**2021 Summer Research Abstracts**

**Bladder epithelial modifications and biofilm formation in chronic urinary tract infections**

Mary Catherine Beard, Elizabeth Swanson, and Orion Rivers

Chronic and antibiotic-resistant urinary tract infections (UTIs) are a widespread complication in human and canine patients and can lead to life-threatening bacteriaemia if left untreated. Certain factors are known to increase the risk of chronic UTIs, such as long-term catheterization and diabetes mellitus. *Enterococcus faecalis* is a multi-drug resistant and biofilm-forming pathogen commonly isolated in these patients with chronic UTIs. Current research lacks understanding in how the urothelium in chronic UTI patients is altered such that biofilm colonization is possible. The objective of this study focused on identifying epithelial morphology of bladder tissue exposed to catheter trauma and/or diabetic-level urine glucose concentrations. It also aimed to determine whether *E. faecalis* biofilms grown *in vitro* were stimulated by high glucose concentrations. Biofilms were grown using a constant-flow bioreactor and measured using confocal laser scanning microscopy. Epithelial morphology was evaluated using scanning and transmission electron microscopy. It was found that catheter damage and/or high glucose concentrations altered the membrane morphology of cells, and significantly disrupted the epithelial barrier. It was also noted that the thickness of *E. faecalis* biofilms grown in glucose-enriched media was less than that of biofilms grown in standard media. Overall, the findings demonstrated that catheterization and diabetic-level urine glucose compromise the protective urothelial barrier, however high glucose concentrations may not be the sole cause of biofilm stimulation in diabetic patients.

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**Spectroscopic Characterization of Collagen and Associated Biomechanical Changes in Alcohol- Induced Osteoporosis in Mice**

Allison Brunner, Gombojav Ariunbold, Lauren Priddy, Haifeng Wang, and Michael Jaffe

Osteoporosis as a disease characterized by decreased bone mass and deterioration of bone microarchitecture of which more than 75 million people worldwide suffer. Secondary osteoporosis is predisposed by risk factors including alcohol abuse. There is little that is known about the role of alcoholism on the collagen content and mineral composition of the bone and its biomechanical properties. The aim of this pilot study is to determine the difference in collagen content of the bone of genetically similar but phenotypically different BXD mice. Raman spectroscopy was used to identify the collagen in the bone, and the Keyence system allowed us to quantify surface roughness of the BXD mice bones. The results are still being collected and data analyzation is an ongoing process. It is suspected that there are collagen content differences between the BXD mice. The data collected from this study is preliminary data to be used in distinguishing biomechanical, histopathological, and spectroscopic properties of a variety of collagen-containing tissue types in a mouse model of osteoporosis secondary to chronic alcohol abuse for future studies.

Student Support: Mississippi State University College of Veterinary Medicine

**Transfection of bovine preputial keratinocytes for expression of antibody against *Tritrichomonas foetus***

Lauren Ellison, Ella Swales, Merrilee Thoresen, E. Heath King, Darcie Sidelinger, Daryll Vannover, Hannah Peck, Philip J. Santangelo, Amelia R. Woolums

mRNA therapy, which induces cells to produce proteins, has recently come into focus as a possible treatment for many diseases. The method is potentially powerful because synthetic mRNA can theoretically code for any protein a cell can produce. *Tritrichomonas foetus*, despite having a large economic impact on cattle production, does not have an approved effective treatment. Synthetic mRNA therapy to induce protective antibodies on bovine preputial epithelium could offer a solution. mRNA was obtained for the TF1.15 and TF 1.17 antibodies that have been shown to bind to 2 different epitopes of a *T. foetus* attachment protein. *An* *in vitro* transfection methodology with bovine primary preputial keratinocytes (PPK) was used to determine the synthetic RNA concentration (1µg, 2µg, or 4µg), time from transfection to *T. foetus* exposure (24 or 48 hours), and mRNA construct (TF 1.15 or TF1.17) that produced a sufficient amount of antibody to bind *T. foetus*. Antibody production was quantified using immunofluorescence (IFA) to count the proportion of PPK producing antibody. IFA was also used to show that antibodies produced by transfected PPK bound to *T. foetus.* Antibody production in PPK cytoplasm was greatest at 24 hours, while more antibody was present in PPK culture supernatants at 48 hours. IFA demonstrated binding of expressed antibody to *T. foetus.* The optimized transfection conditions were 1µg of RNA, introduction of *T. foetus* after 24 hours of transfection, and the TF1.15 antibody construct. Further work to determine if binding of antibody impairs *T. foetus* attachment and pathogenicity is underway.

