2023 VetMed Research Scholars Abstracts

The Unseen Threat: ESBLs & Carbapenem-Resistant Bacteria in eastern Mississippi

Talia Barnes, John Webb, Joo Youn Park, Keun Seok Seo, and Cooper Brookshire

Concern exists about humans and animals sharing antibiotic resistant pathogens with each other, which justifies One Health focused antibiotic resistance studies. Carbapenem-resistant Enterobacterales (CRE) have recently emerged in companion animal veterinary medicine, especially in tertiary referral hospitals. Extended spectrum betalactamase producing Enterobacterales (ESBLs) have been common in veterinary medicine for many years. In Mississippi, carbapenemase producing ST307 *Klebsiella pneumoniae* with blaNDM-5 was recently reported in hospitalized dogs. The objective of this study was to estimate the detection rate of CRE and ESBLs in the East Mississippi general dog population. We hypothesized that the detection rate of CRE would be very low and the detection rate of ESBLs would be high. To test our hypothesis, we collected 274 fecal samples through pooled sampling from residential areas, dog parks, and veterinary hospitals across seven cities in Eastern Mississippi. Using selective media containing ceftiofur (for ESBL isolation) and meropenem (for CRE isolation), we detected 55 ESBLs and 0 CRE. Each isolate was further characterized with chromogenic media, and antibiotic susceptibility testing was used to confirm ESBL and CRE phenotypes. Prevalence was estimated using a pooled sample calculation, with an ESBL prevalence estimate of 22.6% (95% CI 0.17-0.29) and CRE prevalence estimate of 0% (95% CI 0.0-0.01). These data suggest that ESBLs continue to be common in the general dog population, while CRE are very uncommon and may be a localized issue in tertiary referral hospitals.

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Modeling Traumatic Brain Injury

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Traumatic brain injury (TBI) occurs when a violent external assault causes brain damage. Commonly occurring in sports, on the battlefield, and in car accidents, TBI results in the hospitalization of approximately 250,000 individuals and 69,000 deaths in the United States annually. Following injury, the timing of treatment can determine the extent of the physical and cognitive effects on the individual and the socioeconomic costs to society. In addition, the availability of novel therapeutics that can be rapidly administered is also critical. The purpose of this research is to develop novel therapeutics to treat TBI that can be administered as a nasal spray formulation thereby allowing rapid administration quickly after diagnosis of a concussion. The first step of this process is the development of a TBI model that can serve as a template for furthering this research. To meet this goal, we utilized a user-friendly platform weight-drop device that can induce TBI in a rat without surgical or pre-injury manipulations; thus, allowing a true brain injury to be more accurately mimicked. Following anesthesia, 54 adult male rats were administered impact levels of either 2.5J, 2.0J, 1.5J, 1.0J, 0.5J, or Sham (no impact) with 9 rats per group. After impact, anti-sedative and pain medication was administered. At 3, 7, and 14 days, 3 rats per impact level were sampled and the hippocampus and cerebral cortex were collected. Western blot analysis was used to quantify the levels of glial fibrillary acidic protein (GFAP), a marker for astrocyte activation, and of neuron-specific enolase (NSE), a marker for neuronal damage. Maximum NSE levels occurred at 3 days post-impact whereas, Maximum GFAP levels occurred at 7 days post-impact. The levels of both markers increased as the impact level increased but reached a plateau at higher impact levels. Based on these data, an impact level of 2.0J was determined to be the optimum level that will be used in future experiments to test the effectiveness of novel nasal spray formulations to treat TBI-induced damage.

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How Two Cannabinoids Affect the Potency of Dexamethasone: An Investigation into a Potential Adjunct Therapy

Sabrina Dixon, Todd Archer, and Barbara Kaplan

The objective of this study was to examine immune effects of Dexamethasone (dex) in combination with other immune-suppressive agents to determine if dex dosing could be reduced by using adjunct immune suppressive therapies. Our hope is that an adjunct therapy will allow for reduction in the dosage of dex needed to achieve therapeutically relevant immune suppression, which will enhance the comfort of the patient during treatment and improve owner compliance and satisfaction. Two possible adjuncts are the marijuana compounds, cannabidiol (CBD), and delta-9-tetrahydrocannabidiol (THC). We utilized a pre-clinical in vitro model in which interferon-y (IFN-γ) was stimulated within cell cultures to determine whether either cannabinoid (or the combination) had the potential to serve as an adjunct therapy to achieve the same levels of immune suppression. We hypothesized that dex, CBD, THC, and the CBD/THC combination would all suppress IFN-γ, but that the combination of dex with any of the cannabinoids would exhibit more suppression than any treatment alone. Our results showed that both CBD and THC enhanced immune suppression compared to dex alone. Mice responded variably but overall the addition of cannabinoids increased immune suppression. We also observed that the CBD/THC combination treatment suppressed more than either of the individual treatments. In canine PBMCs, immune suppression increased in a concentration-dependent manner with increasing concentrations of dex, and there was enhanced suppression of the cannabinoid treatments with dex as compared to dex alone. This suggests that these cannabinoids have the potential to serve as adjunct immune suppressive therapies for dex with the goal of decreasing the dex dose needed.
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Gene expression post organophosphate poisoning: tight junction, inflammatory, mitochondrial, apoptotic markers

