**2020 Summer Research Abstracts**

**Human cathelicidin LL37 effects on *Mannheimia haemolytica***

Cassandra D. Barber, Amelia R. Woolums, Merrilee Thoresen, and Philip J. Santangelo

Cathelicidins are molecules of current interest due to their antimicrobial and immune stimulating effects plus a potential for prevention and treatment of many diseases. A novel way to combat Bovine Respiratory Disease (BRD), the leading cause of morbidity and mortality in beef cattle, could be through induction of cathelicidin expression at the site of infection through messenger RNA (mRNA) therapy. The hypothesis was that transfected bovine cells will produce human cathelicidin LL37, then export it into the supernatant where it will have in vitro antimicrobial activity against *Mannheimia haemolytica*, a bacterial BRD pathogen. The methodology used was in vitro transfection of bovine cells with mRNA of green fluorescent protein (GFP, a positive control) or LL37 as well as no mRNA (negative control). At 4, 8, 12, and 24 hours, an immunofluorescence assay (IFA) was performed and the supernatants and lysates of the culture were collected, which were then used to treat two concentrations of *M. haemolytica*.  Results yielded successful transfection with GFP and LL37 (as indicated by green fluorescence and cytopathic effects, respectively), inconclusive IFA due to nonspecific staining in negative controls, and no difference observed of any treatments to *M. haemolytica* cultures. Possible reasons for failure of LL37 to inactivate *M. haemolytica* include: LL37 was not able to target *M. haemolytica*, too low of LL37 concentration used, or insertion of LL37 into the eukaryotic membrane, rendering it inactive. Future studies will focus on testing bovine cathelicidins against *M. haemolytica* and other BRD microbes.

Student Support: Mississippi State University College of Veterinary Medicine

**The effects of microcystin-LR on channel catfish immune cell functions**

Courtney Baugher, Beth Peterman, and Lora Petrie-Hanson

Deaths and reduced growth associated with infectious diseases greatly impact the channel catfish industry. Bacterial disease outbreaks often occur when fish are approaching harvest size. Because these diseases are endemic in most catfish ponds, it would be expected that by this point in production the fish would have already been exposed to and developed protective immunity to these diseases. Toxins produced by the microbial community have been reported to directly kill fish in commercial catfish ponds. Cyanobacterial blooms are common in catfish ponds in the summer, and the most common toxin produced by cyanobacteria are microcystins. The goal of this study was to determine if microcystin Leucine-Arginine (MC-LR), the primary compound produced by *Microcystis* algae, changes the immune functions of channel catfish leukocytes by determining the effects of MC-LR on endocytic mechanisms of channel catfish phagocytic cells. The hypothesis of this study was that MC-LR exposure will alter the ability of channel catfish leukocytes to phagocytose particles and *Edwardsiella ictaluri,*the causative agent of Enteric Septicemia of Catfish (ESC). The results demonstrated that MC-LR in concentrations of 0.01 ug/ml and 0.1 ug/ml significantly reduced phagocyte uptake by various endocytic mechanisms. Furthermore, MC-LR negatively impacts the Ca2+ dependent receptor-mediated uptake by phagocytes and negatively impacts uptake functions via the mannose receptor. MC-LR reduces the ability of phagocytes to uptake *E. ictaluri,* and this could increase fish susceptibility to ESC*.* Overall, the findings demonstrated that MC-LR can have an effect on the immune functions of channel catfish by impacting the endocytic mechanisms of phagocytic cells.

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

**Effects of cannabinoids on TNF-α in mouse splenocytes in response to various stimuli as a pre-clinical model for canine immune function**

Carly Campbell, Abbey Fleming, and Barbara Kaplan

Marijuana and its components, cannabidiol (CBD) and D9-tetrahydrocannabinol (THC), have grown in popularity in the last decade, even for use in veterinary medicine. CBD has been promoted as an anti-inflammatory compound that might have a role in treating inflammatory conditions. Here we hoped to associate the anti-inflammatory properties of CBD with a reduction in pain experienced by mice. Initial studies showed CBD inhibited TNF-α production in response to various stimulation conditions with one exception. The one case of CBD-induced TNF-α elevation led us to the concern that TNF- α might not always be anti-inflammatory and analgesic.  Thus, the purpose of our studies was to evaluate the effect of CBD on TNF-α levels in response to various levels and types of immune stimuli. We first examined mouse splenoctyes treated with vehicle (0.1% ethanol) or CBD (10 mM) followed by stimulation with two levels of anti-CD3/CD28 antibodies. Next we compared splenocytes treated with CBD and stimulated with two levels of anti-CD3/CD28 antibodies or Dynabeads. In both experiments, CBD inhibited TNF-α production when stimulation was robust, but CBD had no effect on lower magnitude of stimulation when stimulation alone did not produce TNF-α.  To evaluate the effect of CBD on the pain response, studies were initiated using a von Frey apparatus. Using various filaments, healthy mice were probed to establish a pain evaluation protocol. Overall, these studies showed significant inhibition of TNF-α  by CBD and have allowed us to establish the pain evaluation protocol in mice. Results from these studies can be used to develop similar studies using dog lymphocytes and to examine efficacy of CBD in canine osteoarthritis.