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**Histologic Classification of *Tursiops truncatus* Regarding Age and Freshwater Lesions**

Ian J Evans, Christa Barrett, Debra Moore, Timothy W Morgan

In 2019, the Bonnet Carré spillway and diversions for the Mississippi River released trillions of gallons of contaminated freshwater, from 31 states and 2 Canadian provinces that drain numerous agricultural and industrial sites, into the Mississippi Sound. This caused a marked drop in the salinity of the Mississippi Sound as well as an influx of novel bacteria and fungi that contributed to increased Bottlenose Dolphin mortality and led to the declaration of an Unusual Mortality Event (UME) by the National Oceanic and Atmospheric Administration (NOAA). To better understand the effects of fresh water incursion on dolphin mortality, it is important to determine if certain lesions correlate with known causes of mortality such as sepsis. Equally important is to determine how age correlates with morbidity and mortality in fresh water incursions.  With this in mind, we studied decalcification methods (EDTA and Kristenson's) for *Tursiops truncatus* teeth to determine if these methods result in increased conservation of histologic structure and clarity in the resulting dental specimens to help with aging the specimens by counting Growth Layer Groups present in the dentin. Additionally, we classified freshwater lesions histologically from stranded dolphins from the Mississippi Gulf Coast during the UME in 2019 and tested for any association between the presence of certain freshwater lesions and other known signs of sepsis, such as hepatic extra-medullary hematopoiesis (EMH) or pneumonia.

Student Support: Mississippi State University College of Veterinary Medicine

**Development of Evidence Based Antibiotic Stewardship Recommendations For Empiric Canine Skin Infections**

Kyle Ferguson, Keun Seok Seo, Margaret Khaitsa, Cory Langston, Cooper Brookshire

Antibiograms are usually compiled from bacterial culture and antibiotic susceptibility (BCAS) test results from infections that clinicians submit for testing. These data may be biased towards antibiotic resistant, refractory, or otherwise severe infections. There is a need to perform antibiotic susceptibility testing surveillance for infections that are typically treated empirically. This study aimed to create an antibiogram representative of canine skin infections typically treated empirically that included chlorhexidine susceptibility testing. Chlorhexidine topical therapy is the most commonly recommended treatment for canine skin infections, but routine BCAS tests never assess chlorhexidine susceptibility. Twenty-four staphylococcal isolates were collected from canine skin infections from patients without a history of antimicrobial resistant infections and no history of antibiotic administration in the previous 6 months. All 24 isolates were susceptible to commonly recommended first-line antibiotics. Chlorhexidine susceptibility was determined using a hybrid MIC/Kirby Bauer drop-test; 6 μL of chlorhexidine solution at various concentrations around the epidemiologic cutoff value of 8 μg/ml were placed on Mueller-Hinton agar plates that were inoculated with each isolate. All 24 isolates were inhibited by 8 μg/ml chlorhexidine.  These data suggest that chlorhexidine resistance is uncommon, and currently recommended first line antibiotics are appropriate for empiric treatment when there is a low clinical suspicion of resistance. Owing to reduced selection pressure for extended spectrum betalactamase resistance in bystander bacteria, clindamycin should be considered the preferred first-line therapy.

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

**Pressure-induced wound formation and healing with *Staphylococcus aureus* infection in a diabetic mouse model**

Carly Grabner, Reagan McDevitt, Keun Seok Seo, Elizabeth Swanson, George E. Howell III

