2020 Veterinary Summer Scholars Symposium

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Welcome to the Veterinary Summer Scholars Symposium!

Every year the National Veterinary Scholars Symposium serves as a culminating event for students participating in Veterinary Summer Scholars Programs and provides an opportunity for veterinary student scholars to present their research at a scientific meeting. In 2020 public health and safety concerns around the COVID-19 pandemic led to the cancellation of this in-person event. The pandemic simultaneously illustrates the critical importance of scientific research. The AAVMC, with support from Boehringer Ingelheim Animal Health, is committed to supporting veterinary students who continue the important work of research. The Veterinary Summer Scholars Symposium, held in lieu of the in-person National Veterinary Scholars Symposium, is an online scientific meeting and poster session.

“The global COVID-19 pandemic is a vivid reminder of the importance of scientific research. We remain committed to helping students make the most of their research opportunity during these challenging times.”

— Andrew T. Maccabe, CEO, AAVMC

“This year we have seen first-hand the importance of biomedical research and the vital role it plays in advancing our understanding of infectious diseases such as COVID-19. Boehringer Ingelheim is delighted to continue our long-standing support of veterinary research by sponsoring this virtual Veterinary Summer Scholars Symposium that will enable veterinary students and young researchers to share the results of their summer research while addressing safety and travel concerns at this time.”

— Eric Haaksma, Head of R&D, Boehringer Ingelheim Animal Health

The AAVMC is grateful to the incredible work of the planning team at University of California San Diego and Western University of Health Sciences in preparing for the 2020 NVSS. The next NVSS will be held at Iowa State University in August 2021.

Abstract submission and publication sponsored by the AVMA
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Complete Abstracts

Observational study of gibbons exposed to voluntary triggered auditory enrichment

Tamara Abato-Earwood, Shant Balci, Elizabeth Strain, George Nababan, Kregg Maruyama, Wesley A. Johnson, Carlos Castro Candelas, Curtis Eng, Kristopher Irizarry

College of Veterinary Medicine, Western University of Health Sciences, Pomona, California (Abato-Earwood, Balci, Strain, Eng, Irizarry); College of Engineering, Dept. Mechanical Engineering, CalPoly Pomona, Pomona, California (Nababan, Maruyama, Johnson, Candelas)

As members of the apes (Hominoida), gibbons (family Hylobatidae) are not studied as habitually as their counterparts, the chimpanzees (family Hominidae). TEAM Gibbon’s goal is to create a fortifying program while monitoring the development of enrichment through increased gibbon interaction with the MPEG Audio Layer-3 (MP3) player. This project focused on auditory enrichment, that investigated what factors contribute to voluntary MP3 use by captive gibbons. As part of the study design, the MP3 player was exposed to multiple gibbons, enclosures, species, genders, and ages. Upon a gibbon pushing any of the four colored buttons a specific audio file will play. Overall our study investigated four aspects of voluntary auditory enrichment: 1. Investigated gibbon contact with MP3 player and assessed tactile efficiency of gibbon presses. 2. Determined if certain times within the observational period were associated with increased/decreased interaction with the MP3 enrichment. 3. Compared differences between gibbons (gender/age), enclosures, and the presence of food. 4. Observed over 100 interactions with the MP3 player (classified into two categories, those producing MP3 activation, and those that did not). All of our results were assessed for significance. Within the course of our study, multiple gibbons not only touched the MP3 player but also activated audio sounds. Preliminary data suggests that food presence may decrease MP3 interaction, and sufficient novelty may be attained by changing the MP3 players location, height, and consecutive days within an enclosure. Future work on this project should focus on investigating the role of MP3 height on gibbon interaction and choice of MP3 file in maintaining gibbon interest.

Research Grant: Small Grants Involving Student Projects-TEAM Gibbon
Student Support: WesternU Summer Research Fellowship Program. CVM Student Summer Research Scholar Program

Non-FeLV leukemia in the cat: a retrospective analysis of the phenotypic and morphologic characteristics

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Leukemia is neoplastic proliferation of blood-forming cells that typically arise from either the spleen or bone marrow. Leukemia arising from leukocyte lineages can be loosely stratified into acute or chronic and myeloid or lymphoid leukemia. Unlike people and dogs, there is less information known of characteristics of leukemia in cats. The purpose of this retrospective study was to describe the immunophenotypic features of leukemia in cats, determined by flow cytometry and correlate findings with morphologic data. Records from clinical databases were examined from 2016-2020. Cats with a diagnosis of leukemia or suspected leukemia were included in the initial assessment. Exclusion criteria included any FeLV positive cats. 52 cats were included in the analysis. 24/52 cats were diagnosed with T cell, while 9/52 were diagnosed with B cell leukemia. 19/52 cats had leukemia of undetermined origin or suspected leukemia. Of the T cell leukemia 15/23 were CD4+CD5+, 7/23 were CD8+CD5+ while 2/23 were CD5+CD4-CD8-. The cellular morphology for T cell and B cell leukemia was reported, at this time there is no distinguishing characteristics or adjective for the different phenotypes. Morphologically the cells could range from mature to immature, to small to large in size. Descriptive statistical data is being compiled for each leukemia subtype. Further, treatment and survival data are being collected by contacting the submitting veterinarian for follow up. Survival data in relationship to phenotypic and morphologic characteristics will be compiled. In summary, the findings for our study thus far demonstrate that CD4+CD5+ T cell leukemia is the most common in the cat while B cell leukemia also occurs but with less frequency.

Research Grant: National Institutes of Health and Interdisciplinary Biomedical Research Training Program T35-T35OD011070
Student Support: National Institutes of Health and Interdisciplinary Biomedical Research Training Program T35
The prevalence of enthesophytes in equine tarsal radiographs
Haley Yule Abbott, Jody Lawver, Jose Casillas, Jane M. Manfredi

Department of Pathophysiology and Diagnostic Investigation (Abbott, Manfredi), Department of Small Animal Clinical Sciences (Lawver), Department of Large Animal Clinical Sciences (Casillas), College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

Traditionally tarsal pain has been attributed to osteoarthritis (OA) and osteochondritis dessicans, with less diagnosis of injury to soft tissue structures. With previous studies demonstrating the severity of lameness is unassociated with degree of OA-related radiographic change, there is an interest in evaluating tarsal soft tissue pathology. Recent work detailing the radiographic attachments of soft tissue structures in the tarsus can help practitioners to identify enthesiophytes. The purpose of this study is to determine the prevalence of radiographic abnormalities consistent with tarsal soft tissue injuries and compare this to its inclusion in the medical record. Descriptive statistics were performed. Tarsal radiographs and medical records were reviewed from 326 horses admitted to the Michigan State University Veterinary Teaching Hospital between 5/2015 to 5/2020. The mean ± SD age was 8.3 ± 5.9 years, sex was 175 geldings, 111 mares, and 28 stallions. Common breeds included Quarter Horses (55), Thoroughbreds (41), Standardbreds (53), and Warmbloods (14). Mild to severe soft tissue related radiographic changes were seen in 95% of cases. Commonly affected structures were the medial (192/326) and lateral (192/326) collateral ligaments, suspensory ligament (172/326), and long plantar ligament (157/326). Soft tissue lesions were identified in the medical record in 33.3% of cases and were clinically diagnosed as the primary issue in 30.6% cases. Large animal surgeon reports identified soft tissue radiographic changes in 15.1% of cases as compared to 39.1% of radiologist reports. Our results suggest that soft tissue injuries in the tarsus may be more prevalent than previously believed and could be under-diagnosed in practice.

Research Grant: None
Student Support: NIH Grant 5T35OD016477-19

The impact of a ketogenic diet on cognitive and motor function in female mice
Yael Ad, Zeyu Zhou, Audrey Evangelista, Vaiva Raulinaitis, Jon J. Ramsey, Jennifer Rutkowsky

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Calorie restriction and ketogenic diets have been shown to improve cognitive and motor function in male mice. Both diets induce a metabolic switch from glycolysis to fatty acid beta oxidation and ketogenesis that may contribute to the mechanism of action for both interventions. Little is known about the safety and efficacy of ketogenic diets in females, and it is uncertain if there will be similar effects of a ketogenic diet in females as reported in males. To test whether a ketogenic diet improves cognitive and motor function in female mice, and is safe, 14 month-old female C57BL/6JN were fed an isocaloric control or ketogenic diet for two months and underwent different behavioral tests. The elevated plus maze and the open field tests analyze anxiety levels and exploratory behavior. The Y maze and novel object recognition tests are utilized to assess spatial and working memory and non-spatial recognition memory, respectively. The wire hang, grip strength and rotarod examine motor functions. Through one month on diet, there is not a significant difference in body weight between the mice on the ketogenic diet compared to the mice on the control diet ($P > 0.05$). Since there is no difference in body weight or increases in mortality, we conclude that ketogenic diets do not have a negative impact on either energy intake or body weight.

Research Grant: NIH Program Project grant (PO1AG062817)
Student Support: UC Davis STAR program and School of Veterinary Medicine—Musser/Lider endowment funds
Identification and validation of alleles for cardiac arrhythmias and atrial fibrillation

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Cardiac arrhythmias are an important cause of sudden death (SCD). Most horses with arrhythmias have no underlying structural heart disease, making it virtually impossible to detect those that will develop arrhythmias. Arrhythmias in human athletes without underlying structural heart disease are almost exclusively caused by mutations in ion channel genes. Given the similarities between these forms of arrhythmias in humans and horses, it is likely that mutations are an important cause of arrhythmias and SCD in horses. We used whole genome sequence (WGS) of 6 Standardbred racehorses with atrial fibrillation (AFIB) to identify putative arrhythmia-causing mutations. From there, mutations in biologic candidate genes identified with ClinVar were prioritized. In total, 4,066 mutations were present in the 309 candidate genes. 49% (1,994) were considered putative arrhythmia-causing mutations due to being rare in the general population (534 horses). Of these mutations, 512 were likely to have an effect on phenotype (predicted to have a high or moderate impact). 78 mutations (15%) were determined to be false positives due to poor reference genome annotation or genotyping errors. Additional evidence of pathogenicity was obtained using PROVEAN, SNPS&GO, SIFT, and VEST. In total, 153 mutations (30%) in 118 candidate genes had sufficient evidence to be considered putative arrhythmia-causing mutations. Thirty-six additional mutations that were present at the same site as mutations known to cause cardiac disease in humans were also identified. These mutations will be genotyped in 1,200 Standardbred and Thoroughbred racehorses to test for associations between the mutations, cardiac arrhythmias, and SCD.

Research Grant: USDA NIFA-AFRI, AQHA, UMN Multistate grant, ACVIM Foundation fellowship, T32 Institutional Training Grant, Morris Animal Foundation, AAEP, UMN CVM
Student Support: Morris Animal Foundation D20EQ-606, UMN College of Veterinary Medicine Summer Scholars Program

Effect of glutaminase inhibitors on SARS-COV2 replication

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SARS-COV2 is a novel virus that causes serious respiratory illness in humans. It is a highly contagious positive-sense RNA virus that binds to mammalian Angiotensin-converting enzyme 2 (ACE2) receptor. It has close similarity with bat coronaviruses, which are thought to be the source of the human virus, and has infected more than 12 million people worldwide and also resulted in more than 500,000 deaths globally. Unfortunately, there is no vaccine available and it is still unclear whether an initial infection results in sufficient antibody load to prevent reinfection. Further, treatments are currently limited to mechanical respiration and a small number of drugs that modestly decrease symptoms. Thus, there is a critical need for more effective treatments, hopefully including ones that can act as cure. Upon viral entry into infected host cells, many viruses, including SARS-COV2, hijack cellular metabolism. There is an increased demand for biosynthetic intermediates to support viral replication, which closely mimics the well-known Warburg Effect in cancer cells. Viruses rely greatly on glutamine metabolism as source of nucleotide biosynthesis and fatty acid synthesis needed for virus replication. Because virally infected cells show a heavy reliance on glutamine metabolism (i.e. ‘glutamine addiction’) they may be sensitive to inhibitors that block glutaminase activity, which have already been demonstrated to block the growth of glutamine addicted cancer cells. The goal of this research is to attempt to discover novel glutaminase inhibitors, which might someday be used to block the replication of a SARS-COV2 viruses in host cells.

Research Grant: NIH grants R35 GM122575 and CA2011402
Student Support: Cornell University Veterinary Leadership Program
The translocator protein influence on aryl hydrocarbon receptor-mediated transcription

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The aryl hydrocarbon receptor (AhR), is a ligand-activated transcription factor known to mediate most, if not all, of the toxicity of specific planar aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Recent evidence has shown that a portion of the cellular AhR is found in the intermembrane space of the mitochondria. The role of the mitochondrial pool of AHR (mitoAHR) remains unknown, however, many of the putative endogenous AHR ligands have also been linked to the translocator protein (TSPO). TSPO is localized to the outer mitochondrial membrane and is involved in the cellular stress response. We hypothesize that crosstalk exists between TSPO and AhR and that it modulates AHR-mediated transcription. To test this hypothesis, we analyzed quantitative real-time polymerase chain reaction (QRT-PCR) data from three different mouse cell lines, BV2s, microglia cells, Hepa1c1c7s, hepatoma cells, and MLE12, lung epithelial cells. In addition, these cells were engineered using CRISPR-Cas9 to knockout AHR or TSPO expression. Each of the parental and engineered cell strains were also exposed to various activations (BV2s, only) and chemicals, including TCDD and/or PK11195, a TSPO ligand. Our results suggest that loss of TSPO and AHR can impact the transcription of key genes in certain circumstances. Moreover, preliminary results do support the hypothesis that crosstalk between the AHR and TSPO can impact AHR-mediated transcription. These data suggest that mitoAHR and its interaction with TSPO plays a role in TCDD-induced toxicity.

Research Grant: NIH grant R5R25HL103156-10
Student Support: NIH grant R5R25HL103156-10

Advances in understanding molecular pathogenesis of hemangiosarcoma using cutting-edge genome technologies

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Hemangiosarcoma (HSA) is a highly aggressive vascular neoplasm whose origin remains yet to be fully determined. This malignant neoplasm commonly affects the canine population and arises in the spleen, right atrium, liver, and subcutis. Intra-abdominal tumors have a very poor prognosis given that they are detected at a late stage, thus there is an urgent need for advances in strategies for early detection and effective clinical management. Angiosarcoma (AS) is thought to be an analogous vascular malignancy in humans because the clinical and histological features of this disease are so similar to canine HSA. Due to the rarity of AS in humans, funding for genetic studies to better understand either forms of this disease remain limited. While on a limited scale, previous analysis of HSA genome sequences and structures have provided some insights into the disease; however, the molecular pathogenesis of HSA remains incompletely understood. The aim of this review study is to highlight the improvements on genome technology to study HSA, and how such improvements have helped advance our understanding of HSA. This study will compile the most significant HSA genomic data to date and highlight the most recent advances in genomic understanding of this malignant neoplasm.

Research Grant: Boehringer-Ingelheim
Student Support: None
Effect of sunlight hours and reproductive medication on rate of double ovulation and twin pregnancy in mares

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Photoperiod manipulation and the use of reproductive medications has become common in efficient mare breeding. Twin pregnancies can lead to disastrous outcomes for the mare and foal. In the majority of cases, equine twin pregnancies require two ovulations in one estrus cycle. The cause of twin pregnancies and the source of double ovulation is not completely understood. This retrospective study examined the effect of sunlight hours and reproductive medications on the rate of double ovulation and twin pregnancies. Mare breeding records were analyzed from 2011 to 2019 on 267 mares from across Prince Edward Island with 914 breeding cycles. This study found approximately 11% of the cycles resulted in double ovulations and 4% had twin pregnancies. The former is lower than expected, but the latter matches literature findings. Of the total double ovulations, 40% resulted in a twin pregnancy. The rates of double ovulations and twin pregnancies were initially examined with a binary linear and subsequently a multivariable logistic regression. Deslorelin increased the likelihood of a double ovulation ($P < 0.05$, OR = 1.24) and sunlight (OR = 1.19) and human chorionic gonadotropin (OR = 1.68) had a tendency towards significance ($P = 0.05-0.15$), which correlated with literature findings. No factors had a significant impact on the rate of twin pregnancies. The low incidence of double ovulations and twin pregnancies in this study, show that there were likely insufficient numbers to demonstrate an effect on twin pregnancy rate resulting in a type II error. A study including more breeding cycles is warranted to determine the true impact on twin pregnancy rate.

Research Grant: None
Student Support: Canada Student Jobs

Sensitivity and specificity of cytology for the diagnosis of bacterial sepsis in body fluids

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Bacterial sepsis is a relatively common, life-threatening condition with a high case-fatality rate. The current primary diagnostic tools for septic effusion in animal patients are bacterial culture and fluid cytology. While culture is the gold standard, it can take up to several days for results to be made available to clinicians, which can negatively impact a septic patient’s chance at survival. The aim of this study is to evaluate the diagnostic accuracy of cytology in detecting bacterial sepsis in body fluids, which has not been thoroughly studied. We retrospectively reviewed 10 years of medical records at the Ohio State University’s Veterinary Medical Center for mammalian patients with both cytology and bacterial culture of body cavity fluids (peritoneal and thoracic effusion), blood, joint fluid, or CSF. Preliminary results from 209 cases demonstrate an overall sensitivity of 40.2% and a specificity of 90.2% for the ability to detect bacterial sepsis by cytology. Interestingly, the trend for cytology of thoracic fluid ($n = 25$) is somewhat opposite that of the overall diagnostic accuracy, with sensitivity and specificity of 81.8% and 50.0%, respectively. As more cases are included in the study, we expect to be able to identify subtypes of cases or sample types that demonstrate better or worse diagnostic accuracy of cytology for detection of bacterial sepsis. Perhaps unsurprisingly, the overall data collected thus far indicate that cytology is rather insensitive, but quite specific for this purpose. The results from this study will allow better comparison between diagnostic accuracy of cytology and emerging diagnostic tests for the detection of bacterial sepsis in mammalian patients.

Research Grant: None
Student Support: NIH T35 Training Grant
SHIP inhibition in canine osteosarcoma: evaluation of a novel target

Carley Allen, Anita Rogic, Brian Flesner, Angela McCleary-Wheeler

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Canine osteosarcoma is the most common bone tumor reported in dogs. Despite currently available therapies, the median survival time of dogs affected with osteosarcoma is approximately nine to twelve months with 90% eventually developing metastasis. Numerous cellular signaling pathways become mutated or inappropriately activated within osteosarcoma to promote tumor cell growth and survival, including the PI3K/mTOR pathway. Src homology 2 (SH2) domain containing inositol polyphosphate 5-phosphatase proteins, SHIP1 or SHIP2, have been shown to promote cell survival in several cancer types, but their role in canine osteosarcoma has not been previously evaluated. The objective of this study is to determine the biologic effects of novel SHIP inhibitors (3-AC, K118, K149, K161) on established canine osteosarcoma cell lines. Evaluated parameters include cell viability, apoptosis, and inhibition of the PI3K/mTOR pathway. Based on preliminary viability data using MTS assays, the SHIP inhibitors exhibit a time and concentration dependent decrease in viability. The greatest effect for all inhibitors occurred 72 hours post-treatment. Further apoptosis and pathway specificity experiments are being conducted.

Research Grant: University of Missouri College of Veterinary Medicine
Student Support: Morris Animal Foundation Grant: D20CA-603

COVID-19 and its effects on the human animal interaction

Megan Allen, Jane Manfredi, and Kirk Munoz

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Pets are a vital part of people’s lives, and the COVID-19 pandemic may have impacted how we interact with them. Changes in routines and perceptions of possible transmission of COVID-19 may affect the daily life of the pet. Our hypotheses are that due to COVID-19, non-medically trained (NMT) people would have increased awareness of interactions with their pets, it would have a greater impact on cat and dog owners than horse owners, and it would have a negative impact on those seeking veterinary care during stay-at-home orders. An anonymous email survey, open from May to June 2020, was sent out to staff, faculty and students of Michigan State University’s College of Veterinary Medicine (CVM), as well as to active clients within the last year; and to a random number of non-CVM faculty and staff. Inclusion criteria included owning at least one dog, cat, and/or horse. Questions involved areas such as handwashing, frequency of walks/rides, and access to veterinary care. Statistical analysis included ANOVA, chi square tests, and thematic assessment. Significance was set at $P < 0.05$. There was a 15.3% survey response rate overall (19% medically trained (MT) and 81% NMT). Dog owners had the most marked differences in behaviors during the stay-at-home orders, primarily with not allowing their dog to have direct contact with other dogs and people outside their households ($P < 0.01$). While many people postponed veterinary care (40.7% routine care and 7.4% care for a medical issue), they were able to obtain it if needed. Only 2.5% of people were unable to find veterinary care. Behaviors between MT and NMT did not differ, however there were some differences when comparing pre and during the stay-at-home orders.

Research Grant: None
Student Support: NIH T-35 grant award (NIH T35 OD016477)
**Studies on the host selectivity of the staphylococcal innate immune evasion protein, SPIN**

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The Staphylococcal Peroxidase Inhibitor (SPIN) is a small, secreted protein from Staphylococci that inhibits myeloperoxidase (MPO) activity. SPIN blocks the MPO active site and prevents production of cytotoxic hypochlorous acid (HOCl) within the neutrophil phagosome. *Staphylococcus aureus* is primarily a pathogen of humans and previous studies have shown that its SPIN protein cannot bind MPO from non-human mammalian species. This raised questions as to whether SPIN proteins from Staphylococci whose preferred hosts are other mammals might likewise exhibit strict binding preference for MPO from these hosts. Therefore, the objectives of this study were: (i) to evaluate the interactions between SPIN homologs from Staphylococcal species known to infect dogs and recombinant canine MPO, and (ii) to examine whether these SPIN homologs could bind and inhibit human MPO. Preliminary aims were to prepare recombinant SPIN proteins from *Staphylococcus pseudintermedius* and *Macrococcus canis*, as well as to transflect mammalian cells with vectors that direct expression of recombinant human and canine MPO. The SPIN proteins were purified by affinity and gel filtration chromatographies, and characterized by electrophoresis and mass spectrometry. Production of recombinant MPO from transiently-transfected HEK293(t) cells was assessed by immunoblot using anti-His mAb, anti-human MPO, and anti-myc mAb as primary antibodies. Bands specific for both recombinant human and canine MPO were present, indicating positive transfection of mammalian cells and expression of the desired MPO enzymes. These preliminary results provide evidence that preparation of canine SPIN proteins and MPO was successful for use in further structure/function analyses.

**Research Grant:** NIH R01 GM121511 awarded to Brian V. Geisbrecht  
**Student Support:** Kansas State University Veterinary Research Scholars Program

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**Antimicrobial usage and stewardship education for calf producers**

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Antimicrobials are a necessary for the treatment of bacterial infections in food animals, but antimicrobial-resistant bacteria can emerge. These antimicrobial-resistant bacteria are a critical threat to animal and public health. Therefore, having a group of resources to assess when it is necessary to use antimicrobials as treatment would be beneficial. Antimicrobial usage in neonatal veal calves is frequent due to the inherent susceptibility of the calves to bacterial diseases. The overall goal of the study was to quantify the use of antimicrobials within a veal production system, which has not been done in the United States. Data was collected by collecting antimicrobial containers in labeled disposal bins from the day of calf arrival to slaughter date. Treatment records and nutrition records were also gathered during the time for more specific antimicrobial usage data. The total mg of active substance, total mg/kg of animals at risk of infection, and defined daily doses (DDD) will be calculated for each farm. These metrics will be necessary to measure the impact of antimicrobial stewardship interventions implemented on the select farms in comparison to the controls. It is expected that antimicrobial usage will be lower in the farms intervened with antimicrobial education tools.