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**Descriptive Analysis of Bottlenose Dolphin Strandings in the Gulf of Mexico, 2009-2019**

Annie Carrigan and William B. Epperson

Over the past decade, cetacean strandings occurring among the widely established bottlenose dolphin (*Tursiops truncates*) population in the Gulf of Mexico (GoM) have raised public concern about the sustainability of this species and the environmental impact of human activity in this region. To evaluate dolphin stranding trends, records of 4,286 Bottlenose dolphin strandings between 2009-2019 were obtained from the Marine Mammal Health and Stranding Response Program National Database and used for descriptive analysis to identify temporal and spatial clusters. Areas around Galveston, TX, the Mississippi Sound, and Barataria Bay, LA displayed large stranding clusters. Strandings displayed a strong seasonal pattern. In association with the spring calving season, over 1,700 strandings were recorded, peaking in March. Winter months recorded 1,400 strandings, peaking in February. Only 28% of strandings occurred in the 6 months of summer and fall (June through November). Along with stranding hotspots and seasonal trends, this dataset portrayed several large-scale mortality years, focused largely on the LA and MS coastlines. Compared to the yearly mean 393 strandings, 2011 (512) and 2019 (571) were significantly elevated. Strandings in 2011 were in co-occurrence with the 2010 Deep Water Horizon oil spill. Following 2011, strandings declined to a low of fewer than 300 in 2015. Thereafter, strandings increased, peaking in 2019 (571) in association with hyposalinity due to freshwater incursion from the Mississippi River system. This work indicates that specific geographic regions and seasonal patterns are associated with recent dolphin stranding and may offer information to allow focused effort to enhance well-being and sustainability of Bottlenose dolphin populations in coastal waters of the GoM.

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

**Life-cycle elucidation of *Hysteromorpha* sp. in ictalurid catfish production systems in Mississippi, USA**

Caroline D. Coussens, Ethan T. Woodyard, MacKenzie A. Gunn, D. Tommy King, Brad Richardson, Lauren Easter, Jonah Nguyen, Matt J. Griffin, David J. Wise, and T. Graham Rosser

Ictalurid catfish production is the largest aquaculture industry in the United States, with over half of production localized in Mississippi. Two major challenges facing the industry are the consumption of fish and the introduction of trematode parasites by piscivorous birds. Management of trematode infections is dependent on identifying all hosts in the life cycle, as there are no approved antiparasitic medications. In Summer 2019, the MSU Aquatic Parasitology Lab observed reduced feeding activity in catfish infected with a *Hysteromorpha* species. The purpose of this project was to determine the life cycle of *Hysteromorpha* sp. in Mississippi, so that catfish producers could develop management strategies targeting the snail host. Double crested cormorant (*n*=26) small intestines were screened for adult *Hysteromorpha*, while fish from the original *Hysteromorpha* outbreak were necropsied to recover metacercaria. Snails collected from catfish ponds were screened for the shedding of cercaria. Each life stage was morphologically and molecularly characterized by sequencing mitochondrial and ribosomal gene regions for species identification and life cycle elucidation. Channel catfish fingerlings were challenged with cercaria recovered from shedding snails. Evaluation of mitochondrial cytochrome *c* oxidase subunit 1 data identified our specimens as *Hysteromorpha corti*. Sequence data indicated conspecificity of all life stages and implicated *Planorbella trivolvis* as the snail host for *H. corti*. Molecular analysis revealed the cercariae used in catfish challenges as a separate diplostomid species and future research will focus on identifying these, as well as investigating pathogenicity of *H. corti*.