Raegan Dunwoodie, and Janice Chambers

Organophosphate (OP) anticholinesterases are some of the most neurotoxic synthetic chemicals known. The primary neurotoxic mechanism of OP poisoning is acetylcholinesterase (AChE) inhibition. This leads to excessive synaptic acetylcholine causing hypercholinergic activity, and non-lethal, seizures that can lead to brain damage. This brain damage could result from OP-induced inflammation, blood brain barrier (BBB) leakage, and increased apoptosis from mitochondrial damage. The objective of this project was to examine the impact of OP poisoning on tight junction (TJ), inflammatory, mitochondrial, and apoptotic expression in the piriform cortex, a location involved in the generation of seizures. The genes chosen were Occludin (OCLN), Claudin 5 (CLN5), Nucleoside Diphosphate Kinase A (NME1), FOS Proto-oncogene (FOS), and Serine/Threonine Kinase 1(AKT1). Adult male rats were injected with 0.6 mg/kg NIMP (a surrogate of the nerve agent sarin) or the vehicle. Samples were taken at early (1-2hr) or late (4-day) time points. The piriform cortex was harvested, mRNA purified, and expression levels determined by QRT-RPCR. NIMP caused a short-term decrease in mRNA expression of the TJ structural gene OCLN. Additionally, NIMP caused an increase in mRNA expression at both 1-2 hours and 4-days for CLN5, an intracellular trafficking protein related to neuronal degeneration, and FOS, a genetic marker for neurologic inflammation and increased apoptosis. These results suggest that OP poisoning may have a short-term negative impact on the BBB's structural integrity and may also increase neurological inflammation, degeneration, and apoptosis starting shortly after exposure and persisting for several days and could contribute to brain damage.

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Leukocyte Coping Capacity in Cownose Rays (*Rhinoptera bonasus*): a New Tool to Assess Granulocyte Function

Patrick Farias, Sean Perry, Alexa Delaune, and Justin Stilwell

Leukocyte coping capacity (LCC) is a quantification of the ability for granulocytes to produce a respiratory burst by measuring the release of reactive oxygen species (ROS) in response to stimuli. This technique is used as a proxy to assess the impacts of stressors on immune function in a variety of terrestrial mammalian, avian, and reptile species, but not yet in aquatic vertebrates. The objective of this study was to assess the feasibility of using LCC to quantify ROS release from white blood cells in a cartilaginous fish. The study utilized fifteen, clinically healthy *R. bonasus*, housed in a 350,000-gallon closed recirculating system at the Mississippi Aquarium. We measured the capacity of circulating granulocytes in heparinized whole blood to produce a respiratory burst in vitro via stimulation with phorbol-myristate acetate (PMA). Lucigenin was used to quantify oxidative free radical release in PMA-stimulated and unstimulated samples via chemiluminescence. Chemiluminescence was measured at 5-minute intervals for 60 minutes following blood collection. ROS release was detectable in both control and PMA-stimulated samples. PMA samples were higher than controls over time, allowing for LCC calculation. LCC_{max} occurred 10 minutes following stimulation in all samples. This pilot study demonstrates LCC can successfully quantify ROS from *R. bonasus* granulocytes, a first for cartilaginous fish, and illustrates the potential utility of LCC as a tool to assess granulocyte function in fish. Future directions include LCC validation in *R. bonasus* during stress or pathologic states, establishing reference ranges and normal LCC profiles for healthy *R. bonasus*, and evaluating LCC between free-ranging and captive individuals.