**Research Grant:** Centers for Disease Control and Prevention FY2018-OADS-01 (Project Number 60065874)  
**Student Support:** The Ohio State University Veterinary Scholar Summer Research Program
Point prevalence survey of antimicrobial use in companion animal intensive care units in the United States

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The objective of this study is to conduct a point prevalence survey of antimicrobial use in dogs and cats in veterinary intensive care units (ICU) in the United States. The study itself will be two-pronged. The first aim will be to conduct an internal single day point prevalence study (PPS) in the ICU at Tufts’ Foster Hospital for Small Animals. The survey will be modeled on methods used at the University of Minnesota College of Veterinary Medicine. This will be the pilot study using the data capture system, REDCAP, that will facilitate data collection and analysis. The second aim of this study is to expand to a multi-institutional point prevalence survey of antimicrobial use in dogs and cats in ICUs nationally. The internal study, which will follow similar guidelines as in the multi-institutional study, will serve as a test run for the larger scaled study. Records will be collected for all patients in the ICU at a specific time and date. Demographic data, reason for visit, presenting complaint, co-morbidities, tests completed, and imaging results will be recorded in addition to antimicrobial therapy(ies). REDCAP and descriptive analysis will be completed to gather antimicrobial usage patterns. Recruitment of participating institutions will occur over the summer months along with the start of data collection. At the conclusion of this national study, primary researchers from the involved tertiary institutions will submit a scholarly manuscript. Currently, there is scant antimicrobial usage data in small animal patients in the United States. This data is essential in crafting, benchmarking and monitoring antimicrobial stewardship interventions.

Research Grant: None
Student Support: Boehringer Ingelheim

Reference intervals for metarubricytosis in healthy canines—a one health approach in comparative hematology

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Inappropriate metarubricytosis has been studied in human medicine; however, clinicians still disagree if 1 nRBC/100 WBCs in circulation is appropriate in healthy adults. In veterinary medicine, canine inappropriate metarubricytosis has been associated with heat stroke, splenic dysfunction, chemotherapy treatment, lead toxicity, bone marrow dysfunction, and other diseases. Reference intervals vary for healthy dogs as 0-1 or 0-5 nRBCs/100 WBC. This retrospective study of inappropriate metarubricytosis analyzed a random generated sample of 300 clinical pathology records for 234 dogs (from total = 1700 records for 1280 dogs) during 2013-2018 with 1-5 nRBCs/100 WBC in non-anemic dogs (Hct > 37%). Diseases were categorized with 1-5 nRBCs/100 WBCs and breed occurrence. Findings were 1-5 nRBCs/100 WBCs can be associated with cardiac, respiratory, dental, endocrine (diabetes mellitus, hyperadrenocorticism, thyroid and parathyroid), liver, ocular, urinary tract, gastrointestinal, neurologic, dermatologic, splenic, pancreatic, inflammatory/infectious, and intervertebral disc diseases; cancer, obesity, fractures, and ligament tears. Also, there were healthy dogs (n = 8) and dogs with minor health issues (n = 9). Mixed breed was the most prevalent followed by Labrador Retriever, Beagle, Shih Tzu, Yorkshire Terrier, and Miniature Schnauzer, which may reflect the clinic admission breed population. Mortality data could not be accurately assessed due to loss of case follow up. In summary, 1-5 nRBCs/100 WBCs as a reference interval in healthy canines can be associated with some diseases that overlap, and also, differ from diseases found in the Phase I study of > 5 nRBCs/100 WBCs in non-anemic dogs.

Research Grant: Boehringer Ingelheim Vetmedica Inc. (BIVI)
Student Support: Merial Veterinary Scholar and CVM Summer Scholar Program
The role of host cell calcium in viral propagation and pathogenesis

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Viruses are obligate intracellular pathogens that utilize and depend upon host machinery for propagation. The high rates of evolution and adaptation by viruses has challenged anti-viral therapeutic development by producing a moving target for strategies such as vaccination and virus targeted therapeutics. Thus, approaches that may initially be effective often do not produce durable protection. By contrast, therapeutics designed to target host mechanisms and pathways required by viruses to enter, replicate, or leave a host cell are potentially immutable and thereby durable targets to disrupt viral replication and disease transmission. One key host signal that required by viruses is intracellular calcium. Calcium is a “second messenger” that dynamically regulates host mechanisms hijacked by viruses for their entry, trafficking, replication, and egress from the cell. Recent efforts have established that calcium pumps, transporters, and ion channels in the plasma membrane, mitochondria endoplasmic reticulum, and endolysosomal system play a vital role in propagation of many viruses. Consequently, these represent host therapeutic targets that can be exploited for broad spectrum control of a broad range of virus families and family members. This review of the role of calcium in virus-host interactions seeks to clarify recent research into the underlying mechanisms that govern viral propagation and seeks to highlight areas for future study.

Research Grant: None
Student Support: NIH T35 Training Grant

Analyzing gene expression of *Dirofilaria immitis* in susceptible and refractory strains of *Aedes aegypti*

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The parasite *Dirofilaria immitis* causes heartworm disease, a parasitic infection threatening dogs worldwide. The disease remains a widespread problem due to owner non-compliance with monthly preventatives, significant animal reservoirs, and the emergence of drug-resistant parasite strains. Therefore, it is important to identify new ways to prevent the spread of this potentially life-threatening disease. One possibility being explored in the Povelones laboratory is the prevention of dog infection by disrupting the *D. immitis* lifecycle in mosquitoes. The purpose of this study is to compare gene expression profiles of *D. immitis* developing in infection-susceptible and -refractory strains of *Aedes aegypti* mosquitoes to understand the basis of compatibility with the insect host. In this infection model, *D. immitis* are developmentally arrested in the Malpighian tubules of the refractory strain within the first three days of infection. Malpighian tubule RNA from the first three days of infection of *A. aegypti* was used to map *D. immitis* transcripts. Consistent with their developmental profiles in the different mosquito strains, we found that the total number of *D. immitis* transcripts increased each day in the susceptible strain but remained relatively constant and low in the refractory ones. We have identified 11 (day 1), 354 (day 2), and 508 (day 3) differentially regulated genes between the susceptible and refractory mosquitoes. In the next phase, which is currently ongoing, the use of gene ontology, gene set enrichment analysis, and homology-based analyses could be used to develop novel approaches to prevent *D. immitis* from successfully completing its mosquito life cycle ultimately blocking the transmission of this pathogen.

Research Grant: NIH 5T35OD010919-23
Student Support: NIH/BI Summer Veterinary Research Program
Effects of 2,4-D herbicide exposure on reproductive health of Fathead Minnow (Pimephales promelas)

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Aquatic herbicides are commonly used to control a wide variety of invasive and nuisance plants. One common active ingredient used in commercial herbicide formulations in midwestern states is 2,4-dichlorophenoxyacetic acid (2,4-D). Due to the stability of 2,4-D in aquatic environments, many non-target aquatic species experience prolonged exposure throughout critical life stages that can affect development. However, the impacts of chronic 2,4-D exposure on reproductive systems in juvenile fish are poorly understood. We aim to test whether 2,4-D can impact reproductive endpoints such as GSI, sperm count, sperm malformations, oocyte atresia, and whether 2,4-D can act as an endocrine disrupting chemical by measuring testosterone levels in the blood and testis of the fathead minnow (Pimephales promelas). Understanding the environmental costs of 2,4-D application on non-target organisms in situ will improve risk assessment decisions by management agencies and residents in deciding what concentrations of 2,4-D to apply, whether to use alternative methods/herbicides, or whether to treat whole-lakes at all.

Research Grant: Wisconsin Dept. of Natural Resources (AIRD-104-15)
Student Support: UW School of Veterinary Medicine, Dean’s Office

Plasma miRNA expression changes are associated with cognitive function in people with HIV

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Cognitive impairment (CI) remains common in people with HIV (PWH) on antiretroviral therapy (ART). The clinical presentation and severity is highly variable in PWH suggesting that the pathophysiological mechanisms causing CI are likely complex and multifactorial. MicroRNA (miRNA) expression changes may be one important mechanism of CI as they are gene regulators involved in the development, plasticity, and differentiation of neurons. Here we examined miRNA expression changes in relation to domain-specific and global CI in PWH. Thirty-six PWH receiving care at the Southern Alberta Clinic, Calgary, Alberta, Canada completed neuropsychological (NP) testing and blood draw. Plasma miRNA extraction was followed by array hybridization and quantitative real-time reverse transcriptase PCR (qRT-PCR) to measure and validate miRNA levels, respectively. Random forest analysis was used to identify the top 10 miRNAs upregulated and downregulated in relation to NP performance. Notably, miR-127-3p was related to memory and miR-485-5p to motor function, which are miRNAs that have been identified in Alzheimer’s and Parkinson’s respectively. Using miRNET, genes involved in signaling, cell cycle, and transcription related to executive function, memory, language, and global domains. These findings suggest that miRNA expression changes contribute to both domain-specific and global cognitive vulnerabilities in PWH and can provide insights into the functional mechanisms involved.

Research Grant: P30MH075773 (Haughey, Rubin, Sacktor) and a grant from the Cancer Institutes of Health Research: Canadian HIV-Ageing Multidisciplinary Programmatic Strategy in NeuroHIV (Application Number 398645)
Student Support: NIH T35 OD024982
Prevalence of nasopulmonary mites among sea otters and harbor seals show distinct geographic hotspots

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Regional infestation hotspots of the nasopulmonary mite *Halarachne halichoeri* have been demonstrated within the southern sea otter (*Enhydra lutris nereis*; SSO) population. However, the prevalence and geographic distribution of nasopulmonary mites within the northern sea otters (*E.l. kenyoni*; NSO) and within harbor seals (*Phoca vitulina*), remain poorly understood. In this study, we determined mite infestation prevalence and assessed potential mite hotspots using necropsy data for harbor seals and both live-capture and necropsy data for NSO. Among 1,765 live-capture records of NSO from 1986 through 2012, 69 (3.91%, 95% CI 3.1-4.9%) were infested. Mite-positive otters were found at 2 of 3 northern Pacific regions sampled, but at specific capture locations with mites prevalences ranged from 4.35%-72.13%. In contrast, NSO necropsy data from 2004-2015 showed just 1 of 220 (0.45%, 95% CI 0.08-2.53%). Among 105 harbor seals necropsied between 2016 and 2019 in California, 21 (20.0%, 95% CI 13.47-28.65%) were infested, which is not significantly different from the reported SSO rate of 25.6% (*P* = 0.19). However, the distribution of infested seals differs from that of otters. While the prevalence within harbor seal and SSO populations is similar, factors such as host density, behavior, and susceptibility should still be taken into consideration when examining otters for translocation and rehabilitation. The significant discrepancy between NSO prevalence generated from live-capture and necropsy data (*P* < 0.05) emphasizes the importance of utilizing multiple surveillance methods for population health. Future studies should screen for nasopulmonary mites to develop an accurate understanding of their range-wide prevalence and epidemiology.

Research Grant: None
Student Support: Morris Animal Foundation (#D20ZO-608)

Efficacy of intramuscularly administered alfaxalone, dexmedetomidine, and morphine/fentanyl for feline neuter

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Cats have unique physical and behavioral features that make injectable forms of anesthesia more desirable than inhalants. Injectable anesthesia reduces patient handling and stress, eliminates the need for bulky equipment, and provides superior cardiovascular stability. The current study evaluated utility of a single, intramuscular injection of alfaxalone (2.5 mg kg⁻¹), dexmedetomidine (0.03 mg kg⁻¹), and either morphine (0.5 mg kg⁻¹; group ADM) or fentanyl (0.025 mg kg⁻¹; group ADF) as novel anesthetic protocols for short surgical procedures (30 minutes [min]) in cats, including orchietomy. Due to the Covid-19 pandemic, three (1 ADM, 2 ADF) male, client-owned domestic short hair cats (< 5 yrs; < 7 kg) were used. Drugs were drawn up separately and combined into a single syringe for injection (lumbar epaxial muscles) at T = 0 min (baseline). Induction and recovery were scored based on vomiting, retching, salivation, smoothness, vocalization, and righting reflex. After reaching appropriate anesthetic depth (loss of righting reflex, jaw tone, pupil position, pedal withdrawal reflex) cats were intubated and placed on 100% O₂. The following variables were recorded every 5 min: HR, SpO₂, RR, EtCO₂, BP, jaw tone, MM color, CRT, pedal withdrawal, web pinch response. The cat was aseptically clipped and scrubbed within 10 mins, and castration was performed. 1.5 mL blood is drawn at for blood gas analysis at 15 and 30 min. Atipamezole (0.3 mg kg⁻¹ IM) and robenacoxib (2 mg kg⁻¹ SQ) were administered at 30 min. Both protocols provided effective anesthesia with excellent induction and cardiopulmonary stability. Additional preliminary results will be presented on poster.

Research Grant: Korea Institute of Science and Technology (KIST); BioNet America Inc.; WesternU CVM Office of Research
Student Support: PetSmart
Methods for studying parasitic nematodes in living hosts

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In vivo models of helminth infections provide options for the study of host-parasite interaction and evaluation of anthelminthic drug efficacy without the need to sacrifice host animal subjects. Larvae of helminths such as *Ancylostoma caninum* and *Toxocara canis* can survive in a dormant state of hypobiosis within hosts. The ability to persist in a hypobiotic state makes these helminths reservoirs of human zoonotic diseases, including cutaneous and visceral larval migrans. Identification of a method to study helminths in situ is crucial to develop treatments targeting hypobiotic infections thereby reducing zoonotic disease in humans. In addition, drug resistance has developed within a number of helminth species, creating a heightened need for methods to study helminth survival within a natural host. In recent years, a number of novel *in vivo* models to study helminth infections in a variety of species have been proposed. In this work, we review currently available technologies for the *in vivo* study of helminths and present preliminary data on an implant device that can be used to study hypobiotic stages of parasites without the need to conduct terminal animal experiments.

Research Grant: College of Veterinary Medicine intramural funds
Student Support: NIH T35 Training Grant

Is there a bias toward consideration of adaptationist explanations for behavioral phenomena?

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Researchers often seek to understand biological traits in terms of adaptive functioning, assuming that a trait is selected for a specific function that increases fitness. However, over the years, several critics have pointed out potential issues with this assumption. Namely, Gould and Lewontin in their 1979 seminal paper entitled “The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme” demonstrated that alternative evolutionary explanations to adaptation often exist for the occurrence of a trait. They went on to call out followers of the “Adaptationist Programme” for failing to consider such alternative explanations for phenomena. In this study, we examined ten prominent behavioral research journals to determine if there is a bias toward consideration of adaptationist explanations over alternative explanations for the occurrence of animal behaviors and if the extent of such a possible bias has changed since 1979. Starting with the year 1980, articles every fifth year were examined manually for both adaptationist and alternative keywords, as well as for relevant context. Preliminary results suggest there is a bias in the behavioral sciences toward explaining traits as evolutionary adaptations even when equally or more plausible alternative evolutionary explanations could have been offered. We speculate that this bias may contribute to prejudiced beliefs and misleading conclusions about the origins of behavioral traits, slowing progress in uncovering the true nature of nature.

Research Grant: None
Student Support: Office of the Director, NIH, T35 OD011145
The effects of sequence differences on the spatiotemporal distribution of SIV in a rhesus macaque

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Simian Immunodeficiency Virus (SIV) is a retrovirus, closely related to HIV in humans, that infects certain monkeys and can cause a syndrome similar to Acquired Immunodeficiency Syndrome (AIDS). SIV has previously been shown to persist in viral reservoirs in various tissues, including CD4+ T cells and the central nervous system (CNS), in rhesus monkeys. In a recent study of SIV reservoirs one particular rhesus monkey (subject 107A), had viral reservoirs in the central nervous system, but little to no virus in peripheral tissues; this was different from other research subjects which showed evidence of persistent viral reservoirs in the thoracic and lumbar spinal cord and spleen. We hypothesize that the SIV sequences of 107A may contain tissue-specific DNA signatures that may also reveal the spatiotemporal distribution of the virus in rhesus monkeys infected with SIV (e.g. whether tissue reservoirs are seeded sequentially) and expose heterogeneity in viral dynamics. To investigate this question, we used computational methods to analyze and cluster the SIV sequences from CNS and spleen tissues. Computational analysis of 107A viral sequences revealed sequence differences between the CNS and peripheral tissues, as well as possible genetic signatures. This distinction would be useful to investigate the disease progression in the animal and could help us understand the viral reservoirs in the CNS and peripheral organs and any implications for potential therapeutic resolution.

Research Grant: NIH T35 OD024982
Student Support: Charles River Lab

Active warming and surgical draping for perioperative thermal support in laboratory mice

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Mice have a small body mass and high relative body surface area and are easily influenced by environmental temperatures (Hankenson et al.) Surgical research procedures are commonly performed using mice but can have major effects on their core body temperature. Hypothermia is a common issue when mice undergo anesthetic procedures (Hankenson et al.) The aim of this study is to evaluate perioperative warming options for rodent surgery to improve animal welfare and optimize research experiments. Mice (CD1 strain) were randomized into treatment groups (n = 6; 8 mice per group) for this experiment; treatments included exposure to a small animal critical care incubator set to 38°C for 30 minutes before surgery (Pre), after surgery (Post) or both before and after (Both) and/or surgical draping with transparent cling wrap (Control/Drape, Both/Drape). One group received no interventions (Control). Mice were anesthetized with ketamine/xylazine and a laparotomy performed, leaving the abdominal cavity exposed for 10 minutes. Dependent on treatment group recovered mice were placed in a standard cage on a warm water blanket set to 38°C or in the incubator. Successful recovery occurred when mice were able to right themselves. Rectal temperatures were taken every minute during surgery; subcutaneous temperatures were collected at several points perioperatively from transponders placed at least 72 hours prior to surgery. We predict that perioperative warming and surgical draping will provide superior thermal support to laboratory mice enrolled in surgical procedures.

Research Grant: Boehringer Ingelheim and the Graduate School at Michigan State University
Student Support: Michigan State University Summer Research Program
Infectious disease models for hypothesis testing: parameterization of a COVID19 model

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As SARS-CoV-2 races across the globe we have seen a renewed interest in using infectious disease models as forecasts for how an outbreak will spread. When used as forecasts, these models are credited for supporting public policy initiatives to justify changing the recommendations for ramping up, maintaining, or scaling back social distancing guidelines. However, predictive use of models may be misleading, particularly in the absence of accurate understanding of the principles used for the formulation of the model, its limitations, and the values used for its parameterization. Primarily used as a tool to investigate the nature of an outbreak via hypothesis testing, we took a critical look at a state-transition epidemiological model, referred to as Epidemix, developed in collaboration with the Royal Veterinary College (UK), City University (Hong Kong), EpiInteractive (New Zealand) and the Pan-American Health Organization (PAHO) to assess the evolution of the COVID19 outbreak in Minnesota. By conducting the parameterization of the Epidemix COVID19 Model, we found significant variability in model output using parameters released by various public health organizations. Results highlight the intrinsic limitations of models as predictive tools and reinforces their utility for better understanding disease transmission and outbreak evolution. The experience helped us to understand the use and application of epidemiological modeling to understand the evolution of epidemics in a population, with an application to veterinary medicine and veterinary public health issues.

Research Grant: None
Student Support: University of Minnesota College of Veterinary Medicine Summer Scholars Program

Assessing gibbon interaction with auditory and environmental enrichment

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Gibbons are considered apes, specifically belonging to “smaller/lesser” apes, and are extremely vocal and communicate via song. The mp3 player elicits auditory and mental stimulation, while requiring tactile interaction, offering a cognitively engaging experience. This was done by the gibbons pressing the four different colored buttons on the device, initiating the different categories of sound. In addition to the auditory enrichment, environmental enrichment, which was categorized into stationary perches and moving swings/ropes, was observed in order to compare the efficacy of each. Part of our analysis included testing the hypothesis that items located in the top half of the enclosure received more use/interactions than items located in the lower half. We also hypothesized that the mp3 player was interacted with more readily and frequently when placed near a branch so that they may sit while exploring the device. The enclosures studied, as well as the sounds played on the device, alternated each week. We also compared enrichment interactions between times when food was present and when food was not present in order to observe any differences in their interest in interacting with enrichment. The data was analyzed via video and audio footage, which determined if the artificial enrichment is appropriate in encouraging species-specific behaviors. The project was designed to span 11 weeks with six weeks of on-site work, which began June 22, at the Gibbon Conservation Center in Santa Clarita, California. It is expected that the auditory stimulation will be a positive influence to the gibbons, however, they will be more inclined to interact with branches they can swing from due to their arboreal nature.

Research Grant: Small Grants Involving Student Projects- TEAM Gibbon Student Support
Student Support: WesternU Summer Research Fellowship Program, CVM Student Summer Research Scholar Program
Subtyping influenza A virus antibodies in Northern Elephant Seals

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Birds are reservoirs of all influenza A virus (IAV) subtypes, where some subtypes can infect other species. While the human pandemic H1N1 strain of IAV has been previously isolated from Northern Elephant Seals (NES), not all of the subtypes capable of infecting this species have been identified. Marine mammals may serve as mixing hosts due to their potential to be coinfected with avian- and human-origin IAV, a process that could allow for reassortment to produce new genotypes. The ultimate goal of this project is to define subtype-specific IAV antibody reactivity in NES on the California coast by measuring antibody titers for H1, H3, and H5 in archived ELISA-IAV positive NES sera. These serum samples were collected from NES at The Marine Mammal Center in Sausalito, CA in 2019. A hemagglutination inhibition assay was used to determine the titer of antibodies against H1, H3, and H5 subtypes in each of the 68 serum samples tested. 58/68 ELISA-positive NES serum samples tested positive for H1, with at least a 4-fold higher titers than for either H3 or H5 subtypes. 7/68 samples did not test positive for any H subtype. 3/68 samples had similar titers for multiple subtypes, which may reflect infection with one subtype and cross-reactivity, or alternately, previous infection with multiple H subtypes. These data suggest that most NES were infected by and/or possessed maternal antibodies to the H1 but not H3 nor H5 IAV subtypes. Further validation of the titer difference between animals with known subtype status would be needed to conclude that H1 is the only influenza subtype circulating in these elephant seals.

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Student Support: National Institutes of Health T35 OD010956

Host specificity of avian influenza virus internal genes in wild birds

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Surveillance of wild reservoir hosts of Avian Influenza Virus (AIV), including the Orders Anseriformes and Charadriiformes, is important to understand the distribution and evolutionary change of AIV. This study utilized publicly available curated sequences available through the Influenza Research Database. Six AIV internal gene segments that express 8 or more proteins, which are considered to be highly conserved, were analyzed to determine if the amino acid sequences of these 8 proteins varied amongst species, and to determine if evolutionary changes occurred over time. Isolates were divided into 23 subgroups by avian species, region, and sometimes hemagglutinin subtype. Single nucleotide polymorphism analysis provided an average sequence diversity score for each sequence, in addition to a consensus sequence, which was utilized for multiple sequence analysis. The amino acid sequences from all 8 proteins in wild ducks and shorebirds showed no distinct difference between species, or between region when sequences from Eurasia and North America were compared. Sequences of gull H13 and H16 subtypes from both North America and Eurasia shared more similarities with each other than with duck, shorebird, or gull subtype H1-H12 sequences in their respective regions. Gull H13 and H16 isolates from North America also showed unique patterns in their distribution of Non-Structural Protein 1 subtypes A and B, with 4 unique variants of subtype A found in analysis. High levels of conservation in all 8 proteins between duck and shorebird species were found, and temporal analysis revealed no evolutionary change, supporting that AIV is highly adapted to its’ wild avian hosts.

Research Grant: U.S. Department of Agriculture, ARS CRIS Project 6612-32000-066-00
Student Support: Boehringer Ingelheim Veterinary Scholars Program
**Human cathelicidin LL37 effects on *Mannheimia haemolytica***

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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.