Approximately 25% of type 2 diabetics will develop foot ulcers in their lifetime. Roughly 60% of ulcers will become infected and 15-20% of ulcers will end in limb amputation. Infection of diabetic foot ulcers by *Staphylococcus aureus* is the leading cause of lower limb amputation. Therefore, the present study was designed to explore the wound healing kinetics of a polygenic model of type 2 diabetes, the TALLYHO mouse, during active infection with methicillin resistant *S. aureus* (MRSA). Pressure wound size, as well as the local effects of MRSA on the wounds, was compared between the TALLYHO mouse and a lean control, the SWR mouse. Both mouse strains were subjected to a wounding procedure using ceramic magnets consisting of 3-12 hour on/off cycles then the wounds were inoculated with MRSA (USA300 strain) and followed for 10 days. During wound formation both strains developed similar wound sizes. The TALLYHO had significantly larger wounds compared to the SWR at days 3 and 7 post-inoculation. The TALLYHO in the cohort with more pronounced hyperglycemia had a significantly greater amount of *S. aureus* present at day 10 compared to the SWR.​ At day 10, the TALLYHO had greater expression of the macrophage marker *F4/80* compared to SWR.​ Expression of wound healing markers displayed an overall decrease in TALLYHO compared to SWR, with *Mmp13* being significantly decreased. These data suggest that the diabetic mouse model developed more significant wounds compared to the normal mouse model, as well as a diminished healing response due to the bacterial infection. Future plans for the study will explore the effects of exposure to persistent organic pollutants on the wound healing parameters used in the present study.

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**Immunomodulation from epigenetic reprogramming improves health and production efficiency in aquaculture**

Hannah Knight, Beth Peterman and Lora Petrie-Hanson

The aquaculture industry is reliant on efficient production along with quality of the fish produced. In intensive systems, maintaining and monitoring health is challenging. Implementing new methods of protective immunity would be beneficial to the industry. Exposure to beta-glucan provides a broad-based immunity to certain pathogens that can be equivalent to vaccination. The functional changes are due to epigenetic reprogramming and are termed trained immunity. To analyze this immunomodulation, we injected mutant zebrafish with beta-glucan, a characterized immune stimulant. Two weeks after injection, innate immune cells were isolated, and nucleic acids extracted. RNA-seq and ChIP-seq were performed. 1267 differentially expressed genes were entered in KEGG pathway analysis. Cytokine to cytokine receptor interaction, oxidative phosphorylation, phagosome and arginine and proline metabolism pathways were highly significant. Our findings suggest that innate immune cells do undergo functional changes resulting in increased metabolic responses and shifts to aerobic glycolysis. This provides effective elimination of pathogens and immune regulation. Further, significant upregulation of epigenetic regulators, such as arginine, suggest specific histone and chromosomal changes related to immunomodulation. Our findings demonstrate the strong and focused effects indicative of trained immunity induction. Using beta-glucan as a food additive in the aquaculture industry can also combat antimicrobial resistance, by replacing antibiotic use, and optimizing overall health of production systems.

Student Support: Foundation for Food and Agriculture Research (FFAR)

**Method optimization for studying effects of cannabinoids on canine PBMC immune responses**

Madison Mertz, Todd Archer, and Barbara Kaplan

In recent years, cannabinoid-containing products have grown increasingly popular for their putative anti-inflammatory properties, but limited conclusive data have been gathered to demonstrate their efficacy or consistency. As veterinarians face a clientele which is gaining interest and access to these products, they require further data on the response of the canine patient. It is commonly assumed cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD) are immune suppressive and prior studies from this lab have shown inhibition of interleukin-2 (IL-2) and interferon gamma (IFN-γ) mRNA in canine peripheral blood mononuclear cells (PBMCs) by THC. The goals of this study were to optimize new methods to detect cannabinoid effects on canine PBMCs and to characterize the effect of cannabinoids on canine PBMC immune function. We hypothesized that CBD and THC would downregulate IL-2 and IFN-γ protein production. Our initial results showed that while there was little cross reactivity of human antibodies with canine proteins, human anti-CD3 stimulated cells better than canine anti-CD3. Perhaps most importantly, our data from flow cytometry showed that both CBD and THC induced statistically significant increases or decreases in cytokine production across various conditions. Together these data suggest that cannabinoids must be considered ‘immunomodulatory’, rather than strictly ‘immunosuppressive’, and that the immune response to cannabinoids may be patient- or condition-specific, but more detailed studies are required to further characterize the effects. With better understanding, clinicians can more confidently advise their clients on potential cannabinoid benefits (or lack thereof).