Student Support: Mississippi State University College of Veterinary Medicine

Investigation of factors related to seroprevalence of *Anaplasma marginale* in Mississippi cow-calf herds

Eryn M. Fleming, W. Isaac Jumper, and David R. Smith

Anaplasma marginale, the causative agent of bovine anaplasmosis (BA), is a disease of economic importance to the U.S. beef cow-calf industry. Seroprevalence of *A. marginale* within and between herds in Mississippi is not well described. The objective of this study was to estimate within and between herd seroprevalence of *A. marginale* in cow-calf herds and investigate herd-level risk factors associated with seroprevalence. Blood samples were collected from all mature cows (i.e., ≥2 years of age) in 20 beef cattle herds across MS. A total of 765 serum samples were tested using a commercially available competitive enzyme linked immunosorbent assay (cELISA). Producers were surveyed on herd demographics, herd management, biosecurity, herd health, and bovine anaplasmosis at the time of sample collection. Most herds were commercial (17/20) and the average herd size sampled was 44 head. Of those herds fed free choice mineral, 47% (9/19) fed free choice mineral containing CTC within the last 12 months. Fifty-five percent (5/9) of herds had access to free-choice mineral containing CTC year-round. Overall, 85% (17/20) of herds tested positive by cELISA, and the average within-herd seroprevalence was 39%. Operations that fed chlortetracycline (CTC)-containing mineral free choice within the previous 12 months had greater odds of increased herd seroprevalence compared to those who did not feed CTC-containing mineral free choice (OR=3.349, 95%C.I.=1.288-7.168). Within herd-seroprevalence varied greatly and is influenced by various management factors. This study is the first to report within and between herd seroprevalence of *A. marginale* infection in MS cow-calf herds.

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

Effect of mRNA-induced antibody on adherence of *Tritrichomonas foetus* to bovine preputial keratinocytes

Cassidy Hardy, Merrilee Thoresen, Hannah E. Peck, Daryll Vanover, Philip J. Santangelo, and Amelia R. Woolums

Tritrichomonas foetus (Tf) infection causes bovine pregnancy loss. There are no approved therapies for infected bulls; currently, control is limited to culling infected animals. TF 1.17 is a surface antigen mediating Tf attachment to host epithelium. Synthetic mRNA encoding antibodies against TF 1.17 at the site of infection could be an effective therapy. Experiments were conducted to determine whether Tf attachment was decreased in bovine preputial keratinocytes (PPK) expressing mRNA for antibody to two TF1.17 epitopes (TF1.15 and 1.17). PPK were transfected with mRNA encoding either secreted or anchored antibodies labelled with NanoLuc® and incubated overnight; transfection efficiency was determined by luminescent assay. Optimal incubation time for the Tf attachment assay was determined by adding 1 x 10^6 fluorescently labelled Tf/well to PPK in 24 well plates and incubating for 0.5, 1, 1.5, 2 and 3 h. Unattached Tf were washed off, cells were fixed, and actin and nuclei were stained for imaging. Attached Tf were counted in 3 fields at 20X magnification per slide. Using the same methods, labelled Tf were co-cultured with mRNA transfected and control PPK for 3 h, Tf were quantified in 6 fields per slide and experiments were repeated 3 times. Transfection was successful, with antibody to TF 1.15 and anchored constructs most efficiently expressed. Cells producing anchored antibody to TF 1.17 had fewest attached Tf, but due to high variability the decrease was not different from other treatments or controls. In vitro conditions lack host immune mediators that may be necessary for antibody to decrease Tf attachment. Future experiments will focus effects of expressed antibodies on Tf adherence in vivo.

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***Haemonchus contortus* vaccine implant for sheep**

Darby Harris, and Jennifer Wilson-Welder

Haemonchus contortus, commonly known as the barber pole worm, is a parasitic nematode that resides in the abomasum of small ruminants. These blood-feeding strongyles can be found globally and cause animal suffering and significant economic losses for producers. Given the widespread increase of anthelmintic resistance in *H. contortus* and other gastrointestinal nematodes, new solutions must be generated. Vaccination against these nematodes has been shown to be effective, but the current commercial vaccine requires serial injections to provide sufficient immunity. The goal of the study is to determine if delivering antigenic targets for *Haemonchus contortus* in a novel Vaccine Platform for Extended Antigen Release (VPEAR) implant device will induce a strong humoral immune response which could be used as a means of reducing worm burden and disease caused by the parasitic nematodes in sheep. Four different groups of proteins or peptides are compared against a negative control antigen. Forty sheep had a VPEAR implant device surgically placed subcutaneously on the right fore flank a few centimeters caudal to the elbow joint. The immune response is being measured using antibodies detected by indirect ELISA. After measuring the immune response for 8 weeks, all groups will be challenged with oral gavage of live *H. contortus* larvae. Although this research is still ongoing, the preliminary data shows that a humoral immune response is being generated against Haemonchus contortus antigens.