**Research Grant:** College of Veterinary Medicine  
**Student Support:** College of Veterinary Medicine

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

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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 μg/ml and 0.1 μg/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.

**Research Grant:** Boehringer Ingelheim and USDA NIFA 12683397  
**Student Support:** Boehringer Ingelheim
Development of a comprehensive catalogue of testable canine genetic diseases

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Accurate canine genetic testing is a valuable diagnostic tool in veterinary medicine. It is also an effective way to safeguard the health of the broader canine population when it is made easily accessible to dog breeders. The aim of this project is to develop an online document containing the information necessary to conduct all routine canine genetic disease tests in North America. This will be the first step in achieving the long-term goal of establishing the Atlantic Veterinary College (AVC) as the first canine genetic testing centre in Atlantic Canada. Canine genetic test lists were obtained from thirteen well-established genetic testing centres in North America to create an exhaustive list of all canine genetic disease tests conducted on the continent. Genetic characterization of each disease was performed, identifying the mutation causing each disease so that the final test list would include each individual test without being redundant. All the tests were then categorized by physiologic system, based on how the tests were organized on the websites of origin and with further research done by team members. Each member of the research team was then able to take on the responsibility of one system, allowing each autonomy and uniqueness in their projects. Team members searched through the primary literature for the information required to create detailed documents regarding how each genetic test may be performed at the AVC. The resulting compilation of these documents will be a comprehensive catalogue of testable canine genetic diseases which will ultimately grant the veterinarians and greater public of Atlantic Canada easy access to all the canine genetic disease tests currently conducted in North America.

Research Grant: None
Student Support: Veterinary Student Research Award

Effects of Delta-Protocadherin-19 on neuronal growth and axon guidance

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The central nervous system development culminates with billions of neurons each containing trillions of active connections. Despite these massive numbers and the seemingly overwhelming input the brain performs detailed and precise tasks. How these neurons know how to organize and structure themselves, however, is unclear. Research focused around diseases with disrupted brain development including autism, epilepsy and schizophrenia have identified the Delta-Protocadherins subclass as factor critical to proper CNS organization and development. Another study has identified differing combinations of Delta-Protocadherins expressed within functionally distinct regions of the spinal cord. On top of that It has been shown that that Delta-Protocadherins are homophilic molecules that foster cell to cell adhesion. Using these three ideas we have a model that differing expression of Delta-Protocadherins by neurons in different regions of the CNS are responsible for the organizational development and segregation of neuron. Our experiments find an improved growth rate and axon length for neurons grown on Delta-Protocadherin 19 over PEI. We use a crisscross pattern to simulate choice and found a significant preference for axons to grow on Delta-Protocadherin 19 rather than PEI.

Research Grant: None
Student Support: NIH T35 Training Grant T335OD012199
Tumor-restrictive role for type III collagen in the breast cancer microenvironment

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The development and progression of cancer is influenced by interactions with non-cancer cells and extracellular matrix components, such as fibrillar collagens, in the tumor microenvironment (TME). Our group has previously shown important regulatory roles for type III collagen (Col3) in healing tissue and in laboratory and companion animal models of breast cancer. Using a murine orthotopic model of triple negative breast cancer (TNBC), our data revealed increased primary and recurrent tumor growth with a higher rate of metastasis in Col3 haploinsufficient (Col3⁻/⁺) mice compared to wild type (Col3⁺/⁺) mice. We further evinced that Col3 suppresses formation of a tumor-permissive microenvironment as Col3⁻/⁺ mice tumors had increased desmoplasia, fibrillar alignment, and myofibroblast density relative to Col3⁺/⁺ mice. Notably, application of Col3 biomaterials hindered orthotopic tumor growth compared to control. Our recent preliminary studies immunolabeling human TNBC samples demonstrate elevated Col3 deposition in non-invasive versus invasive regions (P < 0.01). To further characterize patterns of Col3 expression and identify molecular signatures of at-risk patients, we are using in silico analyses of over 1000 breast cancer patients from The Cancer Genome Atlas. Initial results indicate that patients with higher Col3:Col1 bulk tumor expression had improved disease-free and progression-free survival relative to those with higher Col1:Col3 expression (P < 0.05). The combination of these data not only reaffirm a tumor-restrictive role for Col3 in breast cancer, but also suggest that using tissue engineering strategies to promote a Col3-rich TME may improve clinical outcomes by limiting tumor recurrence or progression in patients.

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Retrospective evaluation of sea turtle carapace trauma in strandings to the west coast of Florida using CT

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Carapace trauma is a significant cause of sea turtle strandings and mortality. In Florida, carapace injuries are especially prevalent due to the soaring number of recreational vehicles that are used in coastal waterways. A correlation has been established between the number of recreational boats registered in an area and the number of aquatic wildlife boat strike injuries and deaths. Radiographs traditionally used for evaluation are limited to two dimensions, with significant superimposition with the appendicular skeleton and plastron. Utilization of computerized tomography (CT) expands diagnostic capabilities and allows for additional examination of body systems through 3-D reconstruction. In partnership with a local hospital, every sea turtle that presented to the Clearwater Marine Aquarium from 2006-2019 was evaluated with CT. It was hypothesized that the most common cause of trauma in this population of sea turtles would be attributed to vessel strike injury. The objectives of this retrospective CT imaging study were to evaluate and categorize carapace pathology in wild sea turtles, examine the frequency of spinal cord injuries associated with carapace damage, investigate the relative prevalence of propeller versus blunt carapace trauma, and consider the relationship between carapace trauma with head and extremity trauma. The study determined a 20% incidence of carapace injuries in the population involving blunt and propeller watercraft trauma. These injuries were divided into pathology trauma categories including: injuries with spinal cord involvement, without spinal cord involvement, articulation changes between dorsal scapula and carapace, and occasional invasive papillomas involving the boney carapace.

Research Grant: None
Student Support: None
Effect of proteinase inhibition on glucagon-like peptide-2 concentrations in blood samples from healthy cats

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Glucagon-like peptide-2 (GLP-2) is a 33-amino-acid peptide hormone secreted by gastrointestinal (GI) L-cells in response to enteral nutrition. Actions of GLP-2 include enhancing GI barrier function and increasing GI mucosal growth. Measurement of circulating active (1-33) GLP-2 is challenging due to rapid cleavage by the enzyme dipeptidyl peptidase IV to inactive (3-33) GLP-2. Previous studies evaluating methods of GLP-2 collection showed the use of proteinase inhibitors within the sample as the only method that delayed peptide degradation and resulted in higher measured concentrations. The objective of this study was to compare concentrations of GLP-2 in feline plasma collected with or without proteinase inhibitors. Six healthy, client-owned cats were enrolled prospectively. A pre-prandial blood sample was obtained after at least a 10-hour fast, cats were fed a standardized meal, and a second 1-hour post-prandial sample was collected. Blood was collected into chilled EDTA tubes on ice and immediately centrifuged (temperature controlled), separated, and stored at -80° C. At the time of collection, half of each blood sample was mixed with 10% volume of the proteinase inhibitors Aprotinin and Diprotin A per sample volume. Plasma GLP-2 was measured with a commercial ELISA marketed for cats; all samples were run in duplicate. GLP-2 concentrations were compared between samples collected with or without proteinase inhibition using a paired t test. There was no significant difference in GLP-2 concentration between samples with and without proteinase inhibition (pre-prandial $P = 0.96$; post-prandial $P = 0.55$). Findings may be due to differences in feline GLP-2 degradation or other sample collection variables.

Research Grant: None
Student Support: Chet Peterson Family Scholarship and KSU CVM Office of Research

The effect of platelet lysate on biofilm development

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Infections that occur in tissues containing a biomedical device are a major medical challenge and are complicated by the development of bacterial biofilm rendering the infection difficult to eradicate. Bacteria within a biofilm are protected by an extracellular matrix that inhibits the host’s immune response and the penetration by traditional antibiotics. Because of these concerns there is a pressing need to identify treatment alternatives to antibiotics especially for biofilm-mediated infections. An acellular platelet product known as platelet lysate (PL), manufactured in our lab from donor horses, has shown promising antimicrobial activity by directly inhibiting bacterial growth. We hypothesize that PL will sustain its antimicrobial effects in the presence of biofilm. In a preliminary study we have shown that PL effectively reduces *Pseudomonas aeruginosa* (PA) dependent biofilm formation and enhances its breakdown. We now plan to determine the mechanisms by which PL affects the structural polysaccharides and proteins in biofilm. Using 24-well culture plates, biofilm will be formed in empty plates or plates containing stainless steel (a commonly used surgical biomaterial) and treated with PL or regular growth media. After a 48-hour treatment period, bacteria will be recovered to determine viability by counting colony forming units. Separately, biofilms will be tagged with antibodies and imaged with confocal laser scanning microscopy to evaluate changes in matrix components. We expect that PL will reduce viable bacteria and affect the biofilm matrix composition. These results will provide valuable information for future studies focused on developing PL as a biological antimicrobial treatment against biofilm.

Research Grant: Morris Animal Foundation
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine
Methods development for quantitative assessment of head motion in adult horses

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Current methods used to score sedation level in the standing horse are based on two factors: depth, normally assessed based on the height of the horse’s head above the ground (HHAG), and quality, the horse’s response to stimulation/procedures. While the HHAG measurement provides insight to sedation depth, it does not assess sedation quality, which commonly relies on descriptive scoring systems that are subjective and can be difficult to analyze statistically. Tracking the motion path of a horse’s head over time may represent a objective method to indicate sedation level. The aim of this study is to identify a software that can be used to track and quantify horse head motion, and to use this technique to develop a method for quantifying sedation depth and quality in standing horses. Results from this study review multiple programs used to track object motion. An accurate and successful technique to track the motion of any round object with a user specified color was found using Python and OpenCV. Relying on centroid tracking to find the contour of a given object of interest, a purple dot, and the hue, saturation, and value color space to separate the purple dot from its background, this program can be applicable to track horse head motion in standing horses, given that the animal is fixed with such dot located centrally on the forehead.

Research Grant: Unknown
Student Support: Florida Veterinary Scholars Research Program

Development and analyses of a database of organizations providing animal care in northern Canada

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Previous research done by Veterinarians without Borders/Vétérinaires sans Frontières (VWB/VSF) has demonstrated that a large proportion of northern Canadian communities have little to no access to veterinary care. Due to long distances and high costs of travel, animals in these remote communities are usually not sterilized, leading to population control problems, and they often suffer from preventable and/or treatable diseases, leading to animal welfare issues and shorter life spans. In an attempt to ameliorate these issues, there are a number of organizations that volunteer in some of these northern Canadian communities to provide animal health care on a temporary and piecemeal basis. The purpose of this study was to create a database of these organizations and to record information on their work, including locations, species seen, and services provided, among other parameters. Preliminary results include 42 organizations and 16 veterinary clinics working across seven Canadian provinces and three territories. The majority of these volunteer organizations work with northern Canadian Indigenous communities. These organizations provide varied services, including sterilization, vaccination, deworming, telemedicine and re-homing. Most organizations focus on dogs, however cats and other species are also seen. Overall, this database will allow VWB to better facilitate and coordinate collaboration between groups providing animal care services to underserved northern Canadian communities.

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Student Support: Student Stipend: VetSRA at the Atlantic Veterinary College
A comparison between a commercial and 5 heritage turkey breeds: age effects on weight gain and immune indices

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Currently, commercial turkeys are bred for high feed conversion rate, rapid growth rate, carcass size, white coloring, and ability to reproduce by artificial insemination. The culmination of these selection criteria is a commercial bird with superior feed efficiency and growth ability, however, these birds cannot reproduce naturally nor exist in a free-range environment. Heritage turkeys, native to the United States, have a much slower growth rate, can breed naturally and thrive in free range conditions. The present study compared the growth and select immune parameters of 5 breeds of Heritage turkeys to one commercial line, British United Turkeys (BUT). Birds (n = 20 birds/breed) were weighed and bled at 9, 11, and 13 weeks. Packed cell volume (PCV), total protein (TP) and blood smears were performed from whole blood. Peripheral blood lymphocytes were aseptically enriched from 8 mL of whole heparinized blood, enumerated and cultured with a T cell (Concanavalin A, Con A) and pan lymphocyte (Phorbol myristate acetate and ionomycin, PMA/I) mitogen to assess lymphocyte proliferation. As predicted, the BUT birds consistently outperformed the Heritage breeds throughout the study ranging between a 2.0 - 2.5X higher weight gain (kg). Heritage turkeys consistently had numerically higher PCV and TP values compared to the BUT turkeys. A functional evaluation of the lymphocytes revealed that the Heritage turkeys demonstrated a more robust T cell and pan-lymphocyte proliferation than the BUT birds. Our preliminary data suggest that genetic selection based primarily on production parameters in a commercial turkey line negatively impacts physiological and immune indices.

Research Grant: The Livestock Conservancy, PO Box 477, 33 Hillsboro St, Pittsboro, NC 27312
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Modeling mitochondrial dynamics in response to cartilage injury

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Post-Traumatic Osteoarthritis (PTOA) is a degenerative disease of articular cartilage which develops after joint injury, as a result of cellular dysfunction. Pain and joint dysfunction can be delayed years after injury, and by that stage, no treatments can slow PTOA progression. Therefore, intervention soon after injury may be necessary to modify the disease’s course. Emerging evidence indicates that mitochondria (MT) dysfunction is an important early mediator in the pathogenesis and progression of PTOA. MT dysfunction is directly related to the magnitude of mechanical injury (strain) experienced during impact by the cartilage cells, chondrocytes. Strain also disrupts MT dynamics, which influence whether the cell undergoes apoptosis or survives. The known mechanisms for transmission of mechanical forces through the cartilage to chondrocyte MT include the cell adhesion system, cytoskeleton, and mechanosensitive ion channels. However, the role of mechanical stimuli in MT dynamics and the relative importance of these mechanotransduction pathways in the fate of injured chondrocytes is unclear. Mathematical models of MT dynamics already exist in other tissues and are effective at predicting outcomes, but have not included the effects of mechanotransduction. Our objective is to model the changes in MT dynamics in cartilage after injury with a system of equations, predict what mechanotransduction pathways most influence cell fate, and which variables may be experimentally manipulated to improve outcome after injury. This will inform our approach to developing targeted interventions to prevent ongoing cartilage degeneration after injury and PTOA.

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Student Support: NIH Short Term Training for Students in the Health Professions Award 5T35A1007227
The influence of pain on metastasis in dogs with splenic hemangiosarcoma

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Surgical pain promotes tumor growth in rat models. To assess the translational relevance of that phenomenon, the objective of this study was to determine whether perioperative analgesic selection influences the rate of metastasis in dogs with splenic hemangiosarcoma (HSA). This study was a single institution, retrospective case series of 34 dogs with non-metastatic (i.e., stage I or II) splenic HSA having undergone splenectomy + doxorubicin. Medical records (2010-2020) were reviewed. Recorded data included: use of pre-, intra-, peri-, and post-op. analgesics, and time from surgery until metastasis and/or death. Kaplan-Meier statistics were used to calculate median metastasis-free survival time (MMFST) and median overall survival time (MOST) with respect to local anesthetic use; survival between groups was compared via Log-Rank testing. MMFST for dogs receiving an intra-operative local anesthetic was 115.5 days (95% CI: 31-180) versus 71 days (95% CI: 24-175) for those that did not (P = 0.25). MOST for dogs given a local anesthetic and those not given one, was 155.5 days (95% CI = 59-315) vs. 131 days (95% CI = 45-176) respectively (P = 0.17). Use of intra-operative local anesthetics had no detectable impact on MFST or OST. However, the sample size was relatively small, and there are non-significant differences that are supportive of our hypothesis. Differences in OST/MFST dependent on analgesic usage suggests a correlation between pain and tumor behavior and could influence future analgesic considerations for oncology patients. Future aims are to expand sample size to improve statistical power, as well as analyze pre-, peri-, and post-operative analgesic data.

Research Grant: None
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070

Diagnostics and pathology of ophidian serpentovirus infections

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The following presents a systematic narrative review of all ophidian nidovirus publications, with a goal to compile diagnostic criteria for clinical practice. Nidoviruses were first documented in reptiles in 2014, and are now classified in the Serpentovirinae subfamily of Tobaniviridae and colloquially named serpentoviruses. They occur primarily in pythons (family Pythonidae) and boas (family Boidae), but have also been documented in the shingleback skink (Tiliqua rugosa) and Bellinger River snapping turtle (Myuchelys georgesi). Clinical signs include increased oral mucus secretion, increased respiratory effort, anorexia, weight loss, and reddened oral mucosa. Histological changes include pharyngitis, stomatitis, sinusitis, tracheitis, and esophagitis, all with pronounced epithelial hyperplasia; changes in the lower respiratory tract consist of proliferative interstitial pneumonia. Secondary bacterial bronchopneumonias are common. More severe pathological changes, especially in the lower respiratory tract, occur later in the disease process. Asymptomatic infection can also occur, and a recent screening yielded no correlation between symptoms and positive nidovirus infection. Viral RNA can be identified via PCR, and sampling recommendations include antemortem oral or choanal swabs and tracheobronchial lavage. Postmortem sampling should utilize tissue from the lung and proximal esophagus. Serpentoviruses represent an emerging pathogen that spreads easily in captive snake collections and has a mortality of up to 75%, both of which advocate the importance of clinician awareness and clinical diagnostics.

Research Grant: None
Student Support: Morris Animal Foundation
Assessing agreement between cytologic and histopathologic diagnoses in dogs

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Cytology is routinely used in veterinary medicine to diagnose neoplastic, inflammatory, and other lesions. Yet, there are limited veterinary studies quantifying accuracy, sensitivity, specificity, and predictive values of cytology compared to histopathology. Even fewer follow the Standards for Reporting of Diagnostic Accuracy Studies (STARD) Guidelines, which were developed to increase rigor and transparency in diagnostic studies. The objective of this project was to create a database of canine cases with cytologic and histopathologic diagnoses obtained from the same anatomic site to serve as the foundation for future accuracy studies. The NCSU Veterinary Hospital electronic medical records were searched between 2015-20 for canine patients with cytology and histopathology results from the same site collected within 30 days. Cases were excluded if the location of test sample was not identical. For each site, the primary cytologic and histopathologic diagnoses were identified. Agreement between diagnoses was defined as complete, partial, non-agreement, or non-comparable (e.g. non-diagnostic sample). Of 343 cases, the most frequent sites were liver, lymph node, subcutaneous tissue, skin, bone marrow, spleen, bone, nose, and oral cavity. Overall, cytology and histopathology diagnoses were in complete or partial agreement for 68.2% of cases. Site-dependent differences in agreement were noted. This foundational work will form the basis for future studies that follow STARD guidelines and also serve as a critical training resource for veterinary students and residents.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Smart farming: application of time of flight cameras for sow lameness detection

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Growing public concern about the welfare of gestating sows is driving changes in husbandry practices as producers are being asked to move away from individual to group housing of sows. However, increased incidence of sow lameness is often reported with group housing. Lameness is both a production and welfare problem, but early detection remains challenging as existing metrics are notoriously unreliable and must be done manually. Thus, reliable early detection of lameness promises improved therapeutic outcomes, increased productivity, and improved welfare. This investigation uses time of flight cameras (ToF) to look for correlations between lameness and select features of a sow’s dorsum as she walks, as determined by both an intuitively derived formula and machine learning. Preliminary data tracked the shoulders and hips of sows as they walked as a novel way to evaluate movement- fitting this height to sinusoidal functions. Sound gilts (n = 2) produced larger amplitude coefficients whereas lame (n = 2) produced smaller, as consistent with constrained movement, and therefore a threshold could be determined to delineate between the two. The same preliminary data produced 208 healthy and 429 lame images. Of 500 identified features, which are prevalent in at least 80% of the either set, only 26 features were used to train a Nearest Neighbor Classifier. This classifier has 93.6% accuracy, and was validated with a 10-fold cross validation. Moving forward, sows will be trained to walk 13 [m] between two feed boxes, and the number of laps in 15 minutes will be taken as a measure of lameness. This will be correlated to images taken while running the obstacle course, as well as visual scoring beforehand.

Research Grant: PA Department of Agriculture
Student Support: NIH T35 Training Grant (NIH-BI Summer Research Program)
Behavioural consequences of acute thyroidectomy-induced hypothyroidism in pigs

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Certain swine diseases are associated with a transient hypothyroidism characterized by reduced circulating triiodothyronine (T3) and thyroxine (T4). Two pilot studies were conducted. Experiment 1 examined the rate of onset of hypothyroidism using six 5-week-old pigs in two groups: surgically thyroidectomized (ECTOMY; n = 3) and sham surgery (SHAM; n = 3). Indwelling jugular catheters were placed during surgery. Serum T3 and T4 levels, measured by radioimmunoassay (RIA), decreased rapidly following thyroidectomy from a mean of 94.4 (± 57.0) ng/dl (T3) and 7.3 (± 1.2) µg/dl (T4) pre-surgery to 22.7 (± 10.7) ng/dl and 1.5 (± 0.2) µg/dl, respectively, 48 h post-surgery while levels in SHAM remained constant. Experiment 2 assessed consequences of acute hypothyroidism and included eight 5-week-old pigs: ECTOMY (n = 4) and SHAM (n = 4). Serum T3 and T4 levels were similar between groups prior to surgery but by 4 d post-surgery both were significantly lower in the ECTOMY group (21.4 (± 10.1) ng/dl T3, 0.9 (± 0.2) µg/dl T4) compared to SHAM (97.4 (± 26.4) ng/dl T3, 3.6 (± 0.7) µg/dl T4). Average daily gain and feed and water disappearance were similar between groups pre- and post-surgery (Mann Whitney test). Changes in the postural/behavioural time budget were assessed using video footage (two days, both pre- and post-surgery, 6 h per day) transcribed in three-minute scans. A qualitative behavioural analysis (QBA) was used to score demeanour using a 0 to 10 scale. Changes pre vs post surgery within group were evaluated using the Wilcoxon signed-rank and Sign tests of matched pairs. There were non-significant numerical changes in several behavioural outcomes in both groups, however no clear pattern related to acute hypothyroidism was evident.

Research Grant: Saskatchewan Ministry of Agriculture and Food, Genome Canada, and Genome Prairie
Student Support: Natural Sciences & Engineering Research Council, Boehringer Ingelheim Veterinary Scholars

Expression and localization of fibroblast growth factor receptor (FGFR1) during feline renal development

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Fibroblast growth factor receptor (FGFR) signaling is one of the main signaling mechanisms known to be indispensable for mouse and human renal development. However, the expression and localization of FGF receptors during feline renal development is not known. We hypothesized that FGFRs are expressed in developing feline kidney during embryonic and fetal stages. NCBI sequence similarity search tool, BLAST was used to determine feline homologues of four FGFRs, (FGFR1-4) and the percent identity between protein sequences of feline and human FGFRs. Furthermore, we analyzed the expression and localization of FGFR1 in the fetal feline kidneys by western blot analysis and immunofluorescence. NCBI BLAST results showed that at the amino acid level, human and feline FGFR1, FGFR2, FGFR3 and FGFR4 share 99.15%, 96.32%, 92.73%, and 94.17% similarity, respectively. A FGFR1 antiserum well-characterized to recognize human FGFR1, detected FGFR1 expression in feline fetal kidney extracts. Co-immunofluorescence analysis showed that in the embryonic feline kidney, FGFR1 was expressed in nephron progenitor population, that was identified by NCAM1 expression. However, FGFR1 expression was undetectable in ECAD positive ureteric bud. Similar, FGFR1 expression pattern was observed in nephron progenitors and ureteric bud of feline fetal kidney. Together, our results determine FGFR1 expression and localization in developing feline kidney and suggest that FGFR1 may contribute towards nephron progenitor maintenance and feline renal development.

