2022 Summer Research Abstracts

Clinical utility of standing fecal flotation in hookworm identification in fecal aliquots from shelter canines

Jessica Barber, David Smith, T. Graham Rosser, Hayden Brines, Kimberly Woodruff

Ancylostoma/*Uncinaria* spp (hookworms) are of the most common canine intestinal parasites that are easily transmitted and carry zoonotic potential. Centrifugal Fecal Flotation is preferred for fecal detection but due to practicality, speed, and low economic cost, shelters and veterinary hospitals frequently use the Standing Fecal Flotation. These methods vary in flotation media, centrifugation, and fecal mass. Fecal loop tools are often employed for fecal collection, and generally collect 0.5 or 0.25 grams(g). Variations from the preferred method were the focus of this study, in which the variability in eggs per gram (EPG) of hookworm ova and sensitivity of hookworm detection within varying fecal quantities were investigated. Thirty-one voided samples were collected from apparently healthy canines in shelters. Canines not yet treated for intestinal parasites, or those that had been treated within the previous three days or two weeks, were included. For each sample, aliquots of 2, 1, 0.5, 0.25 g were evaluated for hookworm ova. Average EPG were calculated using the 2 g aliquot based on the presence of hookworms. Results were recorded with Excel and analyzed with SAS Studio 9.4.1. From the 360 fecal floats, 331 were positive. Overall, there was no statistically significant difference in fecal gram size for hookworm ova identification. However, when each aliquot was compared to the 2 g sample standard, statistically significant differences were revealed. Each size aliquot provided a high probability of identifying hookworm ova, contributing to the on-going battle against hookworm infections.

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

Validation of a revised measure of veterinary general decision-making preference

Sarah-Ashlyn Barber, Jesse Grady, Holli Seitz

The Veterinary General Decision-Making Preference Scale (VGDMPS) is a measure of pet owner preference for autonomy when making medical decisions for their pets. It is adapted from the Autonomy Preference Index (API) used in human medical decision-making. Previous attempts to validate the VGDMPS found that clients reacted negatively to the wording of items. The objective of this research was to revise the Veterinary General Decision-Making Preference Scale to make it acceptable to clients and validate it in a non-clinical population. We revised the VGDMPS and assessed face and content validity through cognitive interviews with 5 small animal veterinarians and 11 clients at a veterinary school–based community practice, pausing in the middle of data collection to make further revisions to the instrument. Once the Revised Veterinary General Decision-Making Preference Scale (RVGDMPS) reached acceptable levels of acceptability, clarity, and completeness, we administered the instrument to an online sample of pet owners (n = 229) and administered a follow-up survey to a subsample of pet owners (n = 100). The scale was unidimensional and reached acceptable levels of internal consistency (Cronbach's alpha = .81). Item Response Theory analysis determined that the instrument discrimination parameters for all items are high or very high and that response categories allow for adequate differentiation among differing levels of preference for autonomy. Test-retest reliability was acceptable (ICC = .64, p < .011). The validation of the RVGDMPS provides us with a basis for further veterinary communication research that can be used to examine the effect of client preferences on client satisfaction, adherence, and improved pet health.

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

In vitro model of MOG and OVA peptide stimulated T-cell responses and effects of cannabinoids

Karis Blankenship, Matthew Mitsch, Barbara Kaplan

The plant *Cannabis sativa*, commonly known as marijuana, has a multitude of chemical compounds, known as cannabinoids. Two primary cannabinoids are cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC). These

cannabinoids have been shown to suppress T-cell stimulated cytokine production, specifically in neurodegenerative autoimmune disease in mouse models. However, studying effects of cannabinoids *in vivo* can be limiting due to the systemic nature and costs of *in vivo* models and funding. The goals of this research were to study peptide stimulated T cell cytokine responses in a developed *in vitro* model, as well as to examine CBD and THC treatments causing T-cell cytokine suppression. Two peptides utilized in this research were myelin oligodendrocyte glycoprotein (MOG_{35-55}), a self-peptide located in the myelin sheath of the central nervous system, and ovalbumin (OVA) peptide, a foreign peptide derived from chicken egg whites. We hypothesized that CBD and THC would suppress peptide-stimulated cytokine production. Results showed that both MOG and OVA peptides can drove T-cell cytokine production *in vitro*. CBD and THC also produced immunosuppression, as observed by ELISA to measure cytokine levels. Data suggests that cannabinoids would be more efficacious as a prophylactic as opposed to a treatment for immune-mediated diseases. This study established an *in vitro* mouse model that will allow more complete analysis and further investigation of T cell immunosuppression by CBD and THC, which could provide veterinarians and human doctors guidance on whether cannabinoids provide medical benefit or not.