Student Support: USDA Agricultural Research Service Boehringer Ingelheim Veterinary Scholars Program

Rational design of common epitope vaccine neutralizing Staphylococcal superantigens and cytotoxins

Alyssa Heath, Keun Seok Seo, Nogi Park, Youngkyung Park, and Justin A. Thornton

One of the most prevalent diseases in dairy cattle today is bovine mastitis. A common cause is *Staphylococcus aureus*, an opportunistic pathogen known for its resistance to antimicrobials. Vaccines are an alternative method of treatment; however, studies have failed due to *S. aureus*' evasion of the immune system through various virulence factors. Two important factors are superantigens (SAgs) and cytotoxins, which cause aberrant T-cell activation and lysis of RBCs and WBCs. We hypothesized that immunization with consensus sequences of the receptor binding domain of SAg and cytotoxins will provide cross protection against these toxoids. Sequence alignments showed that the TCR binding domain of SEG, MHC II binding domain of SEI, and stem receptor binding domain of HlgB showed the highest similarity. Recombinant proteins of these binding domains were generated and used for immunizing C57BL/6 mice. Results showed that serum immunized with TCR binding domain was able to neutralize SEC but not SEI. Similarly, serum immunized with MHC II domain was able to neutralize SEI but not SEC. Importantly, SEC and SEI have a higher affinity to TCR and MHC II, respectively, for superantigenicity, indicating that neutralization of a higher affinity interaction between SAgs and TCR or MHC II is important for neutralization. Serum immunized with stem domain of HlgB was able to neutralize HlgB/LukE and LukD/LukE, suggesting cross protection against other cytotoxins. Combined, results suggest that vaccination with consensus sequences can elicit a neutralizing antibody response, providing cross protection against 24 SAgs and 12 cytotoxins. This will be useful to develop an efficacious and affordable bovine mastitis vaccine.

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Effect of vaccination and marketing strategy on pro-inflammatory cytokines in pre- and post-weaned beef calves

Alex Jones, Merrilee Thoresen, Kelsey M. Harvey, Brandi Karisch, Matthew A. Scott, Sarah F Capik, and Amelia R. Woolums

Vaccines induce inflammation to build host immunity. In some management strategies, stocker calves are co-mingled at auction markets. Stress from transportation to feedlots likely increases inflammation. There are no studies explaining common management practices and inflammation. Our objectives were to measure pro-inflammatory cytokines tumor necrosis factor alpha (TNF -α) and interleukin one beta (IL-1β) and compare concentrations to vaccination and marketing strategies. Angus cross beef bull calves (n = 84) were sourced from Mississippi Agricultural and Forestry Experiment Station – Prairie Research Unit (MAFES PRU) in Prairie, Mississippi. Calves were randomized into 4 groups (n = 21); vaccination (VAX) + auction market (AM), vaccination + direct, non-vaccination (NVAX) + auction market, and non-vaccination + direct. VAX calves were first vaccinated with a modified live 5-way respiratory vaccine at an average group age of 92 days; pre-vaccination blood samples were collected from all groups. Seven days later, post-vaccination blood samples were collected from all groups. At an average of 210 days of age all calves were weaned. The direct group remained at MAFES PRU and AM calves were transported to an auction market and later transported to an order buyer facility where they were held for three days. Pre-marketing samples were collected from direct calves and from AM calves' prior to transportation to the auction market. Post-marketing samples were collected from all calves once received at Texas A&M Agrilife Research Unit. Results from our study showed no apparent differences in TNF-a and IL-1b concentrations between VAX or marketing groups, although TNF-a concentrations increased over time in all groups. Further research is needed to understand relationships between common management practices and inflammation.

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Balancing the Equine Hindlimb Digit Using External Landmarks: A New Standard for Equine Podiatry