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Student Support: National Institutes of Health project# 5T35OD010432
Placental pathology after local MCP-1 injection at the nonhuman primate maternal-fetal interface

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Fetal Growth Restriction (FGR) is thought to be at least in part a result of hypoxia at the maternal-fetal interface (MFI), and can result in serious postnatal complications for the neonate. At this time, there are few diagnostic tools available to identify fetuses at risk for FGR. Magnetic Resonance Imaging (MRI) can be a useful tool for determining blood flow and perfusion, and has been previously utilized to study the macaque and human placental intervillous space. Therefore, we sought to induce an inflammatory response and vascular histopathology using an injection of monocyte chemotactic protein-1 (MCP-1) at the MFI in hopes to decrease blood flow to the placenta, to ultimately define the ability of MRI as a diagnostic tool for FGR. The goal of this pilot study is to analyze the effects of MCP-1 at the maternal-fetal interface and begin outlining the appropriate dosage of MCP-1 for future study. Two cynomolgus monkeys were allocated to receive either a saline injection + vehicle or 100 micrograms of MCP-1 into the placenta. Data from CBC’s and dual immunohistochemistry staining for macrophages (CD68/CD163) is being analyzed for immune responses and differing immune cells at the maternal-fetal interface. Analysis is also being conducted on ultrasound measurements (fetal biparietal diameter, head and abdominal circumference, and femur length) as well as fetal necropsy results to begin preliminary judgment if the injection led to inflammation; causing fetal restricted growth.

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Student Support: Boehringer-Ingelheim Summer Scholars Program

Development and validation of a point-of-care RAMP assay for the detection of SARS-CoV-2 in cats

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an emerging pathogen that is being detected on a pandemic-scale within human populations. SARS-CoV-2 is an enveloped, single-stranded RNA virus that has been shown to propagate in different feline species in addition to humans. Cats infected with SARS-CoV-2 can suffer from diarrhea, vomiting, coughing, and shallow breathing among other clinical signs. Preliminary research suggests that the virus could be transmitted to cats from infected humans. More research is still needed to determine the ability of the virus to transmit from cats to humans and cause a zoonotic infection. Therefore, it is important to develop and validate tests to detect SARS-CoV-2 in specimens from cats. Using novel technology, a point-of-care assay was developed to determine the prevalence of the virus in domestic cats to better gauge human exposure. The technology chosen uses recombinase polymerase amplification followed by loop-mediated isothermal amplification within the same reaction unit to specifically amplify and detect a viral gene. This “RAMP” assay was developed and validated using guidelines set by the OIE. The isothermal nature of the amplification reagents for the test, in addition to an extraction-free protocol, will allow it to be used as a point-of-care assay for straightforward application. This test will offer veterinarians a quick, cost-effective, and reliable method to screen for the presence of SARS-CoV-2 in cats using a simple oropharyngeal swab. Packaging of this test into a point-of-care device will be pursued through collaboration with the University of Pennsylvania School of Engineering with the goal to offer it to the general veterinary population.

Research Grant: University of Pennsylvania Research Foundation Award 19-0945
Student Support: NIH T35 Training Grant 5T35OD010919
Does postpartum calcium supplementation interfere with homeostasis of dairy cows?

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Hypocalcemia is a common condition in the immediate postpartum period that affects dairy cows due to an increased loss of calcium through the production of colostrum and milk. This condition is met with detrimental effects to cow health and production, which can be seen clinically as milk fever or subclinically without apparent signs. Our study objective is to evaluate how different postpartum calcium supplementation methods affect blood parathyroid hormone, calcium, and calcitonin concentrations. We will conduct a randomized controlled trial in 64 multiparous dairy cows on a commercial dairy in New York. Cows will be randomly assigned to one of four treatment groups immediately after calving: 1) no calcium supplementation (control), 2) 43 g oral calcium bolus immediately after calving and at 1 day in milk, 3) 43 g oral calcium bolus at 2 and 3 days in milk, or 4) 500 mL subcutaneous infusion of 23% calcium borogluconate. Blood samples will be collected at calving and approximately 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, 120, and 168 h after calving, processed on the farm, and frozen until analysis. Our results will aid in preventing and improving treatment of hypocalcemia in dairy cows by increasing our understanding of the effects of calcium supplementation methods on endocrine function and return to normal calcium status postpartum.

Research Grant: Veterinary Investigator Program T35 NIH OD0010941
Student Support: Veterinary Investigator Program T35 NIH OD0010941

Secondary analysis of contact between adults and household pets

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The COVID-19 pandemic calls for research into human-pet interactions, both to keep pets safe and to better understand potential household transmission events. Using existing survey data, this project characterized direct contact between inner-city adults with asthma and their household pets—dogs (n = 8), cats (n = 10), and other species (n = 9). The application REDCap was used to generate data reports, and figures were then created in Microsoft Excel. Human-animal contact scores were also calculated using Morris’s direct contact paradigm (Morris et al., 2011). Dogs received the highest contact scores, with median score 6 out of 9, while cats and other species received median scores of 4 and 3, respectively. Of the scoring parameters, 50% of both dogs and cats shared a bed with a household member, and hand-licking was reported for 88% of dogs and 60% of cats. 100% of both dogs and cats also contacted other adults in the household, with dogs experiencing the greatest daily contact frequency for all but one demographic group. Ultimately, this project supplements other human-animal contact studies, contributing data for use in future epidemiological models.

Research Grant: NIH T35 OD024982
Student Support: NIH T35 OD024982
Detecting prevalence of digital dermatitis using computer vision software for beef cattle

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Digital dermatitis (DD) is a superficial infectious claw disease localized at the plantar/palmar interdigital space of cattle feet (Dopfer, 2009). Although commonly associated with dairy cattle, beef cattle also suffer from DD as an infectious cause of bovine lameness. Safe, efficient and, early detection that allows for a means of collecting prevalence herd data of DD is a missing piece to beef management in today’s industry. We hypothesized that computer vision models can be used to accurately detect DD and be utilized to obtain accurate records of DD prevalence in beef feedlots. Video footage of cattle feet was obtained at hoof level by a trained veterinarian in beef cattle feedyards. Each video was broken into individual images and labeled using YOLOv3 format (Redmon, et al.) with M-Stage scoring system as defined by Dopfer, et al. (1997). These images served as the dataset for training a real time object detection model with Computer Vision (CV) in a Darknet framework (Redmon, et al.). External and internal validation of model predictions for detecting DD lesions will be conducted to quantify detection accuracy. With the model output a prevalence list of detection results will be created and translated into a treatment list. Digital dermatitis is a global well-being concern in cattle industries. Detection of DD takes time, training, equipment, and can be dangerous for producers and veterinarians. With CV there could be more effective treatment, better decision making about time and frequency of hoof baths in beef cattle and decreased prevalence of DD. Prospects are that this system can be used for the detection of other infectious diseases in food animals.

Research Grant: Program Revenue Funds from Doepfer Lab
Student Support: Boehringer-Ingelheim Summers Scholars Program

RDW to predict patient outcome of critically ill dogs and cats in the OVC HSC—preliminary findings

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Increased red blood cell distribution width (RDW) has been demonstrated as a parameter associated with adverse outcome in humans with critical illness. As a standard component of a complete blood count (CBC), RDW is a commonly measured and readily available parameter in veterinary medicine. Being able to utilize RDW to predict patient outcome in a clinical setting could help with decision making in regards to diagnostics, treatment, and in assessing prognosis. The primary outcome was to determine if RDW could predict patient all-cause mortality. The secondary outcome was to determine if RDW could predict the patients length of hospitalization. The patient population in this retrospective observational study consists of dogs and cats consecutively admitted to the Intensive or Intermediate Care Unit at the Ontario Veterinary College Health Sciences Centre (OVC HSC) between 04/01/2019 to 09/22/2019. The data is currently under analysis. Results will be available soon.

Research Grant: None
Student Support: Boehringer Ingelheim
A modern formulation for a previously used drug and its effect on *Fasciola hepatica*

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Cattle infected with *Fasciola hepatica* have clinical signs of general unthriftiness, low milk production, anemia, hypoproteinemia with a sequela to bottle jaw, hepatic fibrosis, and blocked or calcified bile ducts. Currently, there are limited drugs available in the United States labeled for use against *F. hepatica*. Drugs on the market have limited efficacy on all life stages of *F. hepatica*. The goal of this new drug formulation is to offer a parasiticide that targets all life stages of *F. hepatica* with an overall efficacy of > 90%. Our objective is to isolate *Fasciola hepatica* from animals infected, remove the parasites, and grow a colony of *F. hepatica* for use in testing. This study will determine the efficacy of the new formulation on killing all stages of *F. hepatica*. We hypothesize this new drug formulation will result in higher killing efficacy at all life cycles when compared to other drugs labeled for *F. hepatica* in the United States. In this study, ovine livers infected with *F. hepatica* will be processed in order to remove the adult *F. hepatica*. Eggs harvested from the adults harvested will be grown in vitro into metacercaria. Upon maturation of *F. hepatica* to metacercaria, they will be placed in vivo to be used in efficacy studies for our new formulation. We expect to see an overall efficacy of > 90% on all life stages of *F. hepatica*. Such results would indicate the new formulation to be a new leader in the control of *F. hepatica*.

Research Grant: None
Student Support: Boehringer Ingelheim

Development of ELISA protocol for novel goat coronavirus and survey of exposed herds in California and Oregon

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Coronaviruses are pathogens that lead to respiratory and gastrointestinal disease in a range of animals including humans, cattle, small ruminants, other mammals, turkey, and other avian species. A few studies have investigated the seroprevalence of coronaviruses in small ruminants, such as sheep and goats, and have found only very low levels. The first recorded instance of coronavirus infection in goats within the United States, was in 2017. During two separate goat shows in Northern California, outbreaks of enteric and respiratory disease were observed. The virus was identified by fecal PCR, and sequencing revealed a novel coronavirus closely related to bovine coronavirus. The purpose of this study was to determine the within-herd prevalence of this novel coronavirus in show goats in Northern California and Oregon. Specifically, this study used goat serum samples collected in 2017, 2018 and 2020 from herds with known exposure and lack of exposure, to validate an ELISA protocol for screening goats’ for immunity to this novel virus. This was done using an ELISA kit commonly used for bovine coronavirus modified for use with goat serum. Following validation of this protocol, ELISA analysis was conducted on goat herds with risk of exposure to the novel coronavirus. The results from these analyses indicate that goats in Northern California and Oregon do have significant exposure to this virus. The next steps of this project are to continue to determine within herd immunity to the coronavirus in order to determine to prevalence duration of seropositivity.

Research Grant: Center for Food Animal Health, UC Davis
Student Support: STAR Research Awards Lider and Maddox Scholarship (STAR)
The impact of ischemic stroke on motor function in adult male mice

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Ischemic stroke is a leading cause of death worldwide. Nutrition is a modifiable risk factor for stroke. In particular, dietary deficiencies in one-carbon (1C) metabolism have been linked to an increased risk of stroke. 1C is responsible for the metabolism of homocysteine, which has been shown to increase stroke risk when excess levels build up in the blood. The main component of 1C is folic acid, which is well known for its role in the closure of the neural tube during in utero development. Prior research from our laboratory has shown that maternal dietary deficiencies in 1C metabolism impact brain function and behavior in juvenile offspring. However, the life-long impact of these dietary deficiencies on an adult offspring’s vulnerability to cardiovascular diseases, such as stroke, requires further investigation. The aim of our study was to investigate the impact of maternal dietary deficiencies in 1C on stroke outcome in adult offspring. We induced ischemic stroke in the sensorimotor cortex using the photothrombosis model in adult male mice born to nutrient deficient mothers. To assess stroke outcome, motor function was evaluated using the accelerated rotarod, ladder beam, and forepaw placement task. Brain tissue and plasma was collected and measured for changes in developmental factors. Preliminary data focusing on stroke outcome will be presented. The completion of this study will generate new knowledge, which includes identifying developmental markers to improve current treatment for stroke affected individuals by distinguishing and refining the process of stroke recovery.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

Prevalence of multidrug resistant staphylococci in canine dermatological patients over a two year period

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Methicillin-resistant (MR) and multidrug resistant (MDR) staphylococci are a prominent group of pathogens causing cutaneous infections in companion animals. The treatment of these infections often requires multiple courses of systemic antibiotics increasing the likelihood of selecting for more resistant strains. The objective of this study was to determine the prevalence of MR and MDR staphylococci in dogs with skin diseases seen at the University of Florida over the past two years. Medical records of dogs seen by the dermatology service at the University of Florida from 2018 to 2020 were retrospectively searched in the electronic medical system Cornerstone. Dermatological patients with at least one positive culture for S. pseudintermedius were selected. The results indicate that between April 2018 and April 2020 alone, the percentage of MR S. pseudintermedius isolates have increased from 66.7% to 80%, and MDR isolates have increased from 50.9% to 65%. The number of antibiotic classes these isolates were resistant to also increased from an average of 4 classes in 2018 to an average of 6 classes in 2020. These increases provide supporting evidence of a widespread presence of MDR staphylococci in canine dermatological patients. Broad-spectrum antibiotic use may be a contributing factor to antibiotic resistance in staphylococci. In veterinary medicine, careful consideration should be given in the development of treatment plans for these infections.

Research Grant: Florida Veterinary Medical Association
Student Support: Florida Veterinary Scholars Program
Investigation of virus evolution in SARS-CoV-2 clinical samples from experimentally infected cats

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Understanding mutation rates of RNA viruses, including coronaviruses such as SARS-CoV-2, is starkly important for disease control measures. High mutation rates in RNA viruses usually result in accumulation of genetic mutations, potentially altering viral phenotype. In this study, we evaluated the replication fidelity of SARS-CoV-2 genome after cross-species transmission by analyses of the (a) RNA genome of the human USA-WA1/2020 SARS-CoV-2 after transmission to cats, and (b) uniqueness and quantity of SARS-CoV-2 quasi-species in cat samples compared to original USA-WA1/2020 inoculum. Samples (nasal, rectal, and oropharyngeal swabs, bronchoalveolar lavage fluid, and mucus) were obtained after intranasal/oral challenge of cats with SARS-CoV-2 isolate USA-WA1/2020. SARS-CoV-2-RNA from 29 samples was isolated and sequenced via next-generation sequencing (NGS). Consensus sequences from samples were determined and compared to the reference USA-WA1/2020 sequence to identify nucleotide changes. Regions analyzed are 1,254 base pairs (N gene), 222 base pairs (E gene), and 21,289 base pairs (Orf1ab gene). Consensus sequences deviated from the reference USA-WA1/2020 sequence in the Orf1ab gene by a range of 1-6 nucleotide changes per consensus sequence when compared to reference Orf1ab. No mutations were observed in the N and E gene regions. Identified nucleotide mutations observed after cross-species transmission of SARS-CoV-2 will be further analyzed for their phenotypic impact. This work is crucial in our understanding of mutational changes in the SARS-CoV-2 genome due to cross-species transmission and novel host adaptation, and will aid in the development of effective therapeutics, vaccines and diagnostics for animals and humans.

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Student Support: Elanco Veterinary Scholars Program

Identifying diagnostic sRNA targets in infected hosts using computational approaches

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Early diagnosis of many agriculturally relevant zoonotic diseases has always been problematic since the hosts often remain asymptomatic until it’s too late. The second main problem has been with accurate and sensitive diagnosis often confounded by closely-related and environmental bacteria. We, therefore, focus on developing a computational workflow to identify pathogen-specific diagnostic targets that can be detected in infected hosts. Small RNA (sRNA) is a class of RNA that is used regularly in cellular housekeeping and is critical for gene regulation in immunological and developmental contexts. Upon infection, changes could occur in both host and bacterial sRNA. We are interested in identifying bacterial sRNA that are pathogen-specific, making them excellent candidates for diagnostic biomarkers. Although intra-and extracellular sRNA have been used extensively in cancer detection (host miRNA), few studies used pathogenic sRNA as a diagnostic target. Here, we propose computational approaches to detect pathogenic sRNA in infected host samples. The computational workflow will be used to identify pathogenic sRNA in sequenced sera from white-tail deer (Odocoileus virginianus) infected with bovine tuberculosis (Mycobacterium tuberculosis bovis). We will use existing sRNA databases, host and pathogen reference genomes, measure differential expression, and benchmark against existing sRNA pipelines to help us discern uniquely bacterial sRNA that can be isolated and detected in infected host samples. Taken together, our approach will help us identify sRNA signatures unique to M. tuberculosis variant bovis in Odocoileus virginianus, and any combination of pathogen infected hosts, and facilitate early diagnosis of bacteremia.

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Student Support: NIH Grant R2R25HL103156-10
Effects of cannabinoids on TNF-α in mouse splenocytes as a pre-clinical model for canine immune function

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Marijuana and its components, cannabidiol (CBD) and Δ9-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 splenocytes 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.

Research Grant: None
Student Support: Summer Research Experience, National Institute of Health award number 5T35OD010432

The role of glycoprotein C in horizontal transmission of turkey alphaherpesvirus

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Members of the Alphaherpesvirinae subfamily of the Herpesviridae infect mammalian, avian and reptilian species, and continuously cause problems affecting humans and food animals. Previous research showed that the alphaherpesvirus-conserved glycoprotein C (gC) is essential for horizontal transmission of Marek’s disease alphaherpesvirus (MDV) in chickens. MDV causes a highly contagious infection in chickens that clinically presents with neoplasms and neurological signs. We hypothesized that gC homologs of other avian alphaherpesviruses are also necessary for horizontal transmission. MDV is controlled in the poultry industry by vaccination with modified-live vaccines (MLV) including homologous turkey alphaherpesvirus (HVT). Preliminary data in our laboratory showed that HVT does not spread from chicken to chicken during in vivo studies. This study aims to determine whether HVT gC is expressed during replication in cell culture and whether HVT gC replaced with MDV gC will facilitate horizontal transmission. To test these hypotheses, two viruses were generated using the 2-step Red recombination method: one with an HA epitope in frame at the N-terminus of the HVT gC protein to facilitate its identification, and another in which MDV gC will replace HVT gC. Viruses were characterized in cell culture for replication and then subsequently used in chicken-to-chicken transmission studies. The information provided in this study will allow us to understand more about the HVT vaccine strain commonly used in chickens and its future implications in gene modification research.

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Student Support: Office of the Director, NIH, T35 OD011145
Helping dogs help people, developing canine EGFR CAR T cells to treat osteosarcoma

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Osteosarcoma (OS) is an aggressive tumor primarily affecting adolescent children. For those that do not respond to chemotherapy the prognosis is poor and patient outcomes in OS have not improved in over 30 years. Chimeric Antigen Receptor (CAR) T cell therapy (e.g. CD19 CAR T cell therapy) has been shown to achieve up to 90% complete remission in relapsed B cell lymphoblastic leukemia. In contrast, CAR T cell therapy for solid tumors has been much less effective. There are many barriers in treatment of solid tumors with CAR T therapy, we propose that immune suppressive tumor microenvironment (TME) plays a big proponent in decreasing CAR T efficacy. Use of angiotensin receptor blocker (ARB) losartan in a canine spontaneous tumor model of metastatic OS has been shown to modulate the TME, inhibiting immune suppressive tumor associated macrophages and increasing anti-tumoral cellular immunity. Our lab has shown in mouse tumor studies combination of ARB losartan with the beta blocker propranolol generates additive antitumor activity by modulation of the TME. Epidermal growth factor receptor (EGFR) is highly upregulated and an indicator of poor prognosis in OS. EGFR CAR T cells in mice have shown tumor reduction and an indicator of poor progression in OS. EGFR CAR T cells in mice have shown tumor reduction and increased overall survival, but clinical trials in humans have not shown the same success. A canine OS model replicates clinically relevant variables that are missing in a mouse model such as full immune competency and greater genetic and intertumoral diversity. We will test the overarching hypothesis that TME modulation pharmacologically with repurposed ARBs and beta blockers will decrease immune suppressive signaling in solid tumors, increasing the efficacy of EGFR CAR T cell therapy.

Research Grant: unknown
Student Support: unknown

Case-control study of non-pulmonary soft tissue metastatic disease in dogs with appendicular osteosarcoma

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Osteosarcoma is the most common primary bone tumor in the dog and known to be highly metastatic. Non-pulmonary soft tissue metastases are rare. The purpose of this study was to evaluate whether the treatment administered and other patient factors affect development of non-pulmonary soft tissue metastatic disease in dogs with appendicular osteosarcoma. This was a multi-institutional case control study. Cases and controls were dogs that had appendicular osteosarcoma treated with either amputation, limb-sparing, or stereotactic radiation therapy, and received ≥ 1 dose of intravenous chemotherapy. Cases developed soft tissue non-pulmonary metastatic disease. Controls developed metastases to lungs or bone. Three controls were collected for each case. Information collected included primary tumor treatment, chemotherapy, site of development of first metastases, and any treatment of metastatic disease. Thirty-five cases developed metastases to lungs or bone. Three controls were collected for each case. Information collected included primary tumor treatment, chemotherapy, site of development of first metastases, and any treatment of metastatic disease. Thirty-five cases developed metastases to cutaneous or subcutaneous tissue (18), kidney (4), abdominal organs (10), lymph node (2), and muscle (1). The first site of metastases recorded in cases were lung (17), soft tissues (13), lung and soft tissue (4), and bone, lung, and soft tissue (1). The majority of cases were treated with amputation (34) and one dog had radiation therapy. Seventy-six controls have been identified, the first site of metastases was lungs in 55 dogs, bone in 15 dogs, and lungs and bone in 6 dogs. Treatment for controls consisted of amputation in 73 dogs, radiation therapy in 2 dogs, and limb sparing in 1 dog. Data collection is ongoing, conditional logistic regression analysis will be performed when data are available.

Research Grant: None
Student Support: Signature Oncology Funds
Injury and healing effect on fatigue properties of collagen V haploinsufficient female murine tendons

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Patients with Classic Ehlers-Danlos Syndrome (cEDS), a disorder characterized by mutation in the COL5 genes with COL5a1 haploinsufficiency being the most common, suffer from tendon/ligament fragility and abnormal wound healing. Furthermore, human studies have shown that females have decreased collagen synthesis during repair, potentially exacerbating detrimental changes present in cEDS tendons. Although the hierarchical structure of the tendon has been implicated in changes following cyclic fatigue loading, fatigue properties have not been examined in cEDS tendons. Therefore, the objective of this study was to define the fatigue properties of female murine patellar tendons following injury, as well as the effect of a reduction in collagen V on these properties. This study evaluated genotypic differences and fatigue properties of the patellar tendon in uninjured and injured mice. Overall, fatigue properties of Col5a1+/- tendons were persistently affected to a later time-point post-injury (PI), while the fatigue properties of wild-type (WT) tendons showed minimal differences later in healing. Therefore, collagen V deficient mice have a delayed healing response, with changes persisting to 6w PI, while WT tendon fatigue properties recover by 6w PI. Hysteresis analysis indicates that more energy is lost at the end of fatigue life in Col5a1+/- tendons, while the opposite is true for WT tendons. Overall, this study indicates that collagen V affects the ability of the tendon to heal in a manner that resists microstructural damage associated with cyclic use and plays a crucial role in the tendon healing process.

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Student Support: Unknown

Massage therapy techniques for the prevention and treatment of pneumonia: a protocol for a scoping review

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Background: Massage therapy has been shown to decrease stress hormone levels, reduce anxiety, and improve blood flow and muscle health; one of its applications is to improve respiratory function of patients with respiratory diseases such as acute bronchiolitis, pneumonia, and cystic fibrosis. Pneumonia is an inflammatory lesion of the lungs of aspiratory or infectious origin, which can lead to acute and chronic respiratory signs, decreased lung capacity, and patient mortality. Currently, there is a lack of consensus on what massage methods are being used to treat pneumonia.