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**Microplastics and per- and polyfluoroalkyl substances (PFAS) in bottlenose dolphins along Mississippi’s coast**

Hannah Pray, Chanaka Navarathna, Natalie Hampton, Todd Mlsna, Debra Moore, Stephen Reichley

With global plastic usage increasing annually, plastic pollutants, namely microplastics (≤5 mm), are a growing concern. Microplastics have been shown to harbor and release harmful chemicals, such as per- and polyfluoroalkyl substances (PFAS), acquired from the environment. Microplastics have also been shown to accumulate in top marine predators, such as cetaceans. The aim of this study is to analyze the stomach and intestinal contents of bottlenose dolphins stranded along the Mississippi coast for microplastics and PFOS, a common PFAS. Gastrointestinal contents were digested with 10% KOH in 50% MeOH then analyzed for microplastics using Nile red microscopy, pyrolysis gas chromatography-mass spectrometry (Pyro-GC-MS), and Raman spectroscopy. Filtrate from digested samples were pre-concentrated using solid-phase extraction (SPE) and analyzed for PFOS with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The PFOS extraction and analysis was validated with pike perch fish certified reference material, which had a recovery of 98.6%. The Pyro-GC-MS results for two samples showed the presence of acetamide, which is used as a plasticizer. Raman spectroscopy results also showed characteristic plastic peaks corresponding to those of polystyrene in one sample. Additionally, PFOS was detected in three samples ranging from 95.55 to 1,934.56 µg/kg. The results show that three samples potentially contain microplastics and three samples contain detectable levels of PFOS, while the majority of samples were negative for both microplastics and PFOS. This project is part of a long-term study that will continue in order to better understand microplastics and PFAS within the environment and their impact on bottlenose dolphins.

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**Prevalence of TB and FMDV Among Animals Selected from Slaughterhouses in Mbarara District in Western Uganda**

Braiam Rosado-Ramos, Peter Kalumba, Robinah Sarah Nakabuye, Andrew Rwotomara, Susan Kerfua,Patrick Emudong, Solome Namirimu, Celsus Sente, Cooper Brookshire, Margaret Khaitsa

Bovine tuberculosis (TB) is a public health concern particularly in developing countries, such as Uganda. *Mycobacterium bovis*, has an extremely wide host range, spread by ingestion or inhalation of animal bioproducts like meat and/or milk. The lack of an effective control policy in Uganda and the livestock-wildlife interface raises concerns about other diseases of great economic or public health concern, such as Foot and Mouth Disease (FMD). FMD affects all cloven-footed animals, transmitted via aerosols, fomites and body fluids. The objectives of this study were to investigate the potential of using slaughterhouses in Uganda as bio-surveillance points for TB and to assess the effectiveness of the 2021 FMD outbreak response following a partial animal movement restriction. To estimate the prevalence of TB among cattle, sheep, and goats slaughtered in Mbarara District, we utilized evaluation of gross lesions, Ziehl-Neelsen (ZN) staining, and PCR surveillance. Fifty-six animals from Mbarara district abattoirs were randomly selected, and lung/lymph node smear impressions were performed for ZN analyses. Blood, lung, and lymph node tissues were collected for PCR analyses. The gross lesion evaluation revealed 2 suspicious lung tissue cases (goat and sheep) while the ZN staining revealed six positive cases of TB (3 goats, 2 cows and 1 sheep) from lymph node and/or lung tissues. PCR analyses are currently pending. Forty-seven apparently healthy Ankole beef cattle from a ranch and 15 beef cattle presented for slaughter at the Mbarara municipality abattoir were tested for FMD seropositivity utilizing an FMDV 3ABC ELISA test. Seropositivity rates of 93.6% (95% CI; 0.83-0.98) and 53% (95% CI; 0.3-0.75) were detected from the ranch and slaughterhouse, respectively. None of the 20 sheep and goats tested at the Mbarara municipality slaughterhouse were seropositive for FMD.

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

**A survey of parasites affecting wild fish populations in lakes across Uganda:**

Madison Rawdon, T. Graham Rosser, Stephen Reichley, David Kahwa, Margaret Khaitsa, and Robinah Nakabuye