Peyton Mackey and Ben Nabors

Lameness accounts for up to $1 billion in economic loss for the horse industry annually [1]. Podiatry is an important method for achieving a functional foot and preventing lameness [2], with most of the healthy foot function revolving around the hoof and the distal interphalangeal joint [3]. Podiatry helps to restore balance, embracing “both shape and function of the foot in relation to the ground, as well as to skeletal structures of the digit, both at rest and at exercise” [4]. Recognizing issues with digital balance would be instructive in dictating the required treatment, or podiatry work, needed [5]. However, to this day, the main principle for podiatry revolves around hoof capsule symmetry and dorsal hoof-pastern axis [6], which would be less important because the dorsal hoof angle does not correlate with digital alignment [2]. If external landmarks can be used to determine alignment of the phalanges, then there will be less reliance on radiographs and less disease associated with lameness, leading to less economic loss. This study aims to establish grossly visible landmarks to determine the digital alignment to the ground compared to the hoof wall angle to the ground. While the idea of using the width of the proximal phalanx to draw an imaginary line has been talked about [2], to our knowledge it has not been executed. We completed three phases within our study, comparing photographs to radiographs of pre trimmed, trimmed, and shod digits. We found with a MANOVA Pillai’s trace test that there is a significant difference ($p=0.00000165$) between the hoof wall angle and the digital angle with an alpha value of 0.001. Our partial Eta^2 value was 0.81, which tells us that our angle does influence different measurements.

Student Support: Mississippi State University College of Veterinary Medicine

Comparison of nasal viral shedding and disease severity in calves challenged with Bovine Respiratory Syncytial Virus

Dalton McGeeney, Merrilee Thoresen, Cassandra Barber, Julia Mazanek, William B. Crosby, Santiago Cornejo, Carrie Vance, Florencia Meyer, and Amelia R. Woolums

We are investigating the use of Near Infrared Spectroscopy (NIRS) as a Bovine Respiratory Disease Complex (BRD) diagnostic tool. We have found the biofluids of cattle infected with Bovine Respiratory Syncytial Virus (BRSV) and M. haemolytica have consistently different NIRS spectra than biofluids of healthy cattle. Currently, we are exploring whether spectral differences are related to the viral load and severity of disease. A pilot study was conducted in which groups (n=3) of 6-month-old Holstein bull calves were nebulized with no BRSV, 5x10^3, 5x10^4, or 5x10^5 TCID50 units of BRSV to experimentally create a range of viral loads and disease severity among groups. BRSV F gene copy number was quantified in nasal swabs, collected daily, and tracheal wash samples, collected on day 7, using qPCR to delineate viral concentration. Daily clinical scores were calculated using a previously published scoring system. BRSV was detected on nasal swabs (days 6-8) in all three challenged groups and not the control group. Calves in the high dose group had greater total viral shedding, peak viral shedding, and number of positive samples as compared to other treatment groups, and clinical scores increased in the three challenged groups (days 4 – 8); however, viral shedding and clinical scores were not significantly different from day 0 ($P > 0.05$). On the day of peak virus shedding, clinical score was significantly correlated with BRSV F gene copy number ($P = 0.018$, $r2=0.69$). This challenge did not create a range of disease severity, but calves had a range of viral loads which were related to disease severity. This model will be useful to study the relationship between biofluid NIRS spectra, viral load, and disease severity.

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Establishment of an Implant-Based, Prophylactic Osteomyelitis Rat Model

Emily G. Moles, Luke J. Tucker, Alyssia J. Little, and Lauren B. Priddy

Osteomyelitis is an inflammatory response resulting from the infection of bone by a pathogen, typically *Staphylococcus aureus*, which is prone to antibiotic resistance and biofilm formation. This presents a significant challenge for effectively treating the infection, especially as the concentration of antibiotics needed to treat increases with *S. aureus* biofilm maturation. Few clinically relevant, implant-based models to study preventative measures for osteomyelitis exist. We hypothesized that an *in vivo* model that will allow for the repeatable establishment of infection and treatment in the same surgery can be developed using prior data from our and others' chronic osteomyelitis models and further *ex vivo* experiments. Using an *ex vivo* model, bacterial burden in the rat femur following implantation time (5 or 15 minutes) of a contaminated screw indicated 5 minutes was sufficient to establish relevant and repeatable infection. Four Sprague Dawley rats underwent a surgical procedure involving the same 5-minute implantation time of a contaminated screw, followed by treatment with membranes loaded with natural antimicrobials. At 72 hours, the femur and soft tissue were homogenized for bacterial quantification. The average bacterial counts of the femur and surrounding soft tissue were 6.38 log CFU and 6.22 log CFU, respectively, demonstrating the establishment of a consistent and repeatable model. This research promotes good antimicrobial stewardship by creating a clinically relevant testbed for evaluating treatments aimed at slowing down the development of *S. aureus* biofilms, which may decrease the amount of antibiotics needed to treat osteomyelitis.