Objective: The goal of the future review is to identify what massage techniques are utilized in the treatment of pneumonia in animal and human patients.

Methods: The future scoping review will include published peer-review primary research articles on pneumonic human or animal patients that were treated with massage. No restriction will be applied to the study design, language, year of publication, massage technique, patient age or sex, or pneumonia type. The search strategy included the keywords for the intervention (massage) and the disease (pneumonia) and all available variants. A comprehensive search was conducted in Medline, CAB Abstracts, Scopus, and Biosis. Two consecutive screenings (title and abstract) will be performed by two independent reviewers, then full manuscripts will be screened by a single reviewer. Study details and study, disease, and intervention characteristics will be recorded.

Discussion: The purpose of this scoping review will be to identify the most common massage techniques utilized with pneumonic patients and to recognize if there is a current consensus on massage treatment methods for pneumonic patients.

Research Grant: None
Student Support: UC Davis School of Veterinary Medicine – PetSmart/Lider Endowment Funds UCDSVM STAR Program
Descriptive analysis of Bottlenose dolphin strandings in the Gulf of Mexico 2009-2019

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Over the past decade, marine 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 & the environmental impact of human activity in this region. To evaluate stranding trends, 4286 Bottlenose dolphin stranding records between 2009-2019 were obtained from the Marine Mammal Health & Stranding Response Program National Database & used for descriptive analysis to ID temporal & spatial clusters. Areas around Galveston, TX, the Mississippi Sound, & Barataria Bay, LA displayed large stranding clusters. Strandings displayed a strong seasonal pattern. In association with the spring calving season, over 1700 strandings were recorded, peaking in March. Winter months recorded 1400 strandings, peaking in February. Only 28% of strandings occurred in the 6 months of summer & fall (June-November). Along with stranding hotspots & seasonal trends, this data portrayed several large-scale mortality years, focused largely on LA and MS coastlines. Compared to the yearly mean of 393 strandings, 2011 (512) & 2019 (571) were significantly elevated. Strandings in 2011 were in co-occurrence with the 2010 Deep Water Horizon oil spill. Following 2011, yearly totals 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 MS River system. This work indicates that specific geographic regions & seasonal patterns are associated with recent dolphin strandings & may offer information to allow focused effort to enhance well-being & sustainability of Bottlenose dolphin populations in the GoM.

Research Grant: Boehringer Ingelheim
Student Support: Boehringer Ingelheim

Computed tomographic findings in free ranging chelonians with trauma in central Florida

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Free-ranging chelonians commonly present to veterinary facilities for reported trauma. Radiography is often used to assess lesions in chelonians with trauma, however, the superimposition of shell structures limits the assessment. Computed tomography (CT) is more sensitive detecting these lesions. However, common traumatic CT lesions and prognostic outcomes have not yet been evaluated. The purpose of this study is to create a list of common CT lesions associated with species, type of trauma, and prognostic outcomes. We hypothesize that vertebral lesions will be associated with poorer outcomes. This retrospective study evaluated CT findings in chelonians that presented with trauma to the University of Florida between 2018 to 2020 and had a CT. Lesions were organized into categories of terrestrial and aquatic species. A total of 84 chelonians met the inclusion criteria. Our preliminary results show that terrestrial chelonians with spinal injuries have 13 times the odds of being euthanized after CT review (\( P = 0.0019 \)). Spinal lesions for both populations are significantly (\( P = 0.0057 \)) associated with fewer days in the hospital prior to euthanasia. These results indicate that spinal lesions visualized on CT have a grave prognosis for both aquatic and terrestrial free-ranging chelonians. Overall, these findings support the hypothesis that spinal lesions will have a worse prognosis as compared to other lesions.

Research Grant: None
Student Support: Boehringer-Ingelheim VSP, University of Florida College of Veterinary Medicine
Identifying *Cytauxzoon felis* infections through detection and evaluation of immunodominant *C. felis* antigens

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*Cytauxzoon felis* is a tick-transmitted apicomplexan that infects mononuclear cells and erythrocytes in domestic cats. The mortality rate of cytauxzoonosis in domestic cats is close to 90% with the cats that survive remaining persistently infected for life without displaying clinical signs. These carrier cats can serve as additional infection sources of *C. felis* for naive ticks and further increase the risk of domestic cat exposure to *C. felis*. Our goal is to develop a diagnostic assay to identify carrier cats whose low level of circulating parasitemia lead to inconsistent results with nucleic acid detection methods.

Our experimental approach includes probing protein extracts from *C. felis* infected tissues with serum from carrier cats, identifying immunodominant *C. felis* antigens using LC-MS/MS analysis, followed by expressing, purifying and evaluating select immunodominant antigens by Western Blot and ELISA. Currently, we have developed a real-time quantitative PCR assay against Cf76 to quantify infection levels in *C. felis* infected tissues and probed protein extracts from *C. felis* infected tissues with serum from carrier cats. We are also expressing and purifying Cf76 to use as a positive control to compare with novel immunodominant proteins identified by LC-MS/MS. The ideal immunoassay will detect carrier cats, including those with low levels of parasitemia that may go undetected by using molecular assays, and rapidly diagnose *C. felis* infections earlier in the parasite’s lifecycle so that the veterinarian can make informed decisions regarding patient treatment and welfare.

**Research Grant:** National Center for Veterinary Parasitology Research Grant 2019/2020  
**Student Support:** Morris Animal Foundation Veterinary Student Scholar Program (D20FE-602)

The effects of toll-like receptor 2 and 4 on dissemination of coccidioidomycosis in dogs

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Coccidioidomycosis, commonly referred to as Valley Fever, is endemic to the Southwest United States. The endospored *Coccidioides* spherules can cause primary pulmonary or disseminated disease in infected hosts. Disseminated disease impacts morbidity, mortality, and typically necessitates lifelong antifungal therapy. Toll-like receptors (TLRs) are expressed on cells and can affect the host defense and susceptibility to infections. The cause for dissemination of coccidioidomycosis is unknown and likely multifactorial. A murine study revealed that wild-type TLR4 resulted in a 10-fold increase in splenic *Coccidioides* accumulation. This suggests TLR expression could have an integral role in dissemination; however, there is little information about the immune response or risk factors for dissemination in dogs with *Coccidioides*. Our research looks to bridge the knowledge gap by investigating constitutive and CTS1-stimulated leukocyte TLR2/TLR4 expression in healthy non-immune dogs, dogs with primary pulmonary coccidioidomycosis, and dogs with disseminated disease. We hypothesize that constitutive leukocyte expression of TLR2/TLR4 will be lower in non-immune healthy dogs compared to dogs with coccidioidomycosis (pulmonary and disseminated combined), but dogs with disseminated coccidioidomycosis will have lower leukocyte TLR2/TLR4 expression than dogs with pulmonary coccidioidomycosis. Furthermore, we expect that CTS1-stimulated leukocytes from healthy non-immune dogs will be greater than all dogs with *Coccidioides*, but dogs with disseminated disease will have lower expression than pulmonary-only *Coccidioides*. The results of this study could lead to new therapies to help treat canine coccidioidomycosis.

**Research Grant:** Midwestern University College of Veterinary Medicine  
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Comparison between energy intake and energy expenditure estimated by the FitBark health monitor

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Animal activity monitors are becoming prevalent within the veterinary community and many pet owners are pushing for individualized medicine that has become the standard in human medicine. Fitbark is a canine activity monitor with the ability to monitor activity, sleep, mobility, pain, stress, anxiety, and even skin itching. The purpose of this study is to test the efficacy and validity of the Fitbark activity monitor as it relates to nutritional recommendations when used by veterinarians for treating and preventing certain conditions. Currently the study has 25 dogs enrolled and is on-going. The Fitbark device is worn continuously for 4 weeks while the owner keeps an accurate daily diet log. This information will be used confirm the efficacy of the Fitbark activity tracker so it can be confidently used in disease diagnosis, treatment and prevention, as well as help develop new weight-loss protocols for the ever-growing pet obesity crisis our society is experiencing.

Research Grant: None
Student Support: Rainier Scholar Program

Animals and COVID-19: how veterinarians can bridge the gap in a zoonotic pandemic

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Three out of four emerging infectious diseases are zoonotic, including the novel coronavirus SARS-CoV-2, the infectious agent of COVID-19. The origin of SARS-CoV-2 has been traced to a strain of virus found in bats, which was likely transmitted to humans via the Malayan pangolin, a small mammal found in wet markets throughout China. It is of high importance to explore transmission patterns in both companion animals and livestock species due to the zoonotic origin of SARS-CoV-2. Cats and ferrets have been experimentally infected with SARS-CoV-2 and show signs of microscopic inflammation, viral shedding, and a neutralizing antibody response. SARS-CoV-2 infects mammalian cells by binding a transmembrane protein, ACE2, highly expressed in alveolar type II cells in the lung and throughout tissues of the body. The use of animal models is well known for advancement of medical knowledge, however the typical subjects, mice and rats, cannot become infected with SARS-CoV-2 due to their mutated ACE2 receptor. Less utilized animal models such as ferrets and cats may be superior due to their similar ACE2 receptors and susceptibility to SARS-CoV-2. While no animal to human transmission has been recorded since the initial outbreak, it is important to monitor infections among animals as they can act as a reservoir for disease. Additionally, endangered primates at risk of extinction have been genetically identified as at-risk species for developing COVID-19. Viewing the COVID-19 pandemic from a One Health perspective will not only provide insight into zoonotic disease transmission but also treatment and vaccine strategies from veterinarians that deal with endemic coronaviruses such as feline infectious peritonitis and bovine coronavirus.

Research Grant: None
Student Support: NIH T35 Training Grant 5T35OD010919-23
**Vitamin D regulation in foals experimentally infected with *Rhodococcus equi***

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1,25 (OH), vitamin D₃, the active metabolite of vitamin D, helps regulate skeletal homeostasis through calcium and phosphorus metabolism. In people, vitamin D also modulates the immune response. Immune cells express the vitamin D receptor and contain enzymes that convert inactive vitamin D to the active form. Active vitamin D then regulates production of antibacterial protein synthesis to support clearance of intracellular bacteria like *Mycobacterium tuberculosis*, which causes severe chronic pneumonia in people. *Rhodococcus equi* is an important cause of pneumonia in young foals, and exhibits a similar pathogenesis to *M. tuberculosis* centered on replication within alveolar macrophages. The role of vitamin D in the equine immune system and in *R. equi* infection has not been described. In this study, active and inactive vitamin D concentrations were quantified via ELISA in serum samples from 7 foals with experimental *R. equi* infection from days 0, 3, 7, & 14 post-infection and from 10 age-matched healthy foals. In addition, pulmonary macrophages from 6 healthy foals obtained via bronchoalveolar lavage were cultured with and without a low concentration of virulent *R. equi*. Cell supernatant vitamin D concentrations were quantified before and 2 and 7 days post-infection as above. We hypothesize that *in vivo* and *ex vivo* *R. equi* infection will induce the synthesis of the active metabolite of vitamin D, suggesting vitamin D may have a similar immunomodulatory role in foal pneumonia as in other species. This is the first study to examine vitamin D metabolism during *R. equi* infection in foals. Our results will determine if further investigation of vitamin D as an immunomodulator of leukocytes in equids is warranted.

**Research Grant:** University of Georgia Department of Large Animal Medicine  
**Student Support:** Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

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**Comparison of incisional gastropexy with and without additional full-thickness stomach to body wall sutures**

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Incisional gastropexy (IG) is performed during surgical correction of gastric dilatation/volvulus (GDV) in dogs to prevent recurrent GDV or as a prophylactic procedure in at-risk patients. The purpose of IG is to create permanent adhesion of stomach to the right abdominal wall. One incision is made in the gastric pyloric antrum through the seromuscular layers and a second incision through the transversus abdominis muscle. Then, the two incisions are apposed with a simple continuous suture pattern. One possible cause of IG failure may be ineffective healing due to suture line tension. To alleviate tension on the IG incision a modification has been employed at the University of Missouri Veterinary Health Center (VHC). The modification involves two additional simple interrupted sutures from the stomach to the body wall, one cranial and one caudal to the continuous suture line. The modification sutures are placed full thickness into the stomach to ensure engagement of submucosa, the holding layer of the stomach. A large case series is necessary to determine if the modification reduces IG failures. We will begin by determining if there are detrimental effects of the modification by evaluating VHC medical records from 2005 through 2019 to compare intraoperative and postoperative complications between dogs with and without the IG modification. To date, we have identified 40 cases of the modification which will be compared to at least 40 unmodified cases. Phone call follow-up to referring veterinarians and pet owners will be used to determine outcomes. We hypothesize that there will be no difference in complications between dogs with and without the IG modification.

**Research Grant:** None  
**Student Support:** University of Missouri College of Veterinary Medicine Office of Research
Post hoc analysis of extracellular vesicle procoagulant activity in cats measured with ZYMUPHEN-MP activity

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Thromboembolism is a common and deadly complication of hypertrophic cardiomyopathy in cats. Studies in people suggest phosphatidylserine-exposing extracellular vesicles (PS+EV) play a pathophysiologic role in thrombosis and may serve as biomarkers for thrombotic risk. We recently tested a human assay that measures procoagulant activity (PCA) of PS+EVs for use in cats. PS+EVs were generated by incubating whole blood with LPS. Platelet free plasma was obtained using a centrifugation protocol optimized for use in people. The intra- and inter-assay coefficients of variation were high (28.64 and 54.25%, respectively). Samples with low PS+EV PCA (< 1nM) showed the greatest variability. Residual platelet levels in plasma were higher than in people. Platelets can increase EV formation during freeze-thaw cycles of plasma. We hypothesized that platelet contamination may contribute to assay variability. The aim of the study was to determine the effect of samples with physiologically insignificant PS+EV PCA on assay precision, determine whether a correlation exists between platelet contamination and PS+EV PCA, and further characterize EV production in cats by examining variability of LPS-induced EVs in individual cats. The Mann Whitney U-test was used to compare continuous variables. The Spearman Rho test was used to test correlations. The intra- and inter-assay CV when samples with PS+EV PCA < 1nM were omitted was 12.12% and 22.60% respectively. The correlation coefficient between residual platelets and PS+EV PCA was r = 0.7 (P = 0.23). Similar to people, LPS-induced variable amounts of EV formation in individual cats. Further optimizing platelet clearance from cat plasma may improve precision. This assay shows promise for use in cats.

Research Grant: Western University of Health Sciences College of Veterinary Medicine
Student Support: Boehringer Ingelheim

Replicating Cytauxzoon felis life cycle using Amblyomma americanum and feline embryonal macrophage cells

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Cytauxzoon felis is an apicomplexan parasite transmitted by the lone star tick, Amblyomma americanum. It causes high mortality in domestic cats due to hyperinflammation and rapid disease progression associated with schizont formation and multi-organ failure. There is no effective vaccine to prevent cytauxzoonosis, and treatment is often ineffective if not diagnosed early. Currently, in vivo infection remains the only experimental model to evaluate the pathogenesis of C. felis infection in domestic felids. Thus, the objective of this study was to investigate the development of a novel in vitro cell culture system to study pathogenesis and immune function during C. felis infection by replicating the life cycle of C. felis within A. americanum ticks and feline embryonal macrophage cells (fcwf-4). We hypothesized that merozoites from acutely infected cat erythrocytes could infect and replicate within salivary glands of capillary tube-fed A. americanum ticks, and that sporozoites extracted from the salivary glands of these ticks could infect and replicate within fcwf-4 cells. Our results to date have demonstrated the detection of C. felis DNA in ticks that have been fed blood from acutely infected domestic cats by droplet digital PCR (ddPCR). The study is ongoing and success in ultimately developing an in vitro cell culture system will allow testing of novel vaccines and potential drug therapies without using experimental infection of domestic cats.

Research Grant: Oklahoma State University College of Veterinary Medicine
Student Support: None
T1ρ and T2 mapping detect ischemic injury to the femoral head in a piglet model of Legg-Calve-Perthes Disease

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Legg-Calve-Perthes Disease (LCPD) is a juvenile hip disorder affecting both children and dogs caused by interruption of blood flow to the developing femoral head that can result in joint deformity. Clinically, new imaging techniques are needed to assess the early-stage ischemic injury to better inform treatment decisions. The purpose of this study was to evaluate the sensitivity of T1ρ and T2 mapping to detect ischemic injury to the femoral head in an induced LCPD piglet model. We hypothesized that T1ρ and T2 relaxation times will be increased in femoral heads undergoing ischemic injury compared to unaffected, contralateral hips. Eight 6-week-old piglets underwent surgery to induce unilateral global femoral head ischemia. One week post-operatively, the piglets were imaged in vivo at 3T MRI using 3D T1ρ and T2 mapping. Ischemia was confirmed using contrast-enhanced MRI. The T1ρ and T2 maps of the femoral heads were segmented into four regions of interest (ROIs): secondary ossification center (SOC); articular-epiphyseal cartilage complex (AECC); primary physis; and metaphysis. The median T1ρ and T2 relaxation times in each ROI were compared between the operated and control femoral heads using paired t-tests (P < 0.0125 considered significant). T1ρ and T2 were significantly increased in the operated vs. control femoral heads in the SOC (ΔT1ρ = 19 ± 12 ms, P = 0.0032; ΔT2 = 18.1 ± 7.3 ms, P = 0.0002) and AECC (ΔT1ρ = 12.8 ± 4.1 ms, P < 0.0001; ΔT2 = 11.9 ± 3.7 ms, P < 0.0001). There were no significant differences in the metaphysis and primary physis ROIs. These results support that T1ρ and T2 mapping are sensitive and potentially clinically-translatable techniques to detect early-stage ischemic injury to the femoral head.

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Student Support: University of Minnesota, College of Veterinary Medicine, Office of Graduate Programs

Longitudinal assessment of fecal microbial transfer (FMT) in a case of equine chronic intermittent colitis

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Equine colitis is a devastating condition in horses with a high disease mortality of 25.4% to 35%. Dysbiosis is a crucial factor in the development of equine colitis. Fecal microbial transfer (FMT) is currently adopted to resolve dysbiosis in equine cases. However, there is still a lack of effective therapeutic strategy for 50% of equine colitis cases are due to an unknown etiology. The objective of this case study is to assess serial FMT treatments of a horse with chronic colitis, its health outcomes, and compare the microbiota composition of the recipient, donor, and the prepared fecal juice. An equine patient experiencing chronic intermittent colitis was treated via FMT using fecal juice prepared from a healthy donor horse with a similar diet and residing on the same farm. Treatments were administered five times over two months. Clinical response was assessed via daily stool consistency, mentation and behavior monitoring. Fecal samples from the recipient, donor, and FMT fecal juice were collected roughly two weeks apart prior to each FMT. The profile of the fecal microbiota was compared using 16S rRNA amplicon sequencing. The patient had no detrimental side-effects and demonstrated favorable, but transient clinical response following each FMT. The 16S rRNA sequencing data show that there is significant difference in the gut microbiota between the samples of the recipient and donor, recipient and FMT fecal juice. The fecal juice used for FMT showed a high degree of similarity to the donor feces although the low sample number limits statistical power. Collectively, these data suggest that FMT provided only transient benefit to the diseased horse, and no shifts in microbiome were seen during the treatment period.

Research Grant: University of Missouri Metagenomics Center
Student Support: University of Missouri Veterinary Research Scholars Program, IDEXX-BioAnalytics
Bone lysis and foreign body reaction following polyvinyl synthetic cartilage implantation

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Hallux rigidus or great toe arthritis negatively impacts 1 in 40 people over the age of 50. Hemiarthroplasty using a polyvinyl synthetic cartilage implant reports 90% success however, failure rates of 20% have been reported and the mechanism remains unclear. The purpose of this study was to characterize the pathologic processes at 6 months following hemiarthroplasty using the polyvinyl implant in an ovine model. An osteochondral defect was made in the medial femoral condyle in 6 sheep. In 4 sheep (A-D), a polyvinyl implant was placed per manufacturer recommendations. In 2 sheep the defect was left empty. Implant sheep C&D were euthanized at 1 & 5 months due to unrelenting pain; others were euthanized at 6 months. Post-mortem, joints were grossly evaluated, osteochondral and synovial membrane histology, and computerized tomography (CT) were performed. Gross evaluation of empty defect and implant sheep A&B revealed mild cartilage fibrillation on the femoral condyle and tibial surface whereas sheep C&D had severe fibrillation. On histology, the stromal reaction surrounding the implant was 3-fold wider in sheep C&D compared to A&B, and consisted of severe lymphoplasmacytic inflammation. Similarly, synovium in empty defect and implant sheep A&B had minimal cellular infiltration (1/4) compared to 4/4 in sheep C&D. Quantitative evaluation of CT scans showed that all sheep had some lysis and sclerosis with sheep C&D having 1.8-fold more lysis than the empty defect sheep. The evidence of bone lysis and foreign body reaction indicates an immune response to the implant and urges caution when considering use of polyvinyl implants.

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Student Support: Cornell University (Boehringer-Ingelheim match)

Assessment of companion animals in a veterinary teaching hospital for novel coronavirus (SARS-CoV-2) infection

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The novel coronavirus (coronavirus disease 2019 [COVID-19] or severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) has led to a global pandemic since its original description in late 2019. Companion animals are a point of interest amidst reports of natural infections in dogs and cats following exposure to COVID-19 infected humans. Public health officials, health care providers, and pet owners have become increasingly concerned about whether companion animals may be susceptible to, or involved in the transmission of, COVID-19. Our overall objective is to assess the MSU-VMC patient population for COVID-19 infection, with specific aims to (1) identify COVID-19 infections in dogs and cats through RT-PCR testing of nasal and pharyngeal swabs and through serologic testing with a virus neutralization assay, (2) to characterize clinical signs that are associated with infection, and (3) to identify pet and owner related factors that are associated with infection. Nasal/pharyngeal swabs and serum samples will be obtained from animals undergoing evaluation at the MSU-VMC and analyzed at the Veterinary Diagnostic Laboratory. Owners will be surveyed to obtain information related to both pet and owner health and exposure risk in the preceding 2 weeks. Prevalence rates will be calculated, and a multivariable logistic regression model will be used to identify risk factors for infection. We hypothesize that infections will be documented in cats, and that most infections will be asymptomatic. We further hypothesize that infections will be associated with exposure to humans known to have, or be at high-risk for, COVID-19 infections. This study represents an important step in assessing the susceptibility of pets to COVID-19.

Research Grant: MSU CVM Endowed Research Funds
Student Support: Boehringer-Ingelheim Veterinary Scholars Program, MSU Graduate School fellowship funds
Defining the role of the infant microbiome in mediating AHR outcomes associated with asthma in a mouse model


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The goal of this project is to identify the aspects of the gut microbiome that protect or increase vulnerability to allergic diseases, such as asthma. We hypothesized that during the early stage of life, changes in the gut microbiome of human infants increase the risk of allergic sensitization and allergic diseases. Gut microbiomes were collected from two groups of infants. One group had eczema, identifying them as having potential allergic sensitization and future, and the second group was without eczema, making them less likely to develop asthma. These gut microbiomes were transferred to germ free mice to study their airway allergic responses to house dust mites (HDM). Preliminary data showed that mice with humanized microbiota had greater airway hyperresponsiveness to HDM than mice with an undisturbed microbiota, but the underlying mechanism is unknown. Mast cells are known to influence airway constriction by releasing certain chemicals. Thus, this project examines mast cells in the lungs to determine if they have an impact on lung function when mice are exposed to an allergen. We measured mast cell protease-1 activity (mMCPT-1) in bronchoalveolar lavage samples and are correlating this with the lung function parameters, resistance, and compliance. Literature is being reviewed to identify support for mechanisms linking mast cell activity to lung function. The results of this project will determine if specific chemicals released from mast cells are associated with airway hyperresponsiveness and the gut microbiome.