Parasites affect wild and farmed fish populations on a global scale. Infection can be characterized by reduction in growth, reproduction, and quality of fish products. Many organs including gills, fins, gastrointestinal tract, muscles, and nervous system may be sites of development. Parasitic infections are particularly devastating in cultured fish populations where stressful conditions associated with water quality and crowding exacerbate disease. Our overall goal is to establish baseline prevalence data for parasites infecting wild fish populations in Uganda. Wild caught fish were collected from fish landing sites at Kisenyi, Lake Edward; Rubare, Lake Mburo; and Ggaba, Lake Victoria. Gill parasites were observed in 54.5% (18/33), 0.0% (0/15), and 50.0% (14/28) of fish at lakes Edward, Mburo, and Victoria respectively. Internal parasites were observed in 24.0% (6/24), 13.3% (3/15), and 10.7% (3/28) of fish in lakes Edward, Mburo, and Victoria. Parasites present in the gills included metacercariae and adult trematodes, myxozoan plasmodia, copepods, and motile and sessile ciliates. Parasites present in the gastrointestinal tracts included trematodes, nematodes, and myxozoan plasmodia. Currently, morphological and molecular characterizations are being made of the parasites collected. A *Henneguya* sp. found in the pyloric cecum of a Nile perch has been characterized morphologically and sequenced at the 18S rRNA subunit and is suspected to be a novel species. The molecular and morphological data collected from these parasites can be used in future studies to complete parasitic life cycles as well as develop rapid diagnostic PCR assays for parasites found to be serious pathogens in Uganda’s growing aquaculture system.

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

**Parasites in Captive Felids at the Uganda Wildlife Education Center and Their Zoonotic Implications**

Kiera Reardon, Margaret Khaitsa, Celsus Sente, Delilah Namara, Rebecca Nakato, Rachael Mbabazi, James Watuwa, Victor Musiime, Jackson Bwambale Kananga, David Musingo, James Musinguzi

As endangered animal species continue to decline, zoos are serving an important role by creating reservoir populations of these animals. Keeping animals in captivity causes challenges such as zoonoses and drug resistance in pathogenic organisms. This study investigated occurrence of parasites found in captive felids and their implications on public health. The Uganda Wildlife Education Center (UWEC-Zoo) was the study site, and their animals are acquired through rescue. The hypothesis was that parasite management at UWEC-Zoo is effective thereby resulting in a low prevalence of parasites in their captive felids. Two data sources were utilized: cross-sectional data (June 2021) and retrospective data (2005-2020). For the cross-sectional study, fresh fecal samples were taken from 13 felids (8 lions, 2 tigers, 2 cheetahs, and a leopard). The sampling was repeated twice a week for three weeks. For each fecal sample, wet mounts and sedimentation assays were performed. Retrospective data from 2005-2020 were obtained from UWEC-Zoo and utilized in the study. Felids or feliform species sampled included: serval cats, lions, leopards, hyenas, cheetahs, caracals, and black backed jackals. *Toxocara sp.* and *Cystoisospora sp.* were recovered from the cross-sectional study while retrospective data reported *Ascarid sp., Strongyle sp., Ancylostoma sp., Altenanz sp., Toxocara sp., Amoeba spp., Isospora spp., Taenia spp.,* Tapeworm sp.*, Oesophagostomum sp., and Coccidia spp*. The presence of parasites in captive felids at UWEC-Zoo implied that parasite management was not as effective as previously thought. Also, presence of *Toxocara spp.* with zoonotic potential was a significant finding that warrants further study.

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

**The effect of *Fusarium* spp. on cold-stunned Kemp’s ridley sea turtles**

Jessica F. Sparks, John Thomason, Angela Knight, Debra Moore, Stephen Reichley, Christa Barret, Hossam Abdelhamed, Michelle Banes, and Mark L. Lawrence

*Fusarium* is a filamentous fungus found worldwide that can cause animal and human infections. In sea turtles, it causes sea turtle egg mortalities and infects juveniles and adults. We hypothesized that *Fusarium* spp. cause respiratory infections that have not cleared after administration of antibiotics and carapace lesions in cold-stunned Kemp’s ridley sea turtles (*Lepidochelys kempi)*. In December 2020, twelve cold-stunned Kemp’s ridley sea turtles were rescued and transported to the Institute for Marine Mammal Studies in Gulfport, Mississippi. Of the twelve turtles, two were unresponsive to antimicrobials administered to treat respiratory inflammation; one of these also had a circular, well-demarcated shell lesion. To determine whether *Fusarium* spp. is associated with respiratory infection and carapace lesions, as well as to determine a baseline of the normal flora found on Kemp’s ridley sea turtles, fungal cultures were collected from five different sites of these two turtles. The five sites included bronchoalveolar lavage (BAL), blood, carapace, cloaca, and nasal cavity. The samples taken were inoculated onto three different types of media. For 25 days, fungal growth was monitored macroscopically by phenotypic identification and microscopically by morphology of the conidia. *Fusarium* spp. was identified from the carapace lesion of one turtle, but it was not isolated from BAL. To confirm the identification of the fungus, DNA extraction and PCR were conducted, and sequencing is being conducted. Other fungal species were isolated, with *Penicillium* being the most common species from all sites. Fungal cultures from ten other turtles are pending.