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Measure of agreement among observers utilizing bottlenose dolphin pectoral flipper radiograph aging techniques

Jenna Bordages, Christa E. Barrett, Alison M. Lee, Kathryn Thompson

A readily available, inexpensive, and non-invasive technique to more accurately age bottlenose dolphins is needed in order for stranding networks to continue to promote the conservation of this species. An aging technique assessing bottlenose dolphin pectoral flipper radiographs has been established and is readily available, inexpensive, and non-invasive but it has not been assessed for repeatability or agreement among users. The hypothesis of this study is that there will be good to excellent levels of agreement among four observers of varying experience levels utilizing bottlenose dolphin pectoral flipper radiograph aging techniques. Four observers of varying levels of experience, including a board-certified veterinary radiologist, a marine mammal veterinarian, a veterinary student, and a stranding technician, evaluated 107 bottlenose dolphin pectoral flipper radiographs and scored 16 individual growth plates of the pectoral flippers utilizing the already established pectoral flipper radiograph aging technique. Agreement among the four observers was assessed by intra-class correlation (ICC) using PROC MIXED with SAS on all scored categories. The ICC analyses revealed that all four observers had excellent agreement levels on the total analysis of the 16 osseous locations scored. Results indicate that the published scoring system to age bottlenose dolphins by pectoral flipper radiographs can be used amongst workers of varying levels of experience with similar results; therefore, increasing its value as a tool for all stranding networks.

Student Support: Global Center for Aquatic Health and Food Security, Mississippi State University College of Veterinary Medicine and Mississippi Marine Mammal and Turtle Conservation, Recovery, and Monitoring Program; National Fish and Wildlife Foundation under Mississippi Department of Environmental Quality Agreement No. 18-00081

Veterinary Student Proficiency and Ovariohysterectomy Duration: A Statistical Analysis

Hayden V. Brines, Kimberly A. Woodruff, David R. Smith

Castrations and ovariohysterectomies (OHEs) are among the most common services provided by veterinarians. At Mississippi State University College of Veterinary Medicine, veterinary students are first introduced to these surgical methods during their second year of training and continue to perform these procedures throughout their third and fourth years, specifically during a shelter medicine spay and neuter elective. Student confidence and factors encompassing student surgical procedure have been the focus of previous studies. However, the variability of the student learning is poorly understood. The aim of this study was to determine the number of OHEs veterinary students need to complete to obtain proficient surgical duration. This study utilized Microsoft Excel to record information pertaining to students and their OHE

patients. Linear regression using a proc mixed procedure was used in analysis via SAS software. The average OHE duration for veterinary students started off at approximately forty-four minutes. The rate at which student surgical duration decreased was logarithmic and was statistically significant until their 51st OHE at which point the rate becomes negligible. All in all, this study can be implemented in veterinary colleges to target the ideal number of OHEs to maximize student success and experience, as well as conserve institutional resources.

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

Synthetic mRNA-induced expression of H2 Relaxin by bovine epithelial cells

Caitlyn Burke, Merrilee Thoresen, Daryll Vanover, Heath King, Jean Feugang, Amelia Woolums, Peter Ryan, Philip Santangelo

Relaxin (RLN) is a reproductive hormone that enhances connective tissue remodeling during pregnancy and parturition and has been used therapeutically to reduce the incidence of dystocia in cattle. However, attempts using purified porcine or recombinant human RLN to reduce the incidence of dystocia in heifers present variable results. Human 2 (H2) RLN has a high affinity to the bovine RLN receptor (RXFP1) and given advancements in mRNA therapeutics, H2 RLN mRNA therapy may prove to be a more efficacious treatment for dystocia. Using a H2 RLN-NanoLuciferase (NanoLuc) mRNA construct with a secretion signal, we transfected bovine kidney (BK) and primary bovine epithelial cells (BVEC) with 0.5, 1 or 2 µg synthetic mRNA. At 3, 6, 12, 24 and 48 h post-transfection, cell lysates and supernatants were collected for detection of H2 RLN indirectly via Nano-Glo Assay® (Promega) or directly via ELISA (R&D Systems). Luminescence demonstrated that bovine epithelial cells expressed H2 RLN in cell lysates for all observed time points with a decline only observed at 48 h in the BVEC cells. In contrast, cell supernatants exhibited increasing levels of luminescence, indicating secretion of H2 RLN. Additionally, increasing concentrations of H2 RLN were observed from 6-48 h in supernatants from BK cells transfected with the lowest concentration of mRNA (0.5 µg). Furthermore, the *in vivo* transfection of a 6-month-old dairy heifer with NanoLuc mRNA demonstrates the bovine reproductive mucosa is receptive to transfection resulting in high levels of expression at the ectocervix, the target tissue for H2 RLN. These data provide evidence supporting future *in vivo* transfections with H2 RLN mRNA as a novel approach in reducing dystocia in heifers.