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**Establishment of a Cell Culture Model Using Whole Lung to Study Effects of Cannabinoids**

Abbey Fleming, Barbara Kaplan, Michael Jaffe, and Carly Campbell

Vaping with marijuana or cannabidiol (CBD) is a big trend right now, and many studies show that cannabinoids have an anti-inflammatory effect. Often cannabinoids are studied in single cell type cultures, so we wanted to establish a whole lung ex vivo model to study effects of cannabinoids in the presence of the different cells within the lungs. We became interested in this topic based on previous results in which a single cell suspension of the lung was prepared simply by mashing the tissue through a filter and noting that the cells produced TNF-α in response to lipopolysaccharide (LPS). We therefore hypothesized that cannabinoids would inhibit TNF-α production in this multicellular lung culture. First, we used flow cytometry and found that the most prevalent cell type was macrophages, but there were also B cells, neutrophils, and CD4 and CD8 T cells. Next, we examined TNF-α production in response to different concentrations of LPS and cannabinoids. There was no difference in TNF-α production by 1 or 10 µg/ml LPS. CBD (0.5-10 µM) progressively inhibited the production of LPS-stimulated TNF-α. Interestingly, the cells were more reactive to LPS and cannabinoids only if a centrifugation step was done before cultures, likely because the media was cleared of debris. Flow cytometry also suggested that macrophages produced the most TNF- α and were most sensitive to the effects of cannabinoids. Overall, this method allows for a quick and easy way to obtain a macrophage-enriched cell population in which CBD and THC are anti-inflammatory. We will use this model to look for possible synergistic molecules to use with cannabinoids as anti-inflammatory treatments and/or examine effects of cannabinoids on lung cells from vaping.

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**Effect of Edwardsiella ictaluri Live Attenuated Vaccines on Phagocytic Update and Apoptosis in Catfish B Cells**

Amanda Fowler, Adef O. Kordon, Mark Lawrence and Lesya M. Pinchuk

*Edwardsiella ictaluri*, a Gram-negative and intracellular pathogen, is the causative agent of Enteric Septicemia of Catfish (ESC). Previously, the role of B cells in innate immunity has been reported in several teleost fish, such as zebrafish and rainbow trout. Our research group developed two novel *E. ictaluri* live attenuated vaccine (LAV) strains, *Ei*∆*evpB* and ESC-NDKL1. Recently, the effects of *E. ictaluri* LAV and wild-type (WT) strains on the innate functions of catfish B cells have been demonstrated. In this study, we assessed the roles of *E. ictaluri* LAV and WT strains on phagosome/phagolysosome formation and late apoptotic changes in catfish B cells. We applied PROC FREQ procedure in SAS for windows 9.4, Chi-Square option and Post Hoc Bonferroni Test to describe the differences in the proportion of cells. The level of significance for all tests was set at *P* < 0.0083. Initially, we observed that *E. ictaluri* WT strain induced significantly higher numbers of apoptotic B cells compared to its LAV counterparts by a blind count of B cells with fragmented nuclei. Furthermore, the *Ei*∆*evpB* LAV strain promoted significant increases in the vesicle formation in catfish B cells compared to other treatment groups. Finally, significantly elevated numbers of vesicles containing bacteria were detected in catfish B cells-treated with the ESC-NDKL1 LAV strain compared to its LAV a counterpart and WT strains. Our results show that two *E. ictaluri* LAV strains induced vesicle formation without promoting significant apoptotic changes in catfish B cells suggesting possible role of B cells as antigen presenting cells in protective immune responses against ESC.

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**A Description of Local, Severe Infectious Bovine Keratoconjunctivitis Outbreaks**

Caleb Glover, Amelia Woolums, Roy Berghaus, Isaiah Smith, and Linda Carlson

Infectious bovine keratoconjunctivitis (IBK) is the most important ocular disease of cattle. Prevention and control of IBK can prove challenging and the etiology has recently been questioned. Little has been recently published characterizing IBK at the herd level. The objective was to complete a case series describing disease characteristics and management practices in herds affected by severe IBK outbreaks in West Virginia in 2019. Participants were selected from a single veterinary practice based on history of testing for autogenous vaccine development in 2019. Six cow-calf producers were interviewed. Producers were asked questions pertaining to disease severity and herd health protocols and husbandry. The number of cattle at risk per herd ranged from 36 to 1030. The mean (+/- SD) annual cumulative IBK incidence was 34.6% +/- 31.6% (range, 3.9% - 97.2%). Cumulative incidence was inversely associated with herd size (P = 0.017). Five of 6 producers treated IBK cases only in summer months, while one producer treated IBK in 11 months of 2019. More calves with IBK were treated than cows. Pour-on fly control was used by 4 of 6 herds, while 1 of 6 herds used fly repellant ear tags. Other management was typical of well-managed herds. The results showed a range of disease severities, with one herd experiencing IBK throughout the year. Interpretation of these results is limited due to sample size and study design; however, they suggest that some herds experience severe IBK and that some of the management practices relevant to IBK are conserved between herds. Further study of IBK to identify previously undetermined risk factors is warranted.