Research Grant: NIH grant 1R21AI121748
Student Support: 2020 BRUSH Program R25 HL103156

Histologic evaluation of a canine model of late onset peripheral neuropathy in the Labrador retriever

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Late-onset laryngeal paralysis polyneuropathy (LoLP) is a common inherited peripheral neuropathy in the Labrador retriever population, and has potential to serve as a comparative model for inherited peripheral neuropathies in people. Affected Labradors commonly present between 9 to 13 years with laryngeal paralysis and pelvic limb weakness. Most hereditary peripheral neuropathies are either the result of axonopathies or a dysregulation of myelination. While LoLP has been established as a peripheral neuropathy, the neuropathologic underpinnings are not known. Our aim is to identify the neuropathologic features of peripheral nerve in LoLP-affected Labradors to define whether LoLP is due to an axonopathy or is a disease of myelin dysregulation. Our hypothesis is that LoLP in the Labrador retriever is the result of an axonopathy. Three groups of dogs were used. The affected group was comprised of Labrador retrievers over 9 years of age with a diagnosis of LoLP. The old control group included medium sized non-Labrador retrievers aged 9 and older and the young control group included dogs aged 3 or younger. Peroneal nerve was collected from dogs euthanized for reasons unrelated to this study. Nerves were stained with toluidine blue, embedded resin and sectioned at 1μm. Images were collected at 60x magnification. Average myelin thickness, internal myelin circumference, and axon density were measured, allowing for calculations of g-ratio, neurofilament diameter, and axon densities. Results from this work will allow for determination of the neuropathologic basis of LoLP, which is an important step towards development of LoLP as a comparative treatment model.

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Student Support: NIH T35 OD011078
Assessment of MCIII/MTIII lateral condyle fracture configuration and subchondral bone pathology in racehorses

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Maladaptive stress remodeling of distal epiphysis of metatarsal and metacarpal three (MTIII and MCIII) is among the most common injuries in racehorses and may lead to fracture of the bone. Previous work evaluating limbs of racehorses euthanized after catastrophic injury often found traumatic changes in the articular cartilage and bone of the affected and/or contralateral limb. When condylar fractures do occur, they most commonly involve the lateral condyle. Although treatment is usually indicated, the probability of returning to racing after treatment can be unfavorable if concurrent articular and subchondral bone pathology exist, and it is then likely they will run at a lower level compared to their pre-fracture starts. This study aims to evaluate fracture configuration and accompanying subchondral bone pathology and the effect of disease severity on post-injury performance. Signalment, CT scans and surgery reports from 52 Thoroughbred racehorses who came to the George D. Widener Hospital for Large Animals at the University of Pennsylvania between 2008 and 2020 for lateral condylar fracture of MCIII or MTIII will be compared to the horse’s racing performance pre- and post-injury. CT scans of the fetlock will be evaluated for palmar osteochondral lesions, comminution, fracture height and displacement, concurrent sesamoid fracture, and free joint fragments. Among the horses, median age is 3 (range, 2-8), 14 (27%) were males, 11 (21%) geldings and 27 (52%) females. Regarding fractures, 22 (42%) were complete and 30 (58%) incomplete; 15 (29%) were displaced and 37 (71%) non-displaced. The affected limb was the left fore in 18 (35%) horses, left hind in 12 (23%), right fore in 15 (29%) and right hind in 7 (13%).

Research Grant: None
Student Support: NIH T35 Training Grant T35OD010919-23

Association of tracheobronchial lymphadenopathy with Coccidioides spp. infection

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Coccidioidomycosis is a fungal disease caused by Coccidioides spp. and is endemic to the southwestern region of North America. Radiographic evidence of tracheobronchial lymphadenopathy (TBLN) may be noted in Coccidioides cases; however, these lymph nodes may also be enlarged due to neoplastic or other infectious causes. Our study aim was to evaluate the association of TBLN with Coccidioides in dogs living in an endemic region and we hypothesized that Coccidioides infection would be the most common reason for TBLN in an endemic area. In this retrospective analysis, medical records for patients with coccidioidomycosis or TBLN were reviewed from January 2015-May 2020. Preliminary results showed that 40.9% (38/93) of Coccidioides cases with thoracic imaging had TBLN. 14/38 (36.8%) Coccidioides cases had at least one follow-up thoracic radiograph and 11/14 (78.6%) cases had complete TBLN resolution at a median time of 98 days. The 3 cases that did not demonstrate TBLN resolution had repeat imaging performed at a median of 21 days with no further follow-up. 38/43 (88.4%) dogs with TBLN were diagnosed with coccidioidomycosis. The 5 remaining cases had suspected (2 cases with pulmonary masses) or confirmed (lymphoma, pulmonary carcinoma, multiple malignant neoplasms) underlying neoplasia. Although < 50% of dogs with coccidioidomycosis had documented TBLN, the preliminary findings demonstrate that TBLN in dogs living in an endemic area appears highly correlated with the diagnosis of coccidioidomycosis. Serial monitoring with thoracic imaging demonstrated resolution of TBLN within approximately 3 months of antifungal therapy.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
The affect of Piezo1 on adipogenesis and lipogenesis in rat adipose tissue

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The perivascular adipose tissue (PVAT) surrounds and supports the vascular system; it functions to regulate the vasculature’s homeostasis and secretes materials for the use of the paracrine and autocrine systems, such as adiponectin. The PVAT is composed of adipocytes, which develop from preadipocytes through adipogenesis. The adipogenesis process is composed of cellular determination and differentiation. Adiponectin is a type of adipokine that is released from the adipose tissue only when mature adipocytes are present. PVAT secretes many vasoactive molecules including adiponectin and nitric oxide that support vascular relaxation. When a balanced amount of PVAT is present, it functions to maintain the dilation of the blood vessels. Piezo 1 is a mechanosensitive ion channel, meaning that it is a type of membrane protein that is activated by mechanical stimulation. It is expressed, specifically in mice, in mechanosensitive areas such as the skin, bladder, lung, kidney, and colon. Mechanical forces driven by high blood pressure can activate Piezo 1. The activation of Piezo 1 can potentially inhibit adipogenesis, however, it is unknown if this occurs in the PVAT. Peroxisome proliferator-activated receptor gamma (PPARγ) ligands most commonly aid in preadipocyte differentiation. But when activated PPARγ allows the adipose tissue to better secrete adiponectin. The objective of this research is to explore the relationship between Piezo 1 activation and adipogenesis. We hypothesize that if Piezo 1 is activated, then adipogenesis will be inhibited. If adipogenesis is inhibited, there will be a reduction of adipocytes in number and size. The results are currently pending.

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Student Support: NIH grant R25 HL 103156

Assessing FDA policy effects on antimicrobial resistance in Salmonella Dublin isolates

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In 2012 the Food and Drug Administration (FDA) prohibited the extra-label use of cephalosporin class antimicrobials in food-producing animals to reduce the risk of cephalosporin-resistant zoonotic bacteria. The objective of this study is to assess the prevalence of antimicrobial resistant (AMR) Salmonella enterica serotype Dublin before and after this policy change. We have analyzed Salmonella Dublin isolate antimicrobial susceptibility data collected by the National Antimicrobial Resistance Monitoring System at the slaughter stage between 1998 and 2017. Internationally accepted breakpoint values were used to determine if the isolates were resistant or susceptible. Chi-square tests were performed to compare the proportion of resistance before (2006-2011) and after (2012-2017) implementation of the policy. Survival analysis was used to assess shifts in MIC distributions; growth inhibition is used as the event and concentration of the antimicrobial that inhibits growth is used as the time. The chi-square tests showed statistically significant ($P < 0.05$) increases in the prevalence of resistance to cefoxitin and ceftriaxone, cephalosporin-class antimicrobials, following the implementation of the policy. Chi-square tests also showed statistically significant increases in resistance after the policy for amoxicillin-clavulanic acid, chloramphenicol, ciprofloxacin, nalidixic acid, and streptomycin. There were no significant decreases in resistance. Analysis of the resistance patterns of Salmonella Dublin following the implementation of the FDA policy demonstrate the policy was ineffective at decreasing resistance in Salmonella Dublin.

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Student Support: NIH Leadership T35 AI007227
Phenotypic, molecular, and functional characterization of canine urothelial organoids

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Urothelial carcinoma (UC) or bladder cancer is a devastating disease with the likelihood of recurrence and metastasis in both humans and dogs. Even with treatment, the prognosis for UC is poor due to the late stage present at the time of diagnosis. Dogs with UC can serve as a model for humans because of the close associations between the two malignancies, including microscopic appearance, molecular heterogeneity, biologic behavior and response to chemotherapeutic agents. Tumor organoids are a tool derived from primary tissue that mimic the in vivo environment and physiologic response of the tumor and can be useful to ex vivo cellular and treatment response in individual patients. The aim of this study is to phenotypically characterize canine UC organoids and healthy canine bladder organoids and to compare the expression of specific surface markers between the two. The characterization of the organoids was accomplished by performing immunohistochemistry and RNA in situ hybridization with biomarkers commonly found in UC cells. The UC biomarkers assessed were a marker expressed by all UC cells (CK7), a luminal UC cell marker (FoxA1), and a urothelial stem cell marker (CD44). The results showed expression of CD44 and CK7 in healthy canine bladder organoids, and expression of all biomarkers in canine UC organoids. Overall the results showed there was an upregulation in expression of all biomarkers in canine UC organoids compared to healthy canine bladder organoids. Our results indicate that the increased expression of markers in tumor organoids suggest there is a different phenotype present in UC compared to a healthy bladder. Future studies include utilizing canine UC organoids to test cytotoxic effects of anticancer drugs.

Research Grant: Margaret B. Berry Foundation Grant
Student Support: ISU CVM Summer Scholar’s Research Program

Jiminy Cricket! Exploring the opinion of the veterinary profession on entomophagy and the rearing of insects

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With an increased awareness of an emerging global food crisis, entomophagy, the practice of eating insects, is being discussed as a viable option for feeding the expected population of 9 billion by 2050. Edible insects offer the potential to ensure food security to human and animal populations by acting as a rich and efficiently-obtained source of nutrients. The vast majority of studies on human entomophagy and using insects in animal feed have focused on the opinions of the consumer, the nutritional and health benefits of insect consumption, and the economic and environmental impacts of insect production. Veterinarians play essential roles in ensuring the safety and security of food sources and overseeing the health of livestock. To date, there have been no studies exploring the opinions of the veterinary profession on entomophagy or their investment into the field of entomologic medicine. This aim of this study is to conduct a cross-sectional survey using the Health Belief Model to gauge the opinions of the veterinary profession on entomophagy and the farming of insects as food animals. We hypothesize that the majority (> 50%) of veterinary respondents will have a positive view of entomophagy and insect production medicine in regard to perceived benefit and self-efficacy, a neutral or positive view of entomophagy and insect production medicine in regard to perceived threat and perceived barriers, and a neutral or negative view of entomophagy and insect production medicine in regard to cues to action.

Research Grant: none
Student Support: Boehringer Ingelheim
Muscle dystrophies: proteomic identification of protein biomarker release from necrotic skeletal muscle cells

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Muscle dystrophies are a very serious group of inherited diseases that break down skeletal and cardiac muscle cells causing muscle weakness, atrophy, and death. Although the genes that cause the disease have been identified, it is not yet known exactly what causes the muscle cells to die or why it progresses differently in skeletal vs. cardiac muscle. While novel therapies have been developed, the benefits have been modest. However, earlier disease detection and development of new therapies would be enhanced if good biomarkers were identified. While markers of cardiac necrotic cell death are known, there are many unknowns about what is released during necrotic cell death of skeletal muscle. Therefore, the purpose of this study is to induce necrosis in skeletal muscle cells so that the proteins released from the cell can be mapped and compared to those of cardiac muscle. Rat skeletal muscle cells will be treated with different concentrations of H2O2 for different times to induce necrosis. The proteins released into media will then be identified by proteomics. The resultant maps will be analyzed to discern the most prominent proteins and compared to those generated for cardiac muscle cells. It is expected that the number of proteins released by skeletal muscle cells will increase as the concentration of H2O2 increases. We anticipate that while many of the released proteins will be common to both muscle types, we will also identify skeletal muscle cell-specific markers. By discovering new biomarkers for skeletal muscle cell death our findings would allow for comparisons between the 2 muscle types that would enable earlier detection and the ability to follow both the skeletal and cardiac aspects of the disease in parallel.

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Student Support: An endowment established by IDEXX-BioAnalytics

Evaluating the role of cage density on gut microbiota variability in murine studies

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The gut microbiota (GM) of humans and animals is critical to immune health, susceptibility to disease, and more. Mice are used to investigate the GM and its influences; however, the reproducibility of experiments and translatability to human medicine are sub-optimal. One important consideration in study design is whether the “n” should be the total number of mice or the total number of cages, with each cage being a “biological unit”. Mice are often group-housed (4-5 mice per cage) to minimize costs; contrarily, mice can be housed individually, which increases housing costs and may add unwanted isolation stress. A compromise would be to house 2 mice per cage. The objective of this study is to evaluate the trade-off in statistical power to detect treatment differences and housing costs, based on housing density. Forty-eight C57BL/6 mice were separated into groups of 2 or 4 mice per cage. Half of each group received Baytril as an experimental pressure on the GM to assess the impact of cage density on statistical power. Fecal collections took place weekly from day 0 to week 4, and luminal contents of the jejunum, ileum, and cecum were collected upon euthanasia. DNA was extracted and subjected to next generation sequencing to analyze the richness, diversity, and composition of the GM. We expect that the standard deviation of richness and diversity interval data will be greater in mice housed by 2 per cage compared to those housed 4 per cage. This would indicate that housing mice by 2 per cage would decrease “cage effect” and result in more reliable and interpretable data obtained from contemporary murine studies.

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Student Support: American Society of Laboratory Animal Practitioners Foundation and IDEXX-BioAnalytics Endowment
Modeling the potential spread of African swine fever in North Macedonia

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African Swine Fever (ASF) is arguably the deadliest re-emerging infectious disease of swine. Recent estimates by the European Food Safety Authority (EFSA) found that Southeastern European countries are particularly vulnerable to ASF introduction and spread. North Macedonia is bordered by three countries that have recently confirmed ASF cases in domestic and wild pigs, substantially increasing the risk of introduction to the country. In this study, we aim to develop a spatially explicit agent-based model to better understand potential ASF transmission dynamics considering diverse epidemiological scenarios and control strategies. We use 2019 swine census and movement data collected by North Macedonian veterinary services and the FAO. We simulate ASF transmission pathways including pig movement (using the actual pig trade network in the country), local spread (i.e., assuming a kernel density to simulate nearby transmission by fomites) and wild boar contact (using EFSA wild boar density data). Finally, we incorporate commonly implemented interventions, including surveillance, tracing, and culling. The risk of ASF spread is heterogeneously distributed, being concentrated in Vardarski region. This is likely associated with the combination of high pig density and higher frequency of pig trade. Farms in the eastern part of the country were also at higher risk of transmission due to increased likelihood of contact with wild boar. The implementation of effective surveillance and tracing systems markedly reduced the spread of disease. These results emphasize the importance of early detection, and should help improve the preparedness of early warning systems in North Macedonia.

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Anesthetic efficacy of alfaxalone-dexmedetomidine combination with either hydromorphone or fentanyl in ferrets

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Ferrets are a popular pet and animal model commonly used in biomedical research, but there are few studies establishing safe species-specific anesthetic protocols. Our objective was to generate data demonstrating that a single intramuscular (IM) drug combination of alfaxalone-dexmedetomidine with either hydromorphone or fentanyl may provide analgesia, a smooth induction and recovery, while maintaining minimal cardiorespiratory depression and stable general anesthesia. Randall Selitto algometer and interdigital web pinch were used to determine surgical anesthetic depth. We aimed to have 12 ferrets randomized and placed into: Group 1) 2.5 mg/kg alfaxalone - 0.03 mg/kg dexmedetomidine - 0.2 mg/kg hydromorphone (ADH) or Group 2) 2.5 mg/kg alfaxalone - 0.03 mg/kg dexmedetomidine - 0.03 mg/kg fentanyl (ADF). However, Covid-19 disallowed a full report of the scheduled study and a limited number of ferrets were used. Following anesthetic induction, ferrets were intubated and measured for heart rate (ECG), blood pressure (oscillometry), respiratory rate, etCO$_2$ (capnography), oxygen saturation (pulse oximetry), PVCO$_2$, PVO$_2$, pH, body temperature, color of mucous membranes, CRT, palpebral reflex, jaw tone, and response to mechano-stimulation in 5-minute-intervals for 40 minutes and then reversed with atipamezole (0.4 mg/kg, IM) and naloxone (0.03 mg/kg, IM). Preliminary trials demonstrated that surgical anesthetic depth was achieved in both groups. However, profound pulmonary depression was evidenced by hypoxia and hypercapnia based on pH, PVCO$_2$, etCO$_2$, and SpO$_2$. Cardiovascular stability was maintained throughout anesthesia evidenced by HR, BP, and CRT. Pulmonary depression was reversed with antagonist and recovery was uneventful.

Research Grant: Korea Institute of Science and Technology (KIST); Bionet America Inc.; and WesternU CVM Office of Research
Student Support: Boehringer Ingelheim
Phylogenetic analysis and protein level mapping of mutations in African and European Usutu virus strains

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Usutu virus is an emerging arbovirus in the flavivirus family. Like West Nile virus (WNV), Usutu virus (USUV) is transmitted by mosquito vectors, maintained in avian reservoirs, and can cause disease in humans. Originating in Africa, USUV is now found throughout Europe due to introductions along migratory bird routes. Prior experimentation in mice showed variation in virulence among USUV strains. The aim of this study was to compare USUV strains on a molecular level to identify potential amino acid substitutions which may confer virulence, as well as to assess viral replication in avian cells. A maximum likelihood phylogenetic tree was constructed, and results showed at least 3 introductions of USUV into Europe. Additionally, sequences were scanned for amino acid substitutions G595S and E3425D, reported previously to be unique to a USUV strain isolated from a human with encephalitis. We identified 5 additional strains with both mutations and 2 strains containing G595S alone; however, neither substitution was present in the USUV strains previously tested for pathogenesis in mice. Coding regions from two USUV strains, distinct by 23 amino acids and notably different in pathogenesis in mice, were threaded onto WNV protein models to assess if the mutations alter predicted protein structure. These 3-D models provide insight on how differences at the protein level may relate to changes in function. Currently, we are testing these USUV strains in a wild avian in vitro model to assess if differences in replication in mammals are reflected in reservoir hosts. In the future, we hope to study these USUV strains in vivo in wild bird species, as well as to design viral chimeras to study pathogenic effects of specific point mutations.

Research Grant: None
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Life-cycle elucidation of Hysteromorpha sp. in ictalurid catfish production systems in Mississippi, USA

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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.

Research Grant: Office of Research & Graduate Studies, MSU CVM
Student Support: Boehringer Ingelheim & National Institutes of Health T35 OD010432
Gut microbiome composition of *Eptesicus fuscus*

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The gut microbiome consists of microscopic living organisms that work synergistically with the host to perform a variety of functions, including colonization resistance, synthesis of vitamins, breakdown of dietary fiber, and stimulation of the mucosal epithelium. The healthy microbiome compositions of most domestic species are known and available for reference, however, this data is less common in exotic species. And even when available the accuracy may be called into question, as the accuracy of microbiome data varies with how well the DNA is extracted from the gut microbiota.

The purpose of this study is to describe the composition of the gut microbiome in both captive and wild big brown bats (*Eptesicus fuscus*) using fecal samples and four different DNA extraction methods. These extraction methods include the QIAamp Fast DNA Stool Mini Kit, the Quick-DNA Fecal/Soil Microbe Miniprep Kit, the DNeasy PowerSoil Kit, and an Isopropanol DNA extraction method. We will compare methods in terms of DNA recovery, quantity and quality of nucleic acids, and microbial community composition. We expect to see a difference in microbial community composition between captive big brown bats fed an identical diet and their wild counterparts, which are subject to different environments and diets. Our results will determine level of difference between the microbial community composition of captive and wild big brown bats and potential nutritional implications. Additionally, we will determine which extraction method recovers the highest quality and quantity of DNA in order to establish standardized protocols for assessing gut health status of bat species spatially and temporally.

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Student Support: University of Missouri research grant number 0056825 and USFS research funds

Prognostic indicators for non-human primates with hypothermia

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Hypothermia is a common and critical presentation in veterinary medicine, and is a particular challenge for exotics and laboratory animal practitioners. In nonhuman primates (NHPs), hypothermia disrupts normal physiology and complicates treatment. To date, there are no published reports regarding the presentation or prognosticators of clinical hypothermia in NHPs. We performed a retrospective case review of NHPs presenting as hypothermic (Cynomolgus macaques, Rhesus macaques, and Vervet monkeys; 2005-2020). Data extracted included species, sex, body weight, housing type (indoor/outdoor), body temperatures, re-warming rates, hematology and blood chemistry values.

27% of hypothermic NHPs survived < 48 hours, 21% survived 2-30 days, and 52% survived > 30 days. Cases that survived < 48 hours versus cases that survive > 30 days had lower presenting temperature (29.8 ± 0.9 v. 33.7 ± 0.5°C; *P* < 0.001), chloride (95.2 ± 3.0 v. 104.1 ± 1.1 mEq/L; *P* < 0.01), and sodium (136.2 ± 2.6 v. 143 ± 1.1 mEq/L; *P* < 0.05) concentrations; whereas, blood glucose (307 ± 97.4 v. 119 ± 25.3 mg/dL; *P* < 0.05) and phosphorus (10.4 ± 1.9 v. 6.0 ± 0.8 mg/dL; *P* < 0.05) were higher. Multiple logistic regression with backward elimination was performed to develop a predictive, prognostic index. Presenting temperature (odds ratio [OR] = 0.71) and chloride (OR = 0.83) were significant prognostic indicators (*P* > 0.001). The area under the receiver operator curve for predicting survival was 0.90. The optimal probability cutoff point was 0.41, corresponding to a sensitivity and specificity of 67% and 91%, respectively. The model correctly classified 84% of the cases (n = 31). Overall, presenting temperature and chloride are suggested as useful prognosticators for NHPs experiencing hypothermia.

Research Grant: None
Student Support: National Institutes of Health T35 Veterinary Scholars Program
Novel 3D-printed soft tissue tumor model as an aid to teach surgical oncology principles

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Circumstances requiring off-site learning have highlighted the lack of practical learning experience in surgery rotations. This can be particularly detrimental in understanding the concept of margins and surgical doses in oncologic surgery. This study aims to develop a high-fidelity 3D-printed model of a subcutaneous tumor that will be used to enhance clinical education of surgical oncology principles at the level of a general practitioner, from case-based clinical decision making to demarcation, excision, and inking of the margins. The model will be available for individual practical learning at home and will complement the didactic case-based modules given online during off-site clinical rotations. We hypothesized that this model would be at least as effective as a cadaver model in reinforcing the aforementioned surgical oncology principles. Initial teaching models were created in separate tissue layers using two component silicons of varying shore hardness and 3D printed molds based on computed tomography (CT) images of a canine patient diagnosed with soft tissue sarcoma. A durometer gauge was used to measure tissue hardness of skin, subcutaneous tissues, fascia and muscle from fresh canine cadavers to inform educated decisions on high fidelity materials to replicate live tissue. The goal is to compare this initial layered model with an entirely 3D printed model to maximize efficiency and availability to clinical students. Evaluation of core-competencies will be performed in comparison with clinical year students having received classical clinical training in soft tissue and oncologic surgery and with previous 3rd year students having received classical cadaver laboratory training in surgical oncology.