Student Support: Mississippi State University College of Veterinary Medicine

Chitosan hydrogel and polylactic acid particles loaded with fosfomycin for local treatment of osteomyelitis

Julia M. DiFiore, Luke J. Tucker, Malley A. Gautreaux, Bailey E. Roux, Xavier J. Person, Lauren B. Priddy

Staphylococcus aureus (*S. aureus*) is the most common pathogen in osteomyelitis (OM), an acute or chronic bone infection. *S. aureus* can become resistant to different types of antibiotics delivered systemically and/or orally. Locally administrated antibiotics placed directly at the site of infection and delivered via a sustained release would allow for a higher dose to be used while simultaneously reducing the risks of resistance and toxicity seen with long-term use. Our objective was to evaluate the efficacy of a chitosan hydrogel and/or polylactic acid (PLA) particles loaded with fosfomycin (FOS) to reduce the bacterial load of *S. aureus* in the femur and soft tissue in a rat model of chronic infection. We hypothesized that FOS delivered dually via antimicrobial chitosan hydrogel and PLA particles would reduce the bacterial load compared to FOS delivered via either chitosan or PLA particles alone. Radiographic images of the femur were taken as real-time indicators of infection status and were used to analyze change in relative bone density over time. There were no differences between treatment groups, but relative bone density decreased from day 8 to day 14 but began to stabilize by day 21 in all groups containing FOS. Blood samples were evaluated for the presence of haptoglobin. Though differences between treatment groups were not seen, haptoglobin levels were higher from day 1 to day 14 compared to pre-surgery values, but returned to baseline by day 21. At day 35, bone and surrounding soft tissue samples will be harvested to quantify bacterial load. Tailored biomaterials, such as the

ones used in our study, may allow for increased therapeutic efficacy in future OM cases with challenging pathogens such as *S. aureus*.

Student Support: National Institutes of Health T35OD010432

In Vitro Assessment of Growing Conditions for Fungal Pathogens of Sea Turtles

Catherine DiNicola, John Thomason, Stephen Reichley, Alexis Thompson, Natalie Stilwell

Endangered sea turtles are threatened by a variety of natural and anthropogenic factors, including fungal pathogens in live turtles and nests. Most fungi of sea turtles are considered opportunistic, and it is important to identify the microorganisms present so that therapy can be promptly instituted. Certain fungi remain difficult to diagnose and may require several weeks to isolate using traditional media and growing conditions. The objective was to determine optimal growing conditions for 10 common pathogenic fungi of sea turtles and their eggs. We compared isolate growth rates and characteristics using six types of selective media, two incubation temperatures, and two methods of measurement (manual measurement and digital imaging software). Statistical analysis consisted of repeated measures analysis and Tukey's post-hoc test. Our study revealed a significant effect of media type and incubation temperature on growth rates of all 10 tested fungi. Three types of media (Sabouraud dextrose, potato dextrose, and Rose Bengal) yielded consistent and rapid growth of most fungi, whereas growth was less reliable on inhibitory mold, SABHI, and synthetic mycobiotic agars. Most fungi grew significantly faster at 30°C than 23°C regardless of media type. Thus, the use of certain media and incubation temperatures may enhance the recovery rate of fungal pathogens from sea turtles, and in turn, inform appropriate therapy and reduce the duration of illness and rehabilitation. Our results may also aid the diagnosis of fungal infection in sea turtle nests and in other reptile species, as several of the examined fungi are broad pathogens of ectothermic hosts.

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Exploring antimicrobial peptides as novel mRNA therapeutics for bovine trichomoniasis

Matthew Harjes, Santiago Cornejo, Merrilee Thoresen, Daryll Vanover, Philip Santangelo, Amelia Woolums

Bovine pathogen *Tritrichomonas foetus* (Tf) causes great loss of life and profit on beef farms, and no FDA-approved treatment exists. To address this, a novel mRNA therapy for trichomoniasis is proposed based on transfection of bovine cells with mRNA of antimicrobial peptides (AMPs) that may reduce Tf viability. Our objectives were to 1) test the effect of synthetic AMPs on Tf viability, 2) confirm transfection of bovine cells with mRNA, and 3) test the effect of supernatants and lysates of AMP mRNA-transfected cells on Tf viability. Tf (200,000/ml) were treated with 20 uM BMAP-28 or D-hecate for 0, 0.5, 2, 6, 12, and 24 h and assessed for viability at each point by examining motility using microscopy, metabolism via CellTiter-Glo® 2.0 viability assay, and recovery from AMP treatments. Bovine kidney (BK) cells were treated with 1 ug/well of GFP or nanoluciferase mRNA in a 24-well plate to confirm transfection and subsequently with 0.5 ug/cm^2 of truncated BMAP-28 (Syn-1) or D-hecate mRNA in T25s for 24 h to generate supernatants and lysates. Tf (200,000/ml) were treated for 0, 0.5, 2, 6, 12, and 24 h with the products and assessed for viability. Marked, rapid, and sustained decrease in Tf viability showed upon exposure to synthetic BMAP-28, but not synthetic D-hecate nor products of AMP-transfected cells. Lack of D-hecate effects may be due to inhibition by FBS in media and lack of transfection product effects may be due to low AMP expression. Future work should confirm effects of BMAP-28 on Tf viability and optimize use of D-hecate and AMP mRNA-transfected cell products. This research could lead to development of a trichomoniasis therapy by utilizing AMP mRNA on bovine reproductive epithelium for treatment or prevention.