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Evaluating the Presence of *Cryptosporidium* and *Cyclospora* in Rabbits and Mice

Elora Pierce, Alison Li, Doaa Naguib, Randi Turner, Matthew Valente, Hannah S. Tiffin, Glen Scoles, and Asis Khan

Species of *Cyclospora* and *Cryptosporidium* cause health challenges for humans across the globe. *Cyclospora cayetanensis* and *Cryptosporidium hominis* occur exclusively in humans. Unfortunately, progress in studying host-pathogen interactions and developing new therapeutics has been hampered by the lack of small animal models and in vitro systems for long-term cultivation. This research hoped to identify genetically similar species from fecal samples of *C. hominis* in rabbits and mice and *C. cayetanensis* in mice. 100 rabbit stool samples were received from Egypt and were scanned using a sensitive and specific qPCR assay for *Cryptosporidium*. Of 100 samples, 7 samples were considered positive using qPCR. Separately, PCR and gel electrophoresis on these samples showed a faint band of expected size which confirmed the presence of *Cryptosporidium* in sample 31. Additionally, 17 wild mice samples collected from Beltsville, MD showed that 12 out of 17 samples were positive for *Cryptosporidium* on qPCR. The mice samples were also scanned using two *C. cayetanensis* 18S rRNA-specific qPCRs including BAM19b primers developed by the FDA and Pan-Cyclo 18S rRNA primers. qPCR results indicated 14 out of 17 samples gave a CT value of less than 40. Sanger sequencing was conducted on three of the positive mice samples, which showed a 99% match to *Isospora* and a 95% match to *C. cayetanensis*. Further microscopic examination and sequencing of genetic loci will be conducted in the future to characterize these samples in order to identify and develop a rabbit/mice-adopted animal model for *Cryptosporidium* and *Cyclospora*.

Student Support: USDA Agricultural Research Service Boehringer Ingelheim Veterinary Scholars Program

Evaluation of Safety of AlHV-1ΔORF73/OVHV2-ORF8 chimeric virus vaccine for Malignant Catarrhal Fever in bison

Madeline O. Rech, Katherine N. Baker, Daniela D. More, Maria Herndon, Brett Webb, and Cristina W. Cunha

Malignant catarrhal fever (MCF) is caused by a group of viruses that belong to the family Herpesviridae, genus Macavirus. Ovine herpesvirus 2 (OvHV-2) is the causative agent of sheep-associated MCF (SA-MCF), which is endemic in most sheep populations and can often cause fatal disease once introduced to susceptible species, such as American bison. Domestic sheep are considered the subclinical reservoir, where the virus usually establishes latency. In North America, SA-MCF is one of the most important infectious diseases for bison producers, causing large financial and herd losses. Effective vaccines are necessary to avoid these losses. A vaccine targeting MCF in bison should be non-replicating to reduce transmission risk among individuals. This study aimed to evaluate the transmission risk of a novel SA-MCF vaccine candidate in bison. The vaccine candidate is a chimeric virus containing a recombinant, non-pathogenic strain of alcelaphine herpesvirus-1 (AlHV-1) encoding the OvHV-2 ORF8 to express gB (AlHV-1ΔORF73/OvHV-2-ORF8). This vaccine mixed with adjuvant (Emulsigen®) was given intramuscularly and boosted twice at two-week intervals. No clinical signs or adverse effects were observed in any of the vaccinated animals. To evaluate AlHV-1 shedding, nasal swabs, saliva, and buffy coat samples were collected, total DNA was extracted, and viral genomes detected/quantified using PCR. There was no significant amplification in any of the samples. These results show that the chimeric virus used as a vaccine vector does not cause disease, has a very low transmission/infection risk and therefore is considered safe to give to American bison.

Student Support: USDA Agricultural Research Service Boehringer Ingelheim Veterinary Scholars Program

Diet Manipulation in Macaques With Enteric Disease and the Effects On the Gastrointestinal Microbiota

Gabrielle Roque, Paul Makidon, DVM, PhD, DACLAM, and Keeley McGrew, PhD

In captive import/export macaque facilities, idiopathic diarrhea is a common health condition. Diarrhea incidence varies between 15-39% of captive breeding population which impacts not only the quality of life and welfare of non-human primates (NHP), but also has economic and human disease modelling impacts on the industry. The etiology of diarrhea in this population is multifactorial and includes commonly described pathogens (i.e.. campylobacter, shigella, Yersinia), behavioral stress, dietary issues, and operationally related factors. It is well understood that gut microbiota plays a major role in susceptibility to diarrheal diseases. However, the relationship between enteric disease and microbiota in captive NHP is not well described. Thankfully, there are numerous opportunities for therapeutic intervention. These include; antibiotics, dietary manipulation, fiber, symbiotics, and behavioral manipulation. Standard of care for numerous veterinary species and humans dictate the use of diets that are bland in nature, non-inflammatory, and non-challenging to the intestinal tract. One possible benefit of a bland diet, is the promotion of a “healthier” gut microbiome**.** In this study, we compared a cohort (N=13) of NHPs with diarrhea who were placed on a bland diet vs. a cohort (N=13) of NHPs who were fed Teklad 2050A standard primate chow. In addition to characterizing the microbiome, animals were clinically assessed by a clinical scoring system. Although there was a few differences identified in the clinical scoring between NHPS treated with bland diet vs. standard primate chow, we did observe less remission in the bland diet treatment group. At this point, we are still pending the microbiome analysis. However, remission rates are supportive of the idea that the gut microbiome shifted to a healthier phenotype in the bland diet group compared to the control group. This evidence-based medicine provides a framework for informing future herd management of diarrhea in captive macaques.

