SPARAO exacerbation. Genera within this phylum are obligate biotrophs that parasitize grasses. Interpreted with the association between SPARAO and grass exposure, *we hypothesize that basidomycetes that parasitize grasses in the southeastern US are etiologic agents in SPARAO*. To investigate this hypothesis, two pastures were examined grossly and microscopically for evidence of basidiomycete infection on resident grasses. Primary pasture elicited severe disease in 10 horses with confirmed SPARAO over 7 consecutive summers. Secondary pasture elicited less severe disease in the same time period. Pasture sampling was timed in early July to address documented seasonal disease onset (May to July). Microscopy identified pustules containing basidiomycete urediniospores. In both primary and secondary pasture, extensive infection of *Festuca arundinacea* (tall fescue) by a single basidiomycete species of the genus *Puccinia* was

identified. However, among resident grass species, tall fescue was estimated to be 10 times more prevalent in primary pasture, resulting in higher *Puccinia* exposure in primary pasture. *Puccinia* sensitization is reported in human asthmatics. These findings refine the relevance of basidiomycetes of the genus *Puccinia* to the pathogenesis of SPARAO, and support focused investigations of the associations between exposure, allergic recognition, and clinical disease in horses with SPARAO.

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Comparison of virus isolation from fabric for use in cow-calf herd surveillance of viral bovine respiratory disease

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in many classes of cattle, including weaned dairy calves, feedlot cattle, and beef calves between 3 weeks of age and weaning. Both bacterial and viral agents have been found to cause clinical signs. Because sampling individual calves in cow-calf operations is logistically difficult, this study forms the basis for a possible method of herd surveillance for viral BRD in cow-calf operations. To ensure efficacy in a pasture, the proposed method was first tested in a laboratory setting. Cotton and polyester fabrics were purposely inoculated with Bovine Herpes Virus- 1. Four drying times (0h, 4h, 12h, 24h) and two methods of bacterial control (antibiotics in eluent and 0.22um filter) were compared. At each drying time, four test groups were evaluated; cotton with eluent treated with antibiotics, cotton with filtered eluent, polyester with eluent treated with antibiotics, and polyester with filtered eluent. Virus isolation on on Madin Darby Bovine Kidney cells was used to for virus recovery. At time 0h, no significant difference was found in virus isolated in each treatment group. At time 4h and 12h, no virus was obtained from polyester; virus was isolated from cotton but there was no difference between either bacterial control method. At 24h, cotton with antibiotics as a bacterial control method led to improved virus isolation as compared to the other 3 methods.

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Assessment of gastrointestinal nematode parasitism in dairy calves housed in calf hutches

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Gastrointestinal nematodes (GIN) cause substantial disease and economic losses in the cattle industry. Milk-fed dairy calves housed in individual hutches are presumed not to acquire GIN infection because transmission of GIN requires exposure to a pasture environment and subsequent ingestion of contaminated forage. However, recent assessment of fecal egg counts (FEC) in hutch calves for other research has revealed some calves with high eggs per gram (EPG). We performed a cross-sectional observational study to determine the prevalence of GIN in hutch dairy calves on two calf ranches in Florida to test the hypothesis that calf or group-level factors are associated with presence or magnitude of GIN parasitism. Ninety-five calves from each of two calf rearing operations were sampled in June 2016. Individual fecal samples were collected from 30-90 day old calves and calf weight was estimated by weight tape. The FEC of strongyle-type eggs was determined by the Mini-FLOTAC method. Strongyle-type eggs were found in 9 of 95 (9.5%) samples collected from ranch A and in 28 of 92 (30.4%) samples from ranch B. Calf ranch was the only significant factor associated with the probability of parasitism in a multivariable analysis (tested by multi-level multivariable logistic regression using PROC Glimmix, SAS 9.4) (P=0.0005). Odds of parasitism in calves from ranch B were 4.4 times as great as calves from ranch A. Our results indicate that GIN parasitism does occur in hutch calves and that there is a difference in infection levels based on rearing facility. Further evaluation of the prevalence and impact of GIN parasitism in hutch calves across the Southeast and assessment of factors that contribute to GIN parasitism is warranted.