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Pilot investigation of *Mycoplasma bovis* in Mississippi beef cattle populations using culture and qPCR
Martin A. Holsinger, William B. Crosby, Robert Valeris-Chacin, William B. Epperson, Amelia R. Woolums

Mycoplasma bovis contributes to Bovine Respiratory Disease Complex (BRDC), which has significant welfare and economic impacts on cattle production. The aim of this research was to describe growth characteristics of *M. bovis* isolates and evaluate respiratory shedding in populations of beef cattle at risk for BRDC. Known *M. bovis* isolates were transferred from -80°C to pleuropneumonia-like organism (PPLO) agar, incubated at 37 °C with 5% CO_2, and monitored for growth. To develop selective media for field use, PPLO plates containing ceftiofur (200, 100, 50, 25, and 12.5 µg/ml) were prepared. *Pasteurellaceae* were transferred from -80°C to each media preparation, incubated at 37°C with 5% CO_2, and observed for growth. To assess *M. bovis* respiratory shedding, three populations (n=5, n=9, n=5) of recently weaned beef calves were sampled using double guarded deep nasopharyngeal swabs (NPS), which were streaked onto selective media. Colonies with typical *Mycoplasma*-like morphology were confirmed by *M. bovis* specific PCR. DNA was also extracted directly from NPS for quantitative real-time PCR (qPCR), with C_q<31 considered positive. Known *M. bovis* isolates were first evident in culture at 48-120 hours after plating. Growth of *Pasteurellaceae* was inhibited at all concentrations of antibiotic tested and PPLO with 100 µg/ml ceftiofur was chosen for field use. The prevalence of *M. bovis* across all populations was 42% as determined by culture (20%-80%; *P>0.05*, Fisher's Exact test) and 58% as determined by qPCR (0%-89%; *P*<0.05 Fisher's Exact test). In this study, *M. bovis* growth was faster than previously described, and prevalence of calves shedding *M. bovis* significantly varied among populations when measured by qPCR but not culture.

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Interplay between environmental exposures and *Staphylococcus aureus* infections in diabetic wound healing
Shelby Ladner, Regan McDevitt, Keun Seo, Joo Park, Carol Baker, Alicia Olivier, Elizabeth Swanson, George Howell

There are many known risk factors that contribute to pathogenesis of type 2 diabetes, and recent studies have shown a positive association between exposure to persistent organic pollutants and the increase in prevalence. A common comorbidity of type 2 diabetes are foot ulcers that often become infected, with the most isolated organism being *Staphylococcus aureus*. The goal of our study is to determine potential effects of exposure to organochlorine pesticides on wound formation and resolution in diabetic patients. In our study, we dosed healthy male C57BL/6J and male diabetic Tallyho mice with a mixture of the following organochlorine metabolites: dichlorodiphenyldichloroethylene (DDE), trans-nonachlor, and oxychlordane (referred to as DTO), formed pressure-induced wounds using two circular magnets on the dorsal skin causing ischemic-reperfusion injury, and measured the wounds up to 12 days post wounding. This method was repeated in another cohort with the addition of *S. aureus* infection. The results showed that wound resolution decreased in diabetic Tallyho mice that were treated with DTO. Wound areas were also significantly increased in DTO treated, S. aureus infected diabetic animals at day 1 post wounding/inoculation compared to uninfected vehicle and uninfected DTO treated animals. In conclusion, our study suggests that exposures to environmental pollutants have an impact on diabetic wound healing and can be used in future research for different treatment options or clinical settings to help assess patients for risk of developing foot ulcers and their ability to heal or avoid infections.