Confirmation of chelonid alphaherpesvirus 5 infection in green and Kemp's ridley sea turtles from Mississippi

Sarah Rubelowsky, Justin Stilwell, Caroline Betbeze, Debra Moore, Lyndsey Howell, and Natalie Stilwell

Fibropapillomatosis (FP) is a neoplastic disease associated with chelonid alphaherpesvirus 5 (ChHV5) infection in threatened and endangered sea turtles worldwide. Due to its transmissible nature, high disease prevalence in some areas, and potential for severe disease among affected individuals, FP negatively impacts sea turtle health and creates challenges for rehabilitation programs. To our knowledge, FP remains unreported in Mississippi. Over a 6-month period in 2022-2023, one Kemp's ridley sea turtle (*Lepidochelys kempii*) and three green sea turtles (*Chelonia mydas*) stranded off the Mississippi coast with FP-suggestive lesions. The objectives of this study were to molecularly confirm and phylogenetically characterize ChHV5 strains from the recent Mississippi cases. Conventional PCRassays targeting three ChHV5 genes were performed on genomic DNA from FP lesion, skin, and blood samples collected at necropsy. Positive detections were confirmed using Nanopore sequencing. Results confirmed ChHV5 infection in all four cases as well as another stranded, asymptomatic Kemp's ridley sea turtle from the Mississippi region. Phylogenetic analysis of the concatenated genes revealed that ChHV5 strains from the Mississippi turtles were most closely related to strains from Florida, the Caribbean, and Atlantic seaboard, but were relatively unrelated to strains from Texas, suggesting a possible east-to-west dissemination pattern. These results offer the first confirmation of FP-associated ChHV5 infection in sea turtles from the Mississippi coast. Importantly, the detection of ChHV5 in an asymptomatic Kemp's ridley turtle underscores the need for additional surveillance to understand the prevalence of this pathogen in Mississippi.

Student Support: Mississippi State University College of Veterinary Medicine

Radiographic Validation of External landmarks to Assess Equine Forelimb Digit Alignment

Anna Grace Scrimager and Ben Nabors

Loss of performance use in sport horses costs the horse industry up to $1 billion annually, a quarter of that resulting from forelimb digit lameness. Podiatry is the most effective method to mechanically balance the digit, and directly affects distal limb. Skeletal and soft tissue structures comprising the digit, along with the two principles of locomotion, elastic recoil and concussion absorption, function optimally when balanced. Balance is created when the digital phalanges are aligned, allowing internal structures to absorb concussion evenly and permitting elastic recoil to govern limb locomotion untethered. We hypothesized that external landmarks can be employed to identify a difference in pastern angle (proximal and middle phalanx) and hoof angle (distal phalanx) indicating an unbalanced phalangeal alignment. This would obviate the need for radiographs in the process of achieving balanced horseshoeing. Seven horses were imaged, taking photographs and radiographs of the digit. External landmarks were utilized to assess digital alignment on photographs and validated with radiographic digital alignment. Analysis of measurements via MAN OVA found a significant difference (p=o.007646) between the hoof wall and the digital angle with an alpha value of 0.001. In all seven horses, gross external landmarks directly represented internal digit alignment radiographically.