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The effects of target distance and gun type on accuracy of 8cc darts used to treat beef cattle in remote drug delivery systems

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Remote drug delivery systems (RDDS) have become increasingly popular to treat beef cattle without restraint. However, there are beef quality assurance and animal welfare concerns associated with RDDS usage. Little research has been done to determine the accuracy of RDDS. The objective of this study was to determine the effect of gun type and distance on drop in elevation of an 8cc practice dart. The study was a 2 x 2 factorial design with two levels of gun (CO_2 and air pump) and two levels of distance (9.1 and 13.7 meters). The outcome variable was the drop in elevation measured from the center of the target. Fifteen shots were fired from each combination of gun type and distance for a total of 60 shots. The order of shots from each combination of gun and distance was randomized. Gun muzzle and target were 91 cm above the ground. Five shots were taken from each CO_2 cartridge and recorded to measure drop due to cartridge depletion. Overall there was a significant gun by distance interaction ($P = 0.008$). Darts fired from the CO_2 rifle dropped 11.4 cm (SD = 12.1) and 31.1 cm (SD = 22.7) at 9.14 and 13.7 meters, respectively ($P = 0.0003$). Accounting for distance, the first shot fired from a new CO_2 canister dropped 23.5 cm lower than subsequent firings ($P < 0.0001$). Darts fired from the air pump rifle dropped 27.6 cm (SD = 25.8) and 26.0 cm (SD = 5.5) at 9.14 and 13.7 meters, respectively ($P < 0.0001$). The drop in elevation at the two distances differed by gun type. The distance and variability in elevation drop made no combination of gun or distance reliable for placing a dart within the beef quality assurance injection region.

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Comparing Guarded Nasopharyngeal Swabs to Six Inch Nasal Swabs for Detection of Bovine Respiratory Pathogens in Stocker Cattle

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in stocker cattle. While diagnosis of BRD is usually based on clinical signs, it is sometimes useful to identify bacterial pathogens in affected cattle. Various methods of pathogen identification may be used, but the agreement between commonly used methods of detection has not been well-characterized; this makes it difficult to know whether a technically simple method can substitute for a more technically difficult method. In this study the results from a guarded nasopharyngeal swab (NPS) were compared to the results from a nasal swab (NS) in (n=13) stocker cattle that showed clinical signs of BRD. The proportion of swabs with each growth level by swab type were analyzed by Fisher's exact test. The agreement of growth levels between swab types were assessed by calculating Kappa and weighted Kappa statistics between all pairs of swab types. There was complete agreement between NS and NPS for bacterial culture, all of which were *Mannheimia haemolytica*. Fair agreement was observed for bacterial culture growth by swab type this may be due to low prevalence of samples for growth categories. Total agreement was observed among the swab types to antibiotic susceptibility tests excluding two antibiotics that varied on one NPS sample when compared to NS. It is likely the NS may have an advantage over using a NPS due to decreased sampling time and improved cost efficiency.

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Identification of *Aeromonas hydrophila* virulence genes providing complement resistance
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Aeromonas hydrophila is a Gram-negative water-borne pathogen and the causative agent of hemorrhagic septicemia in channel catfish. Recently, a highly virulent *A. hydrophila* strain has emerged in the Southern United States, causing mass mortalities in catfish farms. This strain can survive in naïve catfish serum, but the molecular mechanisms of complement resistance are not known. Our hypothesis is that challenging random transposon insertion mutants with naïve catfish serum will result in the identification of *A. hydrophila* virulence genes involved in serum resistance. To this goal, a random transposon insertion library has been constructed by using pMAR2xT7 transposon and bioluminescent *A. hydrophila* strain ML09-119. Over 1,500 mutants were challenged with naïve catfish serum, and their bioluminescence was measured for four hours. We identified 94 mutant strains with bioluminescence reduction and further screening of these mutants in quadruplicate resulted in 15 target mutants for transposon end mapping to determine the genes interrupted.

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Effects of microaggregate filters on canine erythrocyte viability and morphology
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Blood transfusions are a life-saving procedure in small animal medicine, and can be administered via gravity, volumetric pump, or syringe pump. In dogs, the red blood cells (RBC) transfused via syringe pump have a significantly shorter post-transfusion survival time compared to RBCs transfused via gravity. One explanation for this decrease in RBC survival is that shearing stresses can damage the RBCs as they pass through a microaggregate filter. The goal of this study was to determine if blood passing through a microaggregate filter, via a syringe pump, would damage the canine RBC. Our hypothesis was that the pressure needed to force cells through a filter would damage the RBC. Whole blood was collected from 8 healthy dogs. Using syringe pumps, blood was administered at 50, 25, and 12.5 mL/hr. Pre- and post-filter blood samples were collected at the beginning and end of a transfusion. Samples were analyzed to determine the erythrocyte osmotic fragility (mean corpuscular fragility, MCF), RBC count, hemoglobin concentration, RBC distribution width (RDW), and RBC morphology. Regardless of the transfusion rate, there was no change in the MCF, RBC count, hemoglobin concentration, and RDW after passing through a filter. Echinocytes were the most common abnormal erythrocyte morphology, but acanthocytes became more frequent during the transfusion. Schistocytes were identified in one sample (post-filter at 12.5 mL/hr). Our study suggests that, regardless of the transfusion rate, the microaggregate filter does not alter the fragility of the canine erythrocyte, but may alter RBC morphology. Alterations in cell morphology could contribute to a decrease in RBC survival when administrating blood with a syringe pump.

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