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Development of an ELISA kit to detect antibodies against Feline Infectious Peritonitis Virus (FIPV)
Dania Cervantes Linares, Jaime Rutter, Youngkyung Kim, Chaeyoung Kim, Joo Youn Park, Carol Baker, Cooper Brookshire, Nogi Park, Keun S. Seo

Feline Infectious Peritonitis Virus (FIPV) causes Feline infectious peritonitis (FIP), one of the most fatal feline infectious diseases. There is no effective treatment or vaccines, and the fatality rate is essentially 100%. Determining the presence of anti-FIPV antibodies in cerebrospinal fluid and serum is helpful. However, antibodies simply demonstrate that the animal has previously been exposed to a coronavirus. Thus, it is necessary to develop a diagnostic tool to determine whether animals maintain protective immunity. The spike (S) protein of FIPV consists of two subdomains: domain 0 (D0) and B (DB), which provide structural support and contain a receptor binding motif for viral attachment and entry to host cells respectively. Thus, the D0 and DB are ideal targets for serological diagnosis and protective immunity. In this study, we constructed HEK293t cells permanently expressing D0 and DB fused with mouse Fc region of IgG1 (D0-mIgG and DB-mIgG, respectively). We established an indirect ELISA using a microfluidic system which curtails the materials and time for ELISA. Our preliminary data showed significant variations in DB-mIgG ELISA results among cat sera collected from animal shelters. These suggest that cats are ubiquitously exposed to FIPV and develop an immune response with different levels of protective immunity. We will continue to analyze 300 cat serums collected from Mississippi State animal shelters to investigate the prevalence of FIPV infections and protective immunity in shelter animals. We will also determine plaque neutralization assay to correlate DB-ELISA results with protective immunity. This will help clinicians in practice, and shelter medicine diagnose cats with FIPV and determine vaccine use.

Student support: Mississippi State University College of Veterinary Medicine

Bovine babesiosis risk of reintroduction into the U.S.

Kayla Mercer and Massaro Ueti

Rhipicephalus (Boophilus) annulatus and *Rhipicephalus (Boophilus) microplus* serve as vectors for the transmission of the apicomplexan parasites *Babesia bovis* and *Babesia bigemina*, the causative agents of cattle fever or bovine babesiosis. *R. annulatus* and *R. microplus* are commonly found throughout Mexico and intermittently found in Texas in counties bordering the Rio Grande. White-tailed deer serve as secondary hosts for these ectoparasites and frequently travel unregulated between these regions. Transovarial transmission of *B. bovis* and *B. bigemina* establishes concern for the potential reintroduction of these protozoa via movement of infected adult *Rhipicephalus* ticks aboard white-tailed deer near the border and subsequent laying of infected eggs in the U.S. The aim of this study was to determine the number of *Rhipicephalus* ticks infected with *B. bovis* and *B. bigemina* crossing the border via white-tailed deer. A minimum of 50 Rhipicephalus ticks per deer were removed from 37 deer crossing the border into Zapata County, Texas. From each deer's assortment of ticks, 16 ticks at various life stages were selected and processed using a grind over liquid nitrogen and Qiagen DNA extraction. Isolated genomic DNA samples were tested for the presence of *B. bovis* and *B. bigemina* using PCR assays to detect kinete-specific protein (ksp). Of the total 598 ticks tested for the presence of *B. bovis* and *B. bigemina*, 2 ticks tested positive for *B. bigemina* and zero tested positive for *B. bovis*. The results indicate that while efforts have been successful to eradicate cattle fever from the U.S., sustained vigilance is necessary in terms of wildlife movement to prevent the reintroduction of these protozoa into the US mainland.

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

Cannabinoid vehicle effects on immune function in dog PBMCs

Matthew Mitsch, Karis Blankenship, Todd Archer, Barbara Kaplan

With more states each year passing laws legalizing the medicinal and recreational use of cannabis, the crucial plight to understand the effects of cannabis becoming increasingly prevalent. Due to the legalization of hemp, unregulated low-dose "nutraceuticals" of CBD such as treats, oils, tinctures, or topicals are being marketed for humans, canines, and felines with buyers wanting relief for their or their pet's anxiety, inflammation, or pain. However, it is currently unclear as to what immunological effects of cannabis treatment or consumption has in canines. This study aims to examine the effects of two plant-derived cannabinoids, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), on immune function in dog peripheral blood mononuclear cells (PBMCs). The

studies will first focus on cannabinoid effects on cytokine proteins; specifically levels of IL-2 and IFN-γ generated from activated canine PBMCs treated with CBD or THC and stimulated with phorbol ester plus calcium ionophore (P/I). Second, these studies will determine if delivery vehicle alters cannabinoid effects by comparing immune function effects of CBD and THC delivered in ethanol or dimethyl sulfoxide (DMSO). The results showed that there was a modest decrease in production**,** expression, and secretion of IL-2 with cannabinoids delivered in DMSO as compared to ethanol. There was also a modest decrease in the population of cells producing IFN-γ both with cannabinoids delivered in DMSO and with increasing concentrations of cannabinoids. Overall, there appears to be a difference in the efficacy of the cannabinoid effects on canine PBMCs depending on the vehicle used to deliver the compounds, suggesting that nutraceutical potency might vary depending on vehicle.