Research Grant: None
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070

Laryngeal cartilage mineralization: effects of age, obesity, exercise, and species differences

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The larynx demonstrates a complex morphology and plays a critical role in swallowing, respiration, and voice production via adduction and abduction movements. Those vital functions depend on neural control and functionality of intrinsic laryngeal musculature and on pliability of the cartilaginous framework. The larynx is a dynamic organ and must remain flexible yet stable to support the structure through movement. The cartilaginous elements that make up the larynx change in size and shape with age and are different between species. Preliminary data suggests areas of calcification and ossification are not homogeneously distributed. In this study, we aim to link shape of individual cartilages with their mineralization patterns to inform our understanding of how pliability changes may affect function. Alterations of the cartilage matrix are visible on CT scans as different distributions of gray values that correlate with mineralization. This mineralization was quantified using the histogram tool in ImageJ software. We investigated a sample of 44 CD-1 mice (Mus musculus) representing different age groups, obesity, and exercise levels. In addition, we included 20 specimens from two other rodent species (Onychomys and Peromyscus). Results demonstrate strong age effects, such as increasing cartilage mineralization up to 3-4 months and a moderate demineralization between 3- and 12-month-old mice. Furthermore, compared to controls, mineralization was increased in the exercised and obese mice. Notable species differences could implicate variations as a result of behavior, genetics, or environment. We also discuss the relevance of our findings in relation to breathing, swallowing, and speech pathologies associated with old age.

Research Grant: National Science Foundation
Student Support: Boehringer Ingelheim Veterinary Scholars Program
The interaction between Cryptosporidium parvum and adaptive immunity in calves

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Cryptosporidium parvum can cause severe diarrheal disease in calves and can be directly transmitted to humans. However, this parasite does not appear to cause disease in adult cattle. The immune response to C. parvum is not well understood, specifically with regard to the differing immune responses in calves compared to adult cows. The aim of this study is to investigate the connection between C. parvum infection and cell-mediated immunity. It is hypothesized that resistance to diarrhea associated with C. parvum in adult cattle is due to naturally occurring changes in cell-mediated immunity as the cows age. Peripheral blood mononuclear cells (PBMCs) were collected from five calves at different ages on days 0, 7, 14, and 21. A sample from one calf on day 14 was cultured via incubation in growth media for 18 hours. The supernatant was collected and will be used as the control sample. The PBMCs were then incubated with oocysts induced to excyst and release sporozoites to increase the efficiency of PBMC infection. The supernatant was collected after 24 hours and cytokine production will be measured using MILLIPLEX MAP to determine levels of IL-2 and IFN-γ produced in response to infection. The PMBCs were collected and stored for qPCR to measure the expression of Fas ligand. This data will be used to evaluate the activity of CD4+ cells and CD8+ cells, respectively.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Is death receptor 5 a pathological or physiological hypertrophic stimulus in mini-swine with heart failure?

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Patients with heart failure (HF) with preserved ejection fraction (HFpEF) have symptoms of HF despite normal resting left ventricular (LV) ejection fraction. Previous studies have shown exercise benefits patients with HFpEF, but the intensity level yielding the most benefit is still controversial. One way exercise may protect the heart is by promoting a hypertrophic remodeling process that is more physiological than pathological in nature. In this regard, tumor necrosis factor (TNF)-related apoptosis induced ligand (TRAIL) and its receptor, death receptor 5 (DR5), present a potential mechanism mediating the cardioprotective effects of exercise. DR5 and TRAIL have been previously shown in mice to contribute to cardiomyocyte hypertrophy without adversely changing cardiac function. Thus, the purpose of this study is to measure gene expression of DR5 and TRAIL in the hearts of sedentary and exercised trained aortic-banded Yucatan mini-pigs. Animals were divided into 4 groups: 1) control sedentary (CON, n = 6); 2) aortic-banded sedentary HF (HF, n = 7); 3) aortic-banded interval trained HF (HF-IT, N = 7); and 4) aortic-banded moderate continuous trained HF (HF-MOD, N = 7). Chronic treadmill exercise training lasted 17 weeks using 2 different intensities 3x’s/week: 1) interval training (5 min. @ 3mph interspersed with 3 min @ 4 mph intervals for 45 minutes; and 2) moderate continuous training (45 min. @ 2.5mph). Quantitative RT-PCR will be used to measure DR5 and TRAIL mRNA in the LV. We hypothesize that the DR5 and TRAIL gene expression will be increased in the HF-IT/HF-MOD groups compared to CON demonstrating the cardiac hypertrophic remodeling previously observed in these animals is of a more physiological phenotype.

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Student Support: Stipend for Nicole Creeden is supported by an endowment established by IDEXX-BioAnalytics
Development of mobile equine asthma management app: survey of 195 horse owners demonstrates need and interest

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Equine Asthma, like asthma in humans, is characterized by lower airway inflammation and bronchoconstriction. The disease has worldwide distribution and affects up to 20% of some equine populations. Exposure to inhaled dust/allergens is a risk factor for development and exacerbation of the disease, making environmental control a vital component of managing affected horses. However, equine practitioners perceive that horse owners struggle with adherence to management recommendations. In human medicine, multiple mobile phone apps have been developed to help people manage asthma by tracking symptoms, providing daily medication reminders, and providing educational materials. Despite similar prevention and control measure recommendations for human and Equine Asthma, no such app exists to help horse owners manage Equine Asthma. The overall goal of this project is to design a cell phone app, intended to increase adherence to asthma control measures, for horse owners with horses affected by Equine Asthma. To better understand the treatments and environmental measures horse owners currently employ, a questionnaire was developed. 86% of respondents with horses in work said their horse’s performance was affected by a respiratory condition. Owners reported difficulty controlling asthma symptoms in their horses, and recurrence of symptoms after treatment was common. 73% of owners reported interest in using an app to help monitor and control their horses’ symptoms. Results of this survey confirm that Equine Asthma represents a significant and recurrent problem for horse owners, many of whom would be interested in using an app. Survey results directly support ongoing efforts to develop an Equine Asthma mobile app.

Research Grant: None
Student Support: Steve Lee Summer Research Stipend, UGA College of Veterinary Medicine

The DMSO reductases of Salmonella influence intestinal colonization and environmental survival

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Salmonella is a leading cause of bacterial food-borne enterocolitis. In the anaerobic environment of the gut, Salmonella utilizes alternate electron acceptors for respiration. Dimethyl sulfoxide (DMSO) can act as an alternate electron acceptor and three putative DMSO reductase enzymes have been identified in gastrointestinal serovars of Salmonella and are homologous to dmsABC from E. coli. The catalytic subunit of each DMSO reductase in Salmonella is encoded by STM0964, STM2530, STM4305. These enzymes catalyze the reduction of DMSO into dimethyl sulfide (DMS) to drive anaerobic respiration. However, despite having acquired three distinct DMSO reductases, the role of DMSO reduction in Salmonella infection remain ill-defined. We hypothesize that DMSO reduction supports Salmonella intestinal colonization and environmental survival. To test this hypothesis, we characterized the phenotypes of a triple DMSO reductase mutant in vitro anaerobic growth, biofilm formation, virulence gene expression, and in vivo competitive infections. We found that the capacity for DMSO reduction improves Salmonella anaerobic growth. In addition, DMSO reduction to DMS acts as a signal to induce biofilm formation in standing cultures. Finally, we found that these metabolites, funneled from DMSO reductases, modulate the expression of virulence genes necessary for invasion and intracellular survival. Lastly, we found that the role of each DMSO reductase has non-redundant effects on anaerobic growth, biofilm formation, and gut colonization. Future work will identify potential regulatory mechanisms for Salmonella to reap the benefits of DMSO reduction in different host and non-host environments.

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Student Support: American Society for Microbiology URF and Foundation for Food and Agricultural Research
The proopiomelanocortin peptide beta-endorphin can mediate the severity of activity-based anorexia in mice

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Proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus are critical regulators of energy homeostasis such that POMC neuron dysregulation may contribute to the development of disorders of energy balance, including eating disorders. Activity-based anorexia (ABA) is a commonly used rodent model of anorexia in which timed, restricted feeding paired with access to a running wheel results in more pronounced reductions in food intake and bodyweight loss, as well as more pronounced increases in wheel running activity. While previous studies have suggested a role for POMC neurons in ABA, the specific contribution of POMC neurons to ABA remains unknown. We report here that Pomp mRNA increases in mice undergoing ABA compared to free-running, ad-libitum fed or sedentary, food-restricted mice. The POMC prohormone is enzymatically cleaved to produce several bioactive peptides, including beta-endorphin. We show that serum levels of beta-endorphin were increased in mice undergoing ABA compared to control animals. We next sought to determine the functional consequence of inhibiting the actions of beta-endorphin. Pharmacological antagonism of the primary target of beta-endorphin, the mu opioid receptor, with naloxone produced a bidirectional effect on wheel running activity preceding food presentation (food anticipatory activity, FAA). In mice considered moderate runners during baseline data collection, administration of naloxone during ABA caused a sharp reduction in FAA. Conversely, administration of naloxone during ABA to mice considered high runners during baseline data collection led to an increase in FAA. Taken together, the results suggest that beta-endorphin can mediate the severity of, ABA in mice.

Research Grant: NIH R01 DK078749
Student Support: NIH F30 DK 117530

Characterizing the intrinsic nervous system of gallbladders from normal dogs and dogs with mucocele formation

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Gallbladder mucocele formation is one of the most common and deadly biliary disease seen in dogs. Hallmark characteristics of gallbladder mucocele formation are abnormal and excessive epithelial mucus secretion and poor contractility. Secretion and contractility are influenced by the intrinsic nervous system of the gallbladder. Currently, our understanding of the local nervous system of the canine gallbladder is limited. The long-term goal of this study is to determine if a dysfunction of the gallbladder nervous system is involved in the pathogenesis of mucocele formation. In order to pursue this goal, the objective of this study was to validate the specificity of antibodies for detection of neurons, glia, and selected neurotransmitters in dogs. Antibody testing was performed on ileum which possesses a well characterized intrinsic nervous system that derives from the same embryonic cells as the gallbladder nervous system. Immunofluorescence was used to evaluate the specificity of antibodies in identifying cell type specific markers and neurochemical coding. Antibodies evaluated were directed against markers specific to endothelium (CD31/PECAM), epithelium (active beta-catenin, beta-catenin), glia (glial fibrillary acidic protein (GFAP), Sox2, Sox10, S100-β), interstitial cells of Cajal (CD117/c-Kit), neurons (HuC/HuD, PGP9.5, Tuj) and (enzymes synthesizing) neurotransmitters (CGRP, galanin, substance P, vasointestinal peptide, nitric oxide synthase, chAT). We have currently validated canine immunoreactive antibodies capable of selectively identifying GFAP, PGP9.5, S100-β, and Tuj. After the validation process is complete, we will move forward in characterizing the intrinsic nervous system of the canine gallbladder.

Research Grant: Morris Animal Foundation Grant D17CA-068
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070
Discovery of new pathways to inhibit in osteosarcoma metastasis

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Osteosarcoma is a rare cancer that affects mostly children and teens. About 20% of patients diagnosed with osteosarcoma have pulmonary metastatic lesions, which result in a 27% 5-year survival rate. Currently, osteosarcoma is treated with surgical resection, chemotherapy and radiation therapy with no major treatment protocol change in the last 30 years. A prior in vivo experiment was conducted using a rat orthotopic, syngeneic, immune competent animal model. Osteosarcoma UMR-106 cells were cultured and injected into the right tibias of Sprague Dawley rats to enable the development of osteoblastic and osteolytic tumors. Six weeks after injection, primary osteosarcoma tumors were evaluated via measurements of right and left tibias. Rats were then euthanized and necropsied for assessment of metastatic pulmonary tumors. Proteomic analysis of primary bone and metastatic lung tumors showed distinct differences. Specifically, the glycoprotein VI (GPVI) pathway was found to be upregulated in the pulmonary metastatic tumors. GPVI is responsible for initiating platelet aggregation by detecting exposed subendothelial collagen resulting from vessel injury. We hypothesize that the use of anti-thrombotic agents specifically targeting GPVI will decrease the metastatic ability of osteosarcoma cells. Using a similar experimental approach as previously described, we would additionally treat rats with either Revacept or ACT017. Revacept is a soluble form of the receptor, acting as a competitive inhibitor for GPVI while ACT017 is an antibody fragment for GPVI. The future experiments could test this hypothesis and evaluate novel treatment options for osteosarcoma patients that could block pulmonary metastasis and increase survival rate.

Research Grant: NCI R21 CA229582
Student Support: NIH T35 OD024982

Pharmacokinetics of Stokes compounding pharmacy extended-release theophylline formulation

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Theophylline is used in veterinary medicine as a bronchodilator to treat canine bronchitis. However, immediate-release formulas require administration four times daily, leading to lower owner compliance and poor management of chronic disease. Additionally, only one commercially available extended-release formulation has been tested in dogs and showed poor bioavailability. These shortcomings led compounding pharmacies to create new formulations for which the pharmacokinetics need to be established. This study will use eight healthy dogs to establish the pharmacokinetics of oral extended-release theophylline compounded by Stokes Pharmacy. The design will be a randomized, two-way crossover with a 7-day washout period between intravenous aminophylline (IVA, 8.6 mg/kg theophylline equivalent), to determine absolute bioavailability, and oral Stokes theophylline formula (STF, 10 mg/kg) administration. Plasma theophylline will be quantified by liquid chromatography and tandem mass spectrometry. Non-compartmental pharmacokinetics analysis will be performed for both IVA and STF. The accumulation ratio and the superposition methods will be used to estimate whether steady state plasma concentrations fall within the therapeutic range established for adult humans (10 - 20 mg/mL), as there are currently no studies determining the canine therapeutic range. We predict that the STF will have similar bioavailability to previously established theophylline products and produce adequate plasma concentrations and durations to be used at a twice-daily regimen. Validating STF will provide another product alternative for treating canine bronchitis, while improving client compliance and disease management.

Research Grant: Stokes Pharmacy
Student Support: Office of the Director, NIH, T35 OD011145
**Impact of climate change on waterborne diseases in companion animals: reconsidering veterinary surveillance**

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Waterborne diseases present substantial health risks to both humans and animals. A wide range of both infectious and non-infectious disease causing agents are associated with water and influenced by environmental conditions. Climate change is increasingly correlated with the severity, frequency, and distribution of waterborne health hazards. Although water associated risks are well studied for people, the impact on animal health is less clear. The objective of this project is to determine if climate change has the potential to alter the incidence of water associated diseases in companion animals, and whether or not the animal health community is prepared to identify such changes. A scoping literature review was conducted to determine if the most common climate-sensitive waterborne diseases in humans have the potential to impact companion animals. Case examples of climate-sensitive waterborne diseases known to occur in dogs, leptospirosis and harmful algal bloom (HAB) toxicosis, were used to identify challenges related to the study of companion animal diseases. This work highlights a critical gap in the animal health community and offers insight into a platform on which a companion animal surveillance system should be built. Given the current projections for global climate change, veterinarians and other animal health professionals need to be ready to respond effectively to evolving waterborne health hazards.

**Research Grant:** None
**Student Support:** Colorado State University, College of Veterinary Medicine and Biological Sciences

**Transcriptomic analysis of the role of tyrosine kinase ITK in Foxp3+ T regulatory cells**

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The tyrosine kinase ITK plays an important role in the activation of T cells. While its role in the development and function of CD4+ effector Th2, Th9 and Th17 cells is well described, its role in the development and function of Foxp3+ T regulatory cells (Tregs) is less well known. Foxp3+ T regulatory cells suppress the function of effector T cells, including Th17 cells, to modulate the immune response and the development of immunopathology. Previous work indicates that ITK negative regulates the development Tregs, such that mice lacking absence of ITK have higher levels of Tregs. Furthermore, while WT Tregs are able to suppress Th17 cells and the development of Inflammatory Bowel Syndrome (IBS) in mouse models, Tregs lacking ITK Tregs are unable to do so. In order to understand the molecular basis for this difference, we performed RNA-Sequence analysis of the transcriptome of FACS sort purified WT and ITK deficient Tregs. We examined differential RNA expression between the two groups and unique genes expressed within each group using bioinformatic software packages CLC Genomics, GenePattern, and Ingenuity Pathway Analysis. We identified 244 and 269 unique genes respectively differentially expressed in WT and ITK deficient Tregs, that may explain the role of ITK in regulating Treg development and/or function.

**Research Grant:** Veterinary Investigator Program T35 OD0010941
**Student Support:** Veterinary Investigator Program T35 OD0010941
Hair analysis for the biomonitoring of pesticide (or chemical) exposure in bats from different land use areas

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North American (N.A.) bats have important roles in ecosystem health through predation of insects. Pesticide exposure of bats can occur through insects consumed and water contamination or through skin contact in their roosting areas. High metabolic rate and storage of fat reserves annually makes them susceptible to long-term chemical accumulation within their bodies. Due to their longevity and foraging habits, bats consume pesticides and other chemicals causing disturbance to natural behaviors, such as hunting, migration, and hibernation, and sometimes death. Many studies assessed chemicals prevalent within bats and how these chemicals alter behavior. These studies historically require organ and other tissues leading to euthanasia of the bats tested. Greater than 30 percent of all species of bats within N.A. are declining; therefore, development of non- or low-invasive methods of toxin analysis are important to reduce further impact on populations. Our objective is to test and validate an analysis using clipped hair from the species *Eptesicus fuscus* (big brown bat). This species is common in Missouri and not listed as threatened. We will use bats submitted to the state rabies lab from throughout Missouri. We hypothesize composition and accumulated chemicals will vary by landscape context. We will select ten samples each from urban, agricultural, and forested areas. Hair will be cleaned, clipped, and tested using solid phase extraction and LC-MS/MS protocols. We expect to find the hair analysis method is equivalent to other tissue analysis methods in determining chemical exposures and accumulations within bats. By validating this non-invasive analysis method, we hope to reduce human impact on species decline.

Research Grant: 0056825 to University of Missouri from Missouri Department of Conservation
Student Support: University of Missouri Veterinary Research Scholars Program (MUVRSP)

Validating respiratory pathogen qPCR for use with feces as a non-invasive diagnostic tool for great apes

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Human respiratory pathogens pose a serious threat to great ape populations, often causing high morbidities and occasional mortalities. Because research and tourism result in frequent contact between humans and apes, quick and safe pathogen identification are essential for species health. Many respiratory pathogens are coughed up, swallowed, and passed in the feces, which are a non-invasive sample that is easy to collect for testing. Although previous studies screening for respiratory pathogens in wild great ape populations have used real-time PCR assays developed for humans, to our knowledge, these assays have not been validated for use with feces. To validate these assays, two different fecal DNA/RNA extraction methods were compared for identification of the respiratory pathogens *Streptococcus pneumoniae* and *human Metapneumovirus* (hMPV) in chimpanzee and gorilla feces. Varying concentrations of pathogens or their nucleic acids were added to fecal samples from clinically normal apes to evaluate assay performance compared to a standard curve. Results showed an effect of extraction method on target amplification and assay efficiency, demonstrating the need for assay validation. Newly validated assays will be used to test fecal samples collected from free-ranging chimpanzees in Gombe National Park, Tanzania during a respiratory disease outbreak of unknown etiology.

Research Grant: Zoological Pathology Program
Student Support: Office of the Director, NIH, T35 OD011145
Assessing students’ confidence and ability to perform nutritional assessments

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Obesity is an important epidemic among companion animal species with significant impacts for animal health. Although veterinarians can play a key role in the prevention and management of obesity, there are concerns that veterinarians may not be given adequate preparation for this role in veterinary school. The aim of this study was to assess the impact of specific nutrition curriculum developed for use in a student led veterinary outreach program on student confidence. The curriculum was previously developed by veterinary students and a veterinary nutritionist and focused on performing nutritional assessments. Pre- and post- surveys were used to assess student confidence and ability. On these surveys, students were asked to rank their confidence in various aspects related to nutritional assessments, answer a question about a short case, and describe their experience using the materials. Students involved in the outreach clinic were also asked to perform a practice nutritional assessment and develop a plan of action based on a fictitious case. Results will be analyzed via the wilcoxon signed-rank test and if significant improvement to confidence and ability is found, the materials will be made available for use by students in the outreach program.

Research Grant: Tisch College Fund for Community Research
Student Support: Lawrence Fund

Characterization of feline triaditis from an anatomic pathology perspective

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Feline triaditis is a multi-organ disease involving inflammation of the small intestines, pancreas, and liver. The pathophysiology and characteristics of triaditis are poorly understood, making it difficult to diagnose and treat appropriately. Moreover, the disease complex of ‘triaditis’ has not been validated as a distinct disease entity by anatomic pathologists. Currently, there are two proposed pathophysiological mechanisms which describe triaditis as an immune mediated disease; or an intestinal disease associated with dysbiosis. Although each mechanism results in different types of inflammatory populations and disease severity, proper diagnosis, and treatment have good prognoses. This study aims to explore the histologic characteristics of feline triaditis in relation to relevant clinical and clinicopathologic parameters to elucidate the pathophysiology of this disease further. A retrospective study surveyed feline patients admitted to the Iowa State University Veterinary Medical Center over the last twenty years. In the study, eighty-two cats were identified to have histologically confirmed inflammation in a minimum of two out of three organs. Thirty-nine of the eighty-two cats were clinically diagnosed, by necropsy or biopsy, with triaditis and majority of the population were male. Lymphocytes and plasma cells were the most common type of inflammatory infiltrate found on histopathology. Additionally, lymphoplasmacytic hepatitis and lymphoplasmacytic enteritis were most common co-morbidities among patients. Our results indicate that histologic and clinical characterization of triaditis could help clinicians better identify and prevent this condition in cats.

Research Grant: none
Student Support: Iowa State University College of Veterinary Medicine Summer Scholar Research Program
Opportunistic survey: diversity and prevalence of hemoparasite infections in wild birds in an urban setting

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Protozoan parasites in the order Haemosporida can significantly impact the health of select bird species. Although infections are generally well tolerated by their natural avian hosts, field and experimental studies have reported that chronic Haemosporida infections can result in reduced reproductive success, host fitness, increased stress, and disease susceptibility. At the Midwestern University campus in the city of Glendale, Arizona, we conducted an opportunistic study investigating the prevalence and diversity of hemoparasites (Plasmodium and/or Haemoproteus) within the wild bird population. Cardiac impression smears were taken from birds found deceased due to building-caused trauma and were analyzed cytologically. Blood parasites were morphologically identified using specific parasite descriptions described in literature and haemosporida identification was confirmed using PCR. We detected the highest prevalence of parasitemia within the white-winged dove (Zenaida asiatica) and mourning dove (Zenaida macroura) populations. These species are highly invasive to the region and were the most significant contribution to our population sample size. Scarce data exists investigating their influence on blood parasite disease transmission and its impact on the health of native bird populations. Our data serves to provide a current report of parasitemia caused by haemoproteus and plasmodium within avian species in the area.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Shelter adoption programs for cats diagnosed with feline leukemia virus or feline immunodeficiency virus

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Approximately 3-5% of cats are infected with FeLV or FIV nationwide, a diagnosis that impacts 60,000 cats in animal shelters each year. Historically, many shelters routinely euthanized positive cats, in conflict with AAFP recommendations against euthanasia based solely on infection status. The public expects shelters to save animals that are not suffering or dangerous to society, and adoption programs are increasingly common in both municipal and private shelters. The purpose of this study was to determine how Florida shelters manage retroviral infections. Shelters were surveyed for policies regarding testing for FeLV and FIV and the outcome of positive cats. In an interim analysis, surveys were received from 99 of the 154 animal shelters known to be operating in Florida, including 44 government shelters, 46 private shelters, and 9 private shelters with government contracts. A total of 85% shelters performed at least some testing, most using combination point-of-care devices for simultaneous FeLV and FIV screening. When shelters separated testing for the two viruses, more cats were tested for FeLV than FIV. Testing was performed for all cats in 39% shelters, some cats in 46% shelters, and no cats in 14% shelters. The most common reason for testing was to screen adoptable cats (79%), while testing cats in trap-neuter-return/return-to-field programs was used by the fewest shelters (14%). The most commonly reported outcome for cats with positive test results was adoption, reported by 52% shelters, followed by transferring to another organization in 50% shelters, or euthanasia in 37% shelters. Most Florida shelters had life-saving options for at least some of their positive cats.