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**Developing an ELISA to measure bovine antibodies for *Tritrichomonas foetus***

Katlyn Harris, Merrilee Thoresen, and Amelia Woolums

*Tritrichomonas foetus* (TF) is the parasitic protozoal cause of bovine reproductive losses. Bulls are asymptomatic carriers of TF, transferring the parasite to cows & heifers who display clinical signs such as, pyometra, metritis, vaginitis, & abortions. TF in a herd leads to reduction in calf crop, which in turn leads to a reduction in monetary return per calf born. Currently, there is no USDA approved treatment for TF infection. Our laboratory is engaged in research to test the ability of messenger RNA (mRNA) therapy to induce production of IgG against the adhesion glycoprotein TF1.17 in bovine reproductive epithelium, to help cattle resist or clear TFinfection. A reliable enzyme-linked immunosorbent assay (ELISA) to measure TF-specific IgG is needed to support research to improve understanding of protective immunity & to confirm efficacy of mRNA therapy to induce antibody production. For this project we had the opportunity to use both the purified TF1.17 antigen & whole TF to coat the plates. For our purified TF1.17 antigen concentration we used 0.8 µg/ml to coat the plates, our positive control TF1.17-vaccinated heifer 1276 serum was 1:1000, negative control ultra-low IgG FBS was1:100, secondary anti-body anti-bovine IgG conjugated to HRP was 1:7500. These concentrations were based on titration and dilution series using both whole TF & TF1.17 antigen coated plates. We spiked smegma with positive control serum, & the results concluded that higher optical densities were observed when using whole TF to coat the plate than when using the TF1.17 antigen. The TF1.17 antigen provides more sensitivity for bovine IgG in smegma. Based on the results of the project, our ELISA did effectively measure bovine antibodies for TF.

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**Exploring State-Level Risks for Introducing Chronic Wasting Disease into Wild Cervid Populations**

Walker Hyche and David R. Smith

Chronic Wasting Disease (CWD) is a fatal, transmissible spongiform encephalopathy affecting members of the deer family, Cervidae*.* The prion agent can remain in the environment for extended periods of time and use multiple routes of transmission. The disease has caused population declines in species such as the whitetail deer (*Odocoileus virgnianus*) and mule deer (*Odocoileus hemionus*). Since the initial introduction of CWD into wild cervid populations in 1981, twenty-four states have diagnosed CWD in wild cervid herds. There is still a knowledge gap regarding the state-level risk factors for introducing CWD into wild cervid populations. The objective of this study was to conduct a nationwide analysis for state-level risk factors for CWD transmission in the United States. Causal loop diagrams were developed to visualize the complex relationships concerning CWD transmission to non-endemic states. A cox proportional hazard survival analysis model was used to test a variety of state-level characteristics to determine risks associated with the time until introduction of CWD into a state. Significance was defined at alpha=0.05. Four factors remained significant in the multivariable model. Carcass bans (HR=0.3098), distance from the CWD epicenter (HR=0.1367), and the presence of alligators (HR=0.3117) were protective of CWD introduction into wild populations. Harvest density indicative of cervid populations per hectare was positively associated with CWD introduction (HR=1.0832 for each 1,000 cervids/h). Understanding risk factors for CWD is essential for continuing efforts to prevent state cervid populations from becoming infected.

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**A System Dynamics Model of Shelter Capacity for Care**

Keegan M. Jones, Robert W. Wills, David R. Smith, W. Cooper Brookshire, and Kimberly A. Woodruff

Shelter animal welfare concerns lead to the development of capacity for care calculators, which produce an optimal animal inventory based on average number of daily adoptions. Their applicability to municipal, open-admission shelters is limited, as these shelters are often tasked with accepting all unowned animals in the community. System dynamics modeling captures a system’s behavior resulting from its structure. The objective of this study was to develop a system dynamics model to observe the effects on the shelter system in response to changes in exogenous parameters, given fixed resources. A causal loop diagram of factors contributing to animal welfare, length of stay, and animal outcomes was developed. A shelter management stock-and-flow model was then constructed to demonstrate endogenous shelter system dynamics by tracking changes in length of stay, shelter population, population health, and proportion of live/dead outcomes. Endogenous model parameters were drawn from previously collected shelter survey datasets. Exogenous parameters included intake and outflow rates, resource use, initial illness incidence, and initial inventory size.

Operating above capacity for care for a set time resulted in an increased number of healthy animals that were euthanized due to resource disparity. Inventory size and animal health oscillated in relation to operating capacity and increasing illness incidence due to increased cost-to-treat ill animals. The shelter model showed that variables beyond adoption rate, intake, and capacity for care may impact shelter conditions and proportion of live animal outcomes. This model may be used to understand complex relationships within shelters and to evaluate management strategies.