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Sheep immune response of *Culicoides* salivary proteins incorporated into inactivated BTV-17 vaccine
Nicholas Mosby, Jaime Rutter, Keun Seok Seo

Bluetongue virus (BTV) is an increasing concern with climate change, urbanization, and global trade. Unfortunately, implementing preemptive control measures proves challenging due to lack of vaccine cross-protection between serotypes and vector control. Research has shown that viral capsid proteins alter in the presence of proteases found in the arthropod saliva, thus creating more infectious sub-viral particles. Given that the primary vector for BTV is the *Culicoides* spp., this study compared the immune effects using an inactivated BTV-17 vaccine, with and without recombinant salivary proteins from *C. sonorensis* in Dorper sheep. Previously, researchers classified important members of *C. sonorensis*'s secretome: D7 odorant binding protein family, Kunitz-like serine protease inhibitor, and maltase. Blood collection occurred prior to treatment, 1 week post-initial dose, and 1 week post-booster dose. qRT-PCR was utilized to amplify cytokines genes depicting TH1 (IL-2, IFN-gamma, TNF-alpha) and TH2 (IL-6, IL-10) responses. The 3 TH1 cytokines analyzed were upregulated for both vaccinations; however, the addition of salivary protein had a 5 and 4 fold increase over the inactivated virus alone when analyzing the IL-2 and TNF-alpha responses, respectively. In comparison, the IFN-gamma results revealed the salivary proteins were upregulated only half that of the inactivated virus alone. For the TH2 response, IL-6 was upregulated a week after both vaccines with salivary proteins, while IL-10 was downregulated for both vaccination groups overall. In summary, the addition of salivary proteins to the inactivated BTV-17 vaccine depicted a proinflammatory response via the measured cytokines.

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Injectable in situ forming implant for sustained release of punicalagin as osteoarthritis therapy
Ashleigh Nicaise, Isaac Miller, Steve Elder

In the treatment of osteoarthritis (OA), intra-articular injection is advantageous because it maximizes drug activity in the joint while minimizing the risk of unwanted side effects to other organs. In situ forming implants have not yet been researched in connection with intra-articular OA therapy but are worth investigating due to the simple assembly and potential for sustained and tunable drug release. Punicalagin is a polyphenol derived from pomegranate (*Punica granatum L.*) and is an ideal candidate for a disease modifying OA drug used in this delivery system because of its anti-inflammatory and chondroprotective properties that could slow the progression of articular cartilage degeneration. Punicalagin inhibits activation of transcription factors in signaling pathways involved with synovial inflammation, as well as binds type II collagen and inhibits collagenase activity. This study aims to assess 1) the release kinetics of punicalagin in implants made from various combinations of polymer and solvent ratios, 2) punicalagin's ability to inhibit degeneration of cartilage in 0.025 mg/mL collagenase over 12 days, and 3) punicalagin's ability to attenuate LPS-stimulated production of IL-1β from human THP-1 differentiated macrophages. The results showed this drug delivery system was able to sustain release of punicalagin over several weeks and will likely extend to months. Punicalagin [100μM] significantly inhibited degeneration of cartilage compared to the control. These findings confirm the potential

benefit of utilizing in situ forming implants for sustained drug release and support further investigation of punicalagin releasing in situ forming implants as an intra-articular therapy for modifying early-stage OA.

Student Support: National Institute of Health T35OD010432

Tissue and plasma enzyme activities in a managed population of golden trevally (*Gnathanodon speciosus)*

Kathryn J. Rapp, Sean M. Perry, Alexa J. Delaune, Justin M. Stilwell

Veterinary care of aquatic species, particularly fish, is limited by a lack of knowledge regarding their unique physiology. Tissue and plasma enzymes are used in veterinary medicine for assessing function and potential damage to specific organs and tracking disease progression. The objective of this study was to identify tissue(s) of origin and plasma concentrations for specific enzymes in healthy golden trevally (*Gnathanodon speciosus*) fish. Our hypothesis assumed enzymes would exhibit tissue specific tropisms with higher activities in one or more tissues compared to others. Six fish were obtained from a managed population to obtain antemortem blood samples. The fish were then euthanized and tissue samples were collected via gross necropsy. Activities were examined for eight enzymes in plasma and ten tissues in each fish. Enzyme activities exhibited significant organ specificities. Aspartate aminotransferase, lactate dehydrogenase, and creatine kinase (CK) levels were highest in skeletal muscle with variably high CK levels in gonads. Alkaline phosphatase levels were highest in the kidney, spleen, and liver. Alanine aminotransferase levels had high specificity for the liver. Gamma-glutamyltransferase was only detectable in the kidney and plasma. Uric acid and blood urea nitrogen were below detectable limits in all samples. This work establishes baseline tissue enzyme origins for golden trevally fish, which will aid clinicians in diagnostic interpretation of serum chemistries and improve veterinary care for understudied fish species.