Student Support: Mississippi State University College of Veterinary Medicine

**Effect of mRNA-expressed antibodies on *in vitro* attachment of *T. foetus* to bovine preputial keratinocytes**

Ella Swales, Lauren Ellison, Merrilee Thoresen, E. Heath King, Darcie Sidelinger, Daryll Vannover, Hannah Peck, Philip J. Santangelo, Amelia R. Woolums

The protozoal parasite *Tritrichomonas foetus* (Tf) infects the preputial epithelium of bulls. Infected bulls are asymptomatic carriers that cause recurring infections in cows, leading to costly reproductive failure. TF1.17, a Tf cell surface antigen, is integral for attachment to bovine preputial epithelium. We hypothesized that transfection of preputial epithelium with synthetic mRNA encoding antibodies against TF1.17 could help bulls resist or clear Tf infection. To establish conditions for this novel treatment, the objectives were 1) optimize fluorescent staining of Tf, 2) determine the optimal number of Tf supporting accurate quantification in cell attachment assays, and 3) assess attachment of Tf to primary preputial keratinocytes (PPK) transfected with mRNA encoding antibody (IgG1) to TF1.17.

Tf grown to 24-hour logarithmic phase were stained with CellTrace™ CFSE or ViaFluor® 405 SE fluorescent stains at 5 μM, 10 μM, 15 μM, or 20 μM. Fluorescence was assessed at 3, 6, 12, and 24 hours. Zero, 250,000, 500,000, or 1,000,000 stained Tf were added to bovine kidney (BK) cells or transfected PPK in triplicate wells of 6-well plates. At 6, 12, 24, and 48 hours unattached Tf were washed off and attached Tf in 6 fields of each well were counted. Ten μM CellTrace™ CFSE provided the best staining for the necessary duration with least cytotoxicity. 500,000 Tf per well was optimal for attachment assays. Tf attachment to transfected cells was not clearly decreased, but attachment variability complicated assessment. Work is ongoing to optimize assessment of the effect of expressed antibody on Tf attachment.

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**Efficacy of chitosan and polycaprolactone biomaterials as antibiotic delivery vehicles in osteomyelitis**

Haley Zetterholm, Luke Tucker, Malley Gautreaux, Xavier Person, and Lauren B. Priddy

Osteomyelitis, an infection of bone, is often caused by *Staphylococcus aureus* and poses a significant threat following traumatic fractures or orthopedic hardware implantation. Current treatment modalities include systemic antibiotic therapy and debridement surgery; there are no local antibiotic therapies that persist long enough to clear infection. Relapses frequently occur, sometimes leading to limb amputation. The aim of this pilot study was to evaluate the ability of chitosan-based local delivery vehicles to reduce bacterial load in a rat osteomyelitis model. Twelve female CD rats were assigned to three groups (n=4): chitosan gel (CH), chitosan-fosfomycin gel (CH-FOS), or chitosan-fosfomycin gel surrounded by a polycaprolactone scaffold (CH-FOS-PCL). FOS dose was 3 mg. Osteomyelitis was induced via placement of an ATCC 6538-GFP *S. aureus*-soaked screw into a bicortical defect in the mid-diaphysis of the femur. Blood and urine samples were taken periodically starting at Day -1. Bone and soft tissue samples were collected at Day 35 to evaluate bacterial load. Four rats (1 CH-FOS, 3 CH-FOS-PCL) experienced spontaneous femur fractures and were euthanized early. Bone CFU/mL was highest in the CH group and lowest in the CH-FOS group, while soft tissue CFU/mL was highest in the CH-FOS-PCL group and lowest in the CH-FOS group, though differences were not statistically significant. ATCC 6538-GFP was not detected in blood at any time point. The plasma was frozen and will be used to measure infection markers. Urine will be evaluated for fosfomycin to determine elution over time. Future work will further examine chitosan-fosfomycin gel as a local antimicrobial therapy.

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