Student Support: Mississippi State University College of Veterinary Medicine

Bridging the gap: overcoming DNA degradation challenges in Sea Turtle genetic analysis with mtDNA targeting

Sarah Thurman, Mark Arick II, Chuan-yu Hsu, Theresa Madrigal, Debra Moore, Beth Peterman, Atilla Karsi

Kemp's ridley sea turtles (*Lepidochelys kempii*) are a critically endangered species found in the Gulf of Mexico. The Institute of Marine Mammal Studies in Gulfport, MS, has played a vital role as a rehabilitation facility for cold-stunned sea turtles stranded along New England shores. These turtles receive veterinary care from MSU veterinarians, are tagged with satellite trackers, and released into the Mississippi Sound. Tracking efforts have revealed that these rehabilitated turtles remain within the Gulf, particularly off the coasts of Mississippi and Louisiana. This evidence suggests that the Northern Gulf Coast now supports a significant population of released sea turtles, emphasizing the importance of understanding this population at a genetic level for effective conservation. To this goal, blood samples were collected from turtles undergoing rehabilitation, and tissue samples were obtained from deceased stranded turtles along the Gulf Coast. The genomic DNA extracted from these samples presented challenges as most exhibited poor quality and varying degrees of degradation. Consequently, mitochondrial DNA (mtDNA) amplification and sequencing emerged as the most viable approach for genetic analysis. Multiple universal primer sets were designed to amplify the complete 16 kb mtDNA or two overlapping fragments. While amplification of the entire 16 kb mtDNA proved unsuccessful, one of the two fragments was successfully amplified using three specific primer sets. We will optimize the conditions to obtain the second fragment required to amplify the entire mtDNA. The assessment of genetic diversity is expected to serve as a foundation for future studies aimed at analyzing migration patterns, understanding reproductive behavior, breeding strategies, and other essential factors contributing to conservation efforts. By delving into the genetic makeup of this rehabilitated and released turtle population, we can gain valuable insights crucial for their long-term survival and effective conservation management.

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

Elucidating ontogeny of adults and metacercariae of *Acanthostomum* species (Digenea, Cryptogonimidae) from *Alligator mississippiensis* and *Lepisosteus oculatus* in the southeastern United States using DNA barcoding

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The genus *Acanthostomum* Looss 1899 (Digenea, Cryptogonimidae) consists of over 20 described trematode species that parasitize fish and crocodilians, causing damage to the gut tissue of their hosts. *Acanthostomum* has a complex 3-host life cycle, including snails, reptiles and fish. Despite a rich history consisting of novel host and geographic records, there are still many questions remaining about their taxonomy and systematics, pathogenesis in each host and their life histories. Morphological characteristics have been used to identify *Acanthostomum* spp., but two areas that require further investigation include (1) elucidating the various life stages and (2) conducting phylogenetic analyses using molecular data to infer evolutionary relationships among different species. Several *Acanthostomum* spp. have been reported from crocodilians globally, but molecular data assessing their diversity and intraspecific variation have not been fully explored. In this study, ribosomal and mitochondrial data were sequenced from adult *Acanthostomum* spp. collected from American alligators *Alligator mississippiensis* and larval metacercariae from the muscle of spotted gar *Lepisosteus oculatus* from the southeastern United States. Hologenophores were prepared for 7 samples by cutting a section from each worm, extracting its DNA, and permanently staining and mounting them. Additionally, a whole worm was processed for DNA analysis. The DNA was used to assess the utility of DNA barcoding for linking individuals to specific species and to infer phylogenies using both ribosomal and mitochondrial DNA in conjunction with morphological characteristics. The results of these analyses revealed that (1) multiple genera previously thought to be distinct may need to be collapsed, (2) a monophyletic crocodilian clade with strong support showed host specificity as an important consideration for taxonomic revisions going forward, (3)

metacercaria fell into clades with strong support, linking the second intermediate larval stage to adults, and (4) possibly two novel species were discovered.

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Ex vivo Comparison of Kinamed SuperCable and Standard Metal Cerclage Wire in a Canine Femoral Fracture Model

Natalie Walls, Seila Day, Steven Elder, and Michael Jaffe

Standard metal cerclage wires are commonly used as an implant in veterinary orthopedic surgery in conjunction with other repair and fixation methods to provide interfragmentary compression of bone fragments. While metal cerclage wire has been used historically there are known complications that may be sustained under physiological conditions. The goal of this study is to evaluate the Kinamed SuperCable orthopedic cable and compare it to standard cerclage wire configurations under cyclic loading until failure. Failure was deemed to occur when a permanent bone-implant construct displacement of greater than or equal to 2mm was seen. It was hypothesized that the Kinamed SuperCable would prove to be superior to standard cerclage wire methods when tested under cyclic 4-point bending for use in veterinary small animal surgery. When the Kinamed SuperCable was compared to standard single loop metal cerclage wire it proved to be superior in both the amount of load (N) and number of cycles it was able to withstand until failure. The Kinamed SuperCable's superior load bearing capabilities make it a practical option as a more effective and efficient way to stabilize small animal orthopedic surgical cases, particularly fractures.

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