Student Support: Mississippi State University College of Veterinary Medicine

**Economic Impact of Removing Arrival Metaphylaxis in the U.S. Stocker and Feedlot Industry**

Alec R. Lucas, Robert W. Wills, Tyler B. McMurray, and David R. Smith

Antibiotic resistance continues to be at the forefront of issues facing animal agriculture. As policies and regulations become more restrictive regarding antimicrobial use, producers, veterinarians, and industry representatives, should cooperatively prepare to use less antimicrobials. The objective of this study was to use stock and flow value-chain models to understand how cattle markets would respond to various antimicrobial use policies based on profitability. Vensim Personal Learning Edition, by Ventana Systems Inc., was used to model the system with causal loop diagrams and stock and flow value-chain models. A metaphylactic intervention was created within each sector of the beef chain to compare how the market would change if metaphylaxis was banned in certain sectors or across the cattle feeding industry. If metaphylaxis was in use, then there was a much higher count of high-risk calves. These high-risk calves were more likely to move directly to the feedlot. Removing arrival metaphylaxis and adding a 5% incentive for calves not treated with antimicrobial increased the number of low-risk calves relative to high risk. The feedlots then preferred the low-risk calves over their high-risk counterparts. If metaphylaxis was only permitted at the backgrounder stage, more high-risk calves moved towards backgrounding operations although low-risk calves were more numerous overall. Low-risk calves marketed directly to feedlot were most numerous when arrival metaphylaxis was removed from the cattle feeding industry. Restrictive antimicrobial use policies might lead to important shifts in beef production sectors. Smaller cow-calf herds, which produce the most high-risk cattle, might be most affected.

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**Assessment of Language Style and Pet Owner Decision Making**

Jessica Menig and Jesse Grady

Development of communication skills and tools are of growing interest in veterinary medicine. Adapting communication styles to pet owner preferences may aid in improving compliance. Our study seeks to determine if language style influences pet owner decision making and its relationship to autonomy preference. 12 recommendation statements for a fecal test were developed and grouped into 2 language styles, paternalistic and non-paternalistic. An online pre-test survey was conducted to assess the recommendation statements for perceived control, choice, paternalism, essentialness, and necessity in attempt to identify 2 contrasting styles of recommendations. The survey was conducted online and with incentivized participation. In the proposed follow up survey, participants would read recommendations of both language styles and respond based on how likely they would be to agree to each recommendation, in addition to completing an autonomy preference survey. The pre-test scores for all variables showed no clear delineation to create 2 groupings of our recommendations. Due to a lack of 2 distinct categories, we did not conduct the phase of the study testing for compliance and autonomy preference. It is plausible that a similar study conducted in a clinic setting would produce different results compared to the online crowd source survey design. Testing for multiple variables within our recommendation statements also brings to focus the need to better identify what factors impact the way pet owners perceive recommendations and furthermore how it influences their decision making.

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

**Osteomyelitis: Animal models review and fosfomycin-loaded chitosan hydrogel therapeutic**

Kylie Roux and Lauren Priddy

Osteomyelitis, or the infection of bone tissue, has become a growing concern in modern healthcare. The standard of care involves aggressive, long-term, systemic antibiotics and surgical debridement. However, treatment is often challenged by antimicrobial resistance (most notably from *Staphylococcus aureus*), failure to penetrate the bone and clear the initial infection, and recurrence of infection. The aims of this project were: (i) to compose a review paper on animal models of osteomyelitis, and (ii) to test the antimicrobial properties of fosfomycin-loaded chitosan hydrogel *in vitro* against *Staphylococcus aureus*. The hypothesis for this project is that fosfomycin-loaded chitosan hydrogel will have enhanced efficacy against *S. aureus*. The review paper served as a comprehensive literature review while the lab was closed due to the COVID-19 pandemic. Upon return to lab, a Kirby-Bauer assay was performed on plates contaminated with *S. aureus* using: PBS, chitosan hydrogel, PBS with high dose (0.05mg) fosfomcyin, chitosan hydrogel with high dose fosfomcyin, PBS with low dose (0.005mg) fosfomycin, and chitosan hydrogel with low dose fosfomycin. PBS and chitosan hydrogel containing the high dose of fosfomycin showed a larger zone of inhibition (ZOI) compared to the other groups. The chitosan gel with the high dose of fosfomycin showed the largest ZOI. There was no difference between the chitosan gel and chitosan with low dose fosfomycin, while both groups had greater bactericidal efficacy than their PBS counterparts, further substantiating chitosan’s innate antimicrobial properties.

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