Student Support: Mississippi State University Global Center for Aquatic Health and Food Security

Creating a database for Kemp's ridley sea turtle strandings in the northern Gulf of Mexico

Hannah F. Renfroe and William B. Epperson

Kemp's ridley sea turtles are the most critically endangered species of sea turtle, and their natural range includes the northern Gulf of Mexico where they represent the majority of strandings. As sea turtles are important sentinel species and bioindicators for the health of the ocean, investigation of strandings, which remain largely unexplained, is pertinent in understanding threats these species face. The objective of this study was to construct a necropsy database for stranded sea turtles in the northern Gulf of Mexico and use the database to assess the ability to determine cause of death, discover relationships within the data, and develop recommendations for future investigators. Gross necropsy reports (n=190) were obtained from stranded sea turtles in Mississippi and Alabama. Microsoft Excel was used to create the database, summarize system findings of each necropsy, translate findings into descriptions of general pathological processes, and identify important systems and processes associated with stranding at a population level. Of the turtles that could be evaluated, most Kemp's ridley sea turtles that stranded between 2019-2020 had no evident abnormalities based on gross necropsy. These turtles were in adequate body condition, had evidence of recent feeding, and over half also had sand present in the respiratory tract, indicating aspiration of a sediment-rich medium prior to death. Commonly affected systems or cavities included the coelomic, digestive, and respiratory. Future recommendations include collecting more histopathology samples, involving veterinary pathologists in necropsies, and collecting stranded turtles more readily to mitigate the effects of decomposition on necropsy interpretation.

Student Support: National Fish and Wildlife Foundation under Mississippi Department of Environmental Quality–Task 3 and Mississippi State University Global Center for Aquatic Health and Food Security

Effect of COVID-19 on the dog populations in Mississippi animal shelters
Emily Sherman, Kimberly Woodruff, David Smith

Anecdotal reports indicated that many animal shelters received less dogs, with an increase in adoptions throughout the COVID-19 pandemic. However, current literature supporting these claims is lacking. The aim of this study was to determine if there is a relationship between the COVID-19 pandemic, changes in operational procedures, intake rates and outcomes of dogs. Dog outcomes were categorized into euthanized, transferred, returned to owner, and adopted for each year between 2017 and 2021. A survey was constructed and administered to 48 qualifying Mississippi animal shelters resulting in a 31.25% response rate. 93.33% reported at least one operational change during the pandemic with 13.33% reporting changes in source of funding. Using Tukey Kramer adjustment for multiple comparison, a statistically significant difference during the COVID 19 pandemic was seen for intake, transfer, and euthanasia. However, no statistical significance was present for adoptions. The rate of intake significantly decreased during 2020 along with euthanasia numbers, whereas transfer numbers significantly rose during 2020. Overall, shelters experienced many significant changes in operation, intake and outcome during the COVID-19 pandemic that were not seen prior to it.

Student Support: National Institute of Health T35OD010432

The future of food: the use of wild-caught mosquitoes as a quality feed protein
Sabrina Swistek, Kortnee VanDonge, Lee Cohnstaedt, Robert Ewing, Morgan Olmstead, Alina Delamota, Brenda Oppert, Phillip Shults

Interest has increased for the use of insects as a high-quality protein source in livestock production. At present, insect protein is used in feedstuff from farm-raised insects such as black soldier fly larvae, crickets, and mealworms. But what if there was a way to harvest wild-caught insect pests from natural environments to convert into feed protein? This would not only serve to provide a sustainable protein alternative but also as a pest control tactic where pest populations are abundant. However, using wild insects as a food source raises concern for potential pathogen transmission to animals, and in turn, presents zoonotic risks. To address these concerns, samples were treated with low-intensity drying (79.4C for 6 minutes) or high-intensity heat (204C for 45 seconds) to quantify surface and gut microbial disinfection methods.
Whole mosquitoes were used as the control treatment. Disinfectant treatments were plated and incubated to determine residual bacterial presence and microbial growth was observed on plates inoculated with whole mosquito (6/12), drying (8/12), and heat treatment (0/12). Shotgun sequencing plates prior to plating will allow broad-spectrum pathogen screening of wild mosquitoes and the use of and Sanger sequencing of plated colonies will help identify specific bacterial risks present in each treatment. This strategy provides the framework for additional research to optimize collection methods, further pathogen detection, and nutritional analysis of dried and heat-treated samples for industrial consideration.

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Evaluating the impact of shared decision making on pet-owner decisions
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Objective – To evaluate what impact shared decision making (SDM) during small animal wellness visits has on pet owner decisions and how it can be improved.
Design – Qualitative study of visit footage in a university small animal primary care setting.
Participants – 37 pet-owning clients and 8 small animal primary care veterinarians
Procedures – Thirty-seven small animal wellness visits seen at Mississippi State University College of Veterinary Medicine's Primary Care service containing decisions for non-core vaccinations and fecal flotations were audio- and video-recorded. Each decision was categorized depending on the decision type (fecal floatation or non-core vaccination) and decision outcome. The recordings of the conversation leading up to each decision were then individually coded using a veterinary-adapted Observer OPTION5 instrument to evaluate the level of shared decision making.

Results – The mean Observer OPTION5 score for non-core vaccination and fecal float decisions was 1.85 (0-20), indicating the use of very little SDM, and was lower than previous studies. The mean Observer OPTION5 score was higher for non-core vaccine decisions than fecal float decisions indicating more SDM surrounding non-core vaccination decisions. There was no statistically significant relationship between SDM level and decision outcome.
Conclusions – The results indicated that there is significant room for increased SDM for fecal floatation and non-core vaccinations during wellness visits. This is especially evident in the fecal floatation Observer OPTION5 scores. SDM is an important communication method that is underutilized in small animal wellness visits.

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Biomechanical evaluation of orthopedic cable compared to standard cerclage wire in a canine fracture model
Nicholas D. Wheeler, Barbara B. Bennett, Steven H. Elder, Michael H. Jaffe

Cerclage wire is frequently used in simple long oblique fracture repairs adjunct to other modalities. Loosening or breakage of the cerclage wire, and displacement of fragments are complications that may be observed as healing progresses due to excessive micromotion and loading of the bone during ambulation. This necessitates the identification of a repair modality for veterinary orthopedic traumas that maintains its integrity throughout postoperative recovery periods. As there is limited veterinary literature assessing orthopedic cable systems, the aim of this study is to evaluate the DePuy Synthes 316L stainless steel orthopedic cable system compared to standard cerclage wire in a twist knot configuration under monotonic tension and 4-point bending until failure. An ex vivo fracture model using paired canine cadaver tibiae was used to evaluate orthopedic cable compared to cerclage wire using identical sized wires and cable. Mechanical testing was performed cyclically under 4-point bending until implant failure. Focusing our monotonic results, we observed that orthopedic cable can withstand 1.76 times more force than cerclage, 1139N and 644.3N respectively. At cerclage wire's maximum load to failure, it's displacement measures 4.6mm whereas at the same force, orthopedic cable's average displacement is 3.7mm. On average, orthopedic cable can withstand forces from 4-point bending 41 times longer than cerclage before failing. Finally, orthopedic cable requires 4934Nmm of work to fail compared to 1755Nmm for cerclage. Our findings suggest the use of orthopedic cable is more advantageous as an adjunct repair modality for oblique long-bone fractures when compared to standard cerclage in a twist knot configuration.

Student Support: Mississippi State University College of Veterinary Medicine

Role of the triacylglycerol hydrolase enzyme carboxylesterase 1d in lung inflammation
Caitlin B. Wonnacott, Abdolsamad Borazjani, Oluwabori Adekanye, Matt K. Ross

Lung injury can be initiated by LPS leading to a buildup of lipid droplets in macrophages that contain polyunsaturated fatty acids (PUFAs). Murine carboxylesterase 1d (Ces1d) is associated with ER membranes in cells and it is the most abundant serine hydrolase in lung. Ces1d has a role in the catabolism of triacylglycerols (TAGs), but there is a knowledge gap regarding its role in tissue injury. We hypothesized that LPS-induced inflammation will increase oxidized (ox)TAGs in lung, and if Ces1d is inactivated the levels of oxPUFAs released from oxTAGs will decrease, thus mitigating inflammation. The objectives were to (1) determine the levels of inflammatory molecules following in vivo exposure to LPS when Ces1d is active and inactive, and (2) examine Ces1d's ability to release PUFAs and oxylipins from synthetic oxTAG in vitro. Intranasal LPS or saline was administered to male wildtype (WT) and Ces1d knockout (KO) mice (n=5/group). BALF and lungs were collected at 6 h and lipid mediators and Il-1β levels determined. PGE_2 levels were unchanged in WT BALF following LPS treatment, whereas they increased 10-fold in KO BALF. This difference also correlated with increases in lung Il1-β in KO lung. When synthetic oxTAG 18:2 (containing 9- and 13-HODE) was incubated with lung membrane fractions from WT and KO mice, the KO lungs exhibited slower rates of HODE release than WT lungs. However, levels of HETEs and HODEs (both oxPUFAs) were unaltered by LPS in lungs of

either genotype, indicating that the absence of Ces1d did not reduce oxPUFAs. Indeed, Ces1d deficiency appeared to make these mice more sensitive to LPS. Thus, we need to revisit our hypothesis to consider other possible TAG hydrolases that might alleviate inflammation.

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