Research Grant: Maddie’s Fund
Student Support: Maddie’s Fund, Department of Small Animal Clinical Sciences
Effect of social stress on CD8+ T cell responses during chronic SIV infection in ART treated pigtail macaques

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Since the beginning of the HIV epidemic in the 1980s, HIV research has been successful in advancing HIV treatment and our understanding of HIV pathogenesis. Despite these advances, there are social stressors that disproportionately impact people with HIV, which are not fully understood and have not been extensively studied in the HIV research field. A previous study conducted by our group found social stress in the form of single housing results in an altered immune response and higher SIV load during acute infection in a pigtail macaque model of HIV infection. Specifically, CD8+ T cells which contribute to viral load control by targeting infected cells, had lower absolute numbers and higher activation levels (elevated CD69 expression) when singly housed. We hypothesize that social stress will alter the CD8+ T cell immune response during chronic SIV infection by resulting in heightened activation in the singly housed macaques compared to socially housed, as an extension of the social stress effect observed in the acute infection stage. To further investigate the effect social stress has on HIV pathogenesis, we are conducting a retrospective analysis to quantify the number of circulating CD8+ T cells and define the level of CD8+ T cell activation. We will use FlowJo software to obtain these data and compare them between singly (N = 8) and socially housed (N = 32) SIV infected macaques using linear mixed effects regression models. These findings will further define the effect of social stress on viral pathogenesis and help guide future HIV studies as they consider socially housing SIV infected macaques to more accurately mimic HIV pathogenesis in this animal model.

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Student Support: NIH T35 OD024982

Identification of E. coli in urine from dogs with signs of urinary tract infection and negative urine culture

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Canine patients commonly present with signs consistent with urinary tract infections (UTIs). In most cases, diagnosis of a UTI is determined based on clinical history (polyuria, hematuria, dysuria, stranguria, and inappropriate urination), urinalysis, and urine culture. In as many as 65% of cases, symptomatic patients will have a negative urine culture result. Human studies have shown that the majority of women who are symptomatic for a UTI and have negative urine culture results will show molecular evidence of Escherichia coli in their urine, as detected via PCR. The purpose of this study is to determine if E. coli DNA is present in the urine of canine patients with UTI symptoms and negative urine culture results. Urine samples that were submitted to a diagnostic laboratory for culture were used in this study. Any potential DNA was extracted from the samples and PCR was used to amplify the uidA gene coding region of E. coli. PCR results were then compared to the reported laboratory culture results, and statistical analysis performed.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Evaluating the impacts of invasive red-eared slider turtles on native yellow-bellied slider turtle health

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Invasive species can pose a major threat to native wildlife by overexploiting, outcompeting, and introducing novel diseases to native species. Here we investigate how invasive red-eared slider turtles (*Trachemys scripta elegans*) impact the health of native yellow-bellied slider turtles (*T. scripta scripta*). These two species hybridize when in contact, further complicating their ecological relationship. Physical examination and blood chemistry evaluation (CHEM8+ with portable iSTAT reader) were performed in the field on yellow-bellied sliders in two ponds: one with red-eared slider contact (*n* = 27) and one without (*n* = 4). Yellow-bellied sliders in contact with red-eared sliders were on average smaller and weighed less than those that were in ponds without contact (length *t*-test, *P* = 0.042, *t* = 2.2; weight *t*-test, *P* = 0.048, *t* = 1.96). Blood chloride and urea nitrogen levels were significantly lower in yellow-bellied sliders in contact with invasive red-eared sliders (than for those not in contact (BUN *t*-test, *P* = 0.02, *t* = 3.15). Total carbon dioxide was significantly higher in yellow-bellied sliders in contact with invasive red-eared sliders than those that were not (TCO2 *t*-test, *P* = 0.02, *t* = -3.79). All other hematology and blood chemistry values were not significantly different between the two ponds. Additional field sampling is ongoing, though these preliminary results suggest a potential effect of red-eared slider presence on yellow-bellied slider body size. Further sampling, particularly of yellow-bellied slider populations not in contact with invasive red-eared sliders, will reveal additional insight into the impacts of invasive red-eared sliders on native species health.

Research Grant: Robert J Koller Endowment for Aquatic Animal Research
Student Support: Morris Animal Foundation Summer Scholars Award

Intestinal microbiome of broiler chickens with clinical and subclinical necrotic enteritis


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Necrotic enteritis (NE) is a form of enterotoxaemia in poultry caused by *Clostridium perfringens*. There are multiple predisposing factors to the disease including, immunosuppression and factors that change the gut microbiota composition. The prevalence of NE is increasing due to the withdrawal of prophylactic antimicrobial use in Canada. Therefore, there is a need for the development of new control and prevention strategies. The long-term objective of this study was to understand the pathogenesis of NE in order to develop effective control strategies. The specific objective of this study was to evaluate changes associated with the intestinal microbiome of broiler chickens who develop subclinical or clinical NE following *C. perfringens* challenge. We have successfully developed a subclinical and clinical NE animal model. The model consists of inoculating birds with infectious bursal disease virus to induce immunosuppression and/or increasing the dietary protein content to 28% to promote bacterial colonization upon challenge with *C. perfringens*. Challenged birds show distinct gross and histopathological lesions of NE. Using this model, intestinal contents were collected from healthy birds, and birds with clinical and subclinical disease to study the degree of dysbiosis of the microbiota. Extraction and purification of DNA from the samples has been completed and the library is being prepared for sequencing. Currently, 16S rRNA gene amplicon sequencing is being performed by next generation sequencing technology. Subsequently, metagenomic analysis will be performed using various bioinformatics software to assess differences in the composition of the microflora between healthy and diseased birds.

Research Grant: Poultry Science Cluster III, Chicken farmers of Saskatchewan and Saskatchewan Agriculture Development Fund
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Evaluating the role of NLGN4X in interferon signaling and immune environment in triple negative breast cancer

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Identifying molecular mechanisms that influence therapeutic responses remain pertinent in ensuring full therapeutic potential against triple negative breast cancer. In preliminary studies, Neuroligin 4X was identified as a protein highly expressed in breast cancer cell lines MDA-MB-231 and MCF-7. In TNBC patients, its high expression correlated with low survival probabilities. RNA-seq analysis of MDA-MB-231 cells silenced for NLGN4X produced more than 2,000 differentially regulated genes. Gene set enrichment analysis (GSEA) of these genes demonstrated upregulation of an antigen presentation pathway (APP) and interferon signaling (IFN) signaling when NLGN4X was silenced. According to the GSEA, the most upregulated genes in the IFN signaling pathway were IFIT3, IFIT5, IRF7, and IFIT2. RNA-seq analysis also showcased a downregulation in genes within the nonsense mediated decay (NMD) pathway with the most downregulated including SMG5, PNRC2, SEC13, and UPF3B. Analysis also presented diversity in unfolded protein response (UPR) gene expression with upregulation in DNAJB6, CLGN, and INHBE, and downregulation in the genes DNAJB9 and HSP90B1. The cancer genome atlas dataset of 104 TNBC patients showed downregulation of both APP and IFN signaling in patients with high NLGN4X expression. The genes significantly downregulated in the TNBC patients included IFNG, IFNA, IFNL1, CCL5, CXCL9, and CCL4. NMD and UPR pathway regulations correlating with NLGN4X knockdown have not yet been attained. The bioinformatic research presented in this study aided in the overall identification of NLGN4X’s role in TNBC; and laid the foundation for future studies aimed at experimentally isolating NLGN4X’s role in certain oncogenic pathways.

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Student Support: National Institutes of Health project # 5SC2-CA211028-03 and HRSA #D34HP00001

DGA-3, a novel compound with potent antifungal activity to treat fungal infections

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A quarter of the world’s population is afflicted with fungal disease, ranging from superficial dermatophytosis to invasive fungemia. The main threatening species: Candida, Cryptococcus, Aspergillus, and Pneumocystis are particularly devastating, causing 1.4 million fatalities per year. These agents are increasing in incidence and prevalence, especially in immunocompromised groups. While the number of people with life-threatening fungal infections continues to increase, current therapies are antiquated, limited in number, toxic to host tissues, and can be ineffective. In addition, multiple studies have documented a rising incidence in common clinical fungal strains that are resistant to conventional antifungal treatments. The need for novel antifungal drugs increases every year. The purpose of this study is to identify and characterize novel antifungal compounds for potential development as therapeutics. Our screening identified that DGA-3, a novel small molecule inhibitor, has potent antifungal activity without causing mammalian cell cytotoxicity. The effect of this compound on fungal virulence are currently being investigated. This study identified a novel compound with potent antifungal activity that can be potentially developed as a novel antifungal drug to treat drug-resistant fungal infections.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Effects of local delivery of gentamicin on tissue interface with a collagen scaffold

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Surgical site infections are a common complication in orthopedic surgeries due to the development of biofilms on foreign material used to stabilize injuries and facilitate healing. These biofilms are difficult to eliminate using systemic antibiotics due to enhanced resistance leading to concern of systemic toxicity when high doses of antibiotics are necessary. This has led to the development of products that not only serve to support the healing of injuries but also can serve as local delivery vehicle for antibiotics. The goal for these scaffolds is to increase local drug concentration, enhancing bactericidal ability while reducing the risk of systemic toxicity. Typically, these scaffolds release antibiotics with an initial burst effect followed by a prolonged low-level release. These characteristics create concern that 1) delivery of concentrated doses of antibiotics in tissues may cause an adverse effect at the tissue-scaffold interface, and 2) that sub-therapeutic systemic concentrations of antibiotics may drive emergence of antibiotic resistance. This study aimed to examine the effects of local delivery of gentamicin via a collagen tissue regeneration device on the tissue-scaffold interface using a rat mandibular defect model; and determine if systemic, prolonged release of sub-therapeutic concentrations of gentamicin would adversely affect the gastrointestinal flora and emergence of resistance genes.

Research Grant: CVM Research Funds
Student Support: Boehringer Ingelheim

Phenotypic plasticity of cutaneous squamous cell carcinoma mediated by cyclooxygenase-2

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Several types of cancers, including cutaneous squamous cell carcinomas (cSCCs), often up-regulate the enzymatic activity of cyclooxygenase-2 (Cox-2) to increase the production of tumor-promoting inflammatory prostanooids. Cox-2 (also known as Ptgs2) thus plays a role in the regulation of tumor development and progression and possibly metastasis, which is common for squamous cell carcinomas. Ptgs2 has been found to be over-expressed in cSCCs that originate from hair follicle stem cells (HFSCs) which act as cancer cells-of-origin when tumor suppressor p53 function is lost and KrasG12D is expressed. Using Cre-Lox recombination to generate Cox-2 knockouts, Ptgs2β/β mice were crossed to Krt15-CrePR; LSL-KrasG12D; p53β/β; Rosa-LSLTdTomato mice. The probability of tumor free survival was significantly improved in the Ptgs2 knockout mice compared with the wildtype. The Ptgs2 knockout tumors grew slowly, developed a rough surface and also possessed distinct phenotypes compared with wildtype tumors. Ptgs2 wildtype mesenchymal-like cSCCs expressed high levels of vimentin with little to no levels of E-cadherin whereas Ptgs2 knockout tumors conversely expressed high levels of E-cadherin with an absence of vimentin. Histologically, HFSC-originating cSCCs showed mesenchymal-like spindle cell carcinomas. Treatment with celecoxib, a Cox-2 inhibitor, caused the elongated spindle-shaped cells to become large and often multi-nucleated, demonstrating a change in cell morphology in vitro and possibly indicating induction of cellular senescence. Overall, the findings demonstrated that Cox-2 is required for efficient tumor formation and its expression correlates with aggressive cSCC phenotypes.

Research Grant: Cornell Stem Cell Program Seed Grant and Cornell Center for Vertebrate Genomics Seed Grant
Student Support: Veterinary Investigator Program T35 ODO010941 NIH
Influence of COVID-19 on access to care for cats and dogs

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The need for social distancing imposed by COVID-19 has accelerated the need for veterinary telemedicine to triage patients and provide some level of care. The first objective of this project was to determine the impact of COVID-19 on veterinary telemedicine, in terms of use frequency and types of applications. We hypothesized the number of practices offering telemedicine and the frequency of tele-appointments within each practice would be increased. The second consequence of COVID-19 on veterinary medicine revolves around concerns over disease transmission from and between pets. Cats are more susceptible than dogs to Coronaviridae and a few case reports have documented feline infections with COVID-19 abroad. The second objective of this project was to explore differences in care and owners’ perception between cats and dogs. We hypothesized that veterinarians would report a higher number of visits related to COVID-19 in cats than dogs. This study targeted small animal veterinary practices throughout the United States that are affiliated with Western University of Health Sciences College of Veterinary Medicine as preceptors of the third- and fourth-year curriculum. We deployed an electronic survey to identify how clinics have been affected by the coronavirus pandemic. Our database has 381 practicing veterinarians across 34 states. We have had 30 respondents thus far, and anticipate approximately 200 responses. The complete results will be presented during the poster session. We expect these results to allow us to better understand how this pandemic is affecting access to veterinary care for cats and dogs. We also expect our results to provide valuable insight into the scope of application of veterinary telemedicine.

Research Grant: PetSmart Charities
Student Support: None

First steps to understanding the epidemiology of ticks and tick-borne agents in northwestern Zimbabwe


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Canine monocytic ehrlichiosis, Heartwater Disease, and Anaplasmosis are devastating animal diseases caused by *Ehrlichia* and *Anaplasma* bacteria transmitted by ticks. Few studies address the ecology of ticks, their pathogens, and their hosts in northwestern Zimbabwe, which is home to a mosaic of rural farms, forest reserves, and Victoria Falls National Park. To gain a better understanding of the epidemiology of tick-borne pathogens in the region we investigated the roles of tick host use and tick occurrence with the presence of a pathogen. Specifically, we tested hypotheses that (1) certain ticks and hosts are more likely to be positive than others, (2) positive ticks are more likely to be found infesting multiple host species, and (3) positive ticks are more likely to co-occur together on the same host than negative ticks. We collected ticks from domestic and wild hosts and then screened those ticks for *Ehrlichia* and *Anaplasma* by amplifying the groEL gene common in both bacteria using molecular methods. A total of 463 ticks representing 12 species were collected from 8 host species; 136 of the ticks were groEL positive. Confirmation sequencing of some of the positive amplicons identified a variety of pathogens including several *Ehrlichia* and *Anaplasma* species. There was a significant tick and host effect as some tick species were more likely to be positive than others. Positive ticks were both more prevalent and abundant on domesticated hosts. Generalist ticks were not more likely to be positive and a host infested with more than one tick species was not a reliable indicator of finding a positive tick. These findings suggest that specific hosts and ticks are responsible for tick-borne infections.

Research Grant: Unknown
Student Support: Center of Excellence Summer Research Experience
**Trichomonas gallinae** in raptors: is there historical evidence for a trophic switch?

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Trichomonosis is a deadly parasitic infection of the upper gastrointestinal tract of birds caused by *Trichomonas gallinae* and other members of the protozoan family *Trichomonadidae*. Trichomonosis has historically circulated within Columbiformes and infected avivorous Falconiformes and Strigiformes. *T. gallinae* was first identified in columbids in the 19th century, however, has been known by falconers for centuries as “frounce” in raptors that predated on pigeons. The present review of the published literature on trichomonosis in raptors worldwide was compiled as a comprehensive resource to determine if host-pathogen dynamics has shifted in recent decades. Current literature suggests that trichomonosis is occurring in a wider variety of raptor species in recent years. Urbanization and changing habitats have altered predator diets and caused outbreaks in raptor species that are historically less affected by trichomonosis. Additionally, the emergence of trichomonosis in Passerines since the finch epidemic strain appeared in 2005 has revealed a new transmission route for infection, which places a wider array of raptor species at risk. Identification of *Trichomonas spp.* has historically been accomplished using light microscopy; however molecular characterization and genotyping of trichomonad isolates in recent years has greatly increased surveillance and elucidated host-pathogen dynamics. Continued molecular characterization of isolates will be necessary in order to understand transmission routes, pathogenicity and virulence of trichomonosis outbreaks in birds of prey.

**Research Grant:** Natural Sciences and Engineering Research Council of Canada  
**Student Support:** AVC Veterinary Summer Research Award

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**Influenza A virus effect on alveolar fluid clearance and CDP-choline as potential treatment for ARDS in mice**

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The bronchoalveolar epithelium clears excess fluid by an active, ATP-dependent Na+ transport process, which can be measured across the whole lung as alveolar fluid clearance (AFC). Maintenance of AFC is important to alveolar gas exchange. Impaired AFC has been shown to correlate with worse outcomes in patients who develop Acute Respiratory Distress Syndrome (ARDS). We have shown that ARDS development in mice infected with influenza A virus (IAV) is associated with impaired AFC. IAV infection reduces mitochondrial function in alveolar type II (ATII) cells and inhibits synthesis of the liponucleotides(LipoNT), which are essential precursors for phospholipid synthesis. We have shown that post-infection treatment of IAV-infected mice with lipoNT significantly attenuates IAV-induced hypoxemia and improves mitochondrial function in ATII cells. Since AFC is dependent on ATP, and mitochondrial function is dependent on normal phospholipid metabolism, we hypothesized that IAV-infected mice treated with lipoNT will have increased AFC when compared to untreated infected mice. To test this, we mock-infected or infected C57BL/6 mice intranasally with 10,000 IAV. At 6 days post-infection, mice were anesthetized and intubated to measure AFC. 300μL of 5% BSA/saline solution was instilled into the lungs via a tracheal cannula, followed by 200μL of air. Mice were then mechanically ventilated on 100% O2 for 30 minutes. Following ventilation, fluid was aspirated back from the lungs. Protein concentration was quantified with a BCA assay. AFC rate was calculated from the ratio of initial to final protein concentration. We found that IAV infection impaired AFC and this effect was reversed by daily LipoNT treatment over the course of infection.

**Research Grant:** R01-20120000052R2  
**Student Support:** Boehringer Ingelheim Veterinary Summer Scholar
Evaluation of regurgitant fraction in dogs with myxomatous mitral valve disease

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Mitral valve regurgitation (MR) secondary to myxomatous mitral valve disease (MMVD) is the most common heart disease affecting dogs. Conventional echocardiographic assessment of MR severity involves linear measurements of left heart chamber size. Echocardiographic estimates of regurgitant fraction (RF) represent a more direct assessment of MR severity. However, studies evaluating RF in dogs with MMVD are limited. The inodilator drug pimobendan has been shown to prolong the onset of heart failure in dogs with subclinical MMVD and cardiomegaly, but its effects on RF are unclear. Therefore, we sought to: 1) describe the relationship between RF and left atrial (LA) size, 2) determine the short-term effect of pimobendan on RF, and 3) assess the reproducibility of RF in dogs with MMVD. Dogs (n = 81) with varying severities of MMVD (n = 30 ACVIM stage B1; n = 38 stage B2; n = 13 stage C) were prospectively enrolled in this study. A subset of 10 dogs with subclinical MMVD received pimobendan for 7-10 days and 9 dogs were included in the RF reproducibility analysis. Regurgitant fraction was estimated using the total stroke volume method quantified by Simpson’s method of discs. Left atrial size was quantified by the LA-to-aortic root ratio (LA/Ao). A significant non-linear relationship was identified between RF and LA/Ao ($R^2 = 0.6; P < 0.0001$) and pimobendan significantly ($P = 0.002$) reduced RF (mean percent decrease of 32 ± 23%). Reproducibility studies are still ongoing. Our results suggest RF quantifies MR in a manner that is different (multidimensional) than LA size, and that pimobendan can reduce RF in dogs with subclinical MMVD. Assessment of MR severity using RF likely has clinical utility and may aid management of dogs with MMVD.

Research Grant: American College of Veterinary Internal Medicine and Center for Companion Animal Health at the University of California, Davis, School of Veterinary Medicine
Student Support: Boehringer Ingelheim Animal Health (BI)

Barriers to care access, and the resulting health effects in a low-income community during COVID-19

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Throughout the duration of the COVID-19 pandemic, veterinary practices have either reduced their caseload to include only emergency visits, or have shut down entirely to prevent the spread of the virus. While this phenomenon has impacted veterinary care access for all pet owners, those from low-income communities are especially impacted, as they often cannot afford to see a vet outside of a subsidized practice. The present study seeks to quantify the effect of Tufts at Tech Community Veterinary Clinic’s pandemic response and subsequent closure on its clients’ access to veterinary care. To adopt a One Health perspective, the researchers also collected data concerning client access to human medical care, as many Tufts at Tech clients have chronic health conditions themselves. Furthermore, the survey-based cross-sectional study examines the physical and mental consequences for both the pet and the pet owner in the event of blocked care access. Participants were randomly selected from a pool of recent clients, and were either emailed or contacted via phone until the target number of 200 clients was achieved. The survey was made available in both English and Spanish, and clients who completed the questionnaire received credit for a free physical exam on their Tufts at Tech account. Descriptive statistics will be analyzed and reported based on the findings from the survey. This data will help to guide and improve future emergency response plans, with the goal of ensuring adequate care access for all marginalized people and their pets.

Research Grant: PetSmart Charities, Tisch Student COVID Response (TSCR) Summer Program
Student Support: PetSmart Charities, Tisch Student COVID Response (TSCR) Summer Program
Estimating North American public and canine health burdens of *Campylobacter* and *Salmonella* infections in dogs

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Campylobacter is the most common bacterial cause of human gastroenteritis worldwide, affecting an estimated 20,000 Canadians and 1.5 million Americans each year. *Salmonella* causes an estimated 1.35 million infections in the United States and 12,000 cases in Canada each year, with the incidence believed to be much higher than reflected by laboratory-confirmed cases. Exposure to household dogs is now a well-recognized risk factor for infection with these enteric pathogens. Both pathogens also represent a significant canine health risk, with documented outbreaks of both *Salmonella* and *Campylobacter* occurring in populations of dogs. Campylobacteriosis in particular may be an under-recognized cause of diarrhea in dogs. Studies on *Campylobacter* and *Salmonella* shedding among healthy dogs indicate a high prevalence of both pathogens, with an estimated 22% to 75% of dogs positive for *Campylobacter* and 1% to 20% positive for *Salmonella*. With a growing number of dogs kept in households in both Canada and the United States, dog owners are likely at increased risk for salmonellosis and campylobacteriosis. The potential risk of human *Salmonella* or *Campylobacter* infection due to contact with dogs is currently unknown, and no studies have thus far estimated the number of human campylobacteriosis and salmonellosis cases attributable to canine contact. The approach used by Hale et al. (2012), who estimated the number of human enteric illnesses, hospitalizations, and deaths due to contact with animals and their environments in the U.S., was applied to estimate the public health burden of canine-transmitted campylobacteriosis and salmonellosis in both Canada and the United States.

**Research Grant:** None  
**Student Support:** Cornell University Veterinary Leadership Program

Associations of cobalamin in small animal diseases of cats and dogs

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Serum Vitamin B12, cobalamin, is utilized by intestinal bacteria and absorbed in the ileum. Disorders of cobalamin are gradually being recognized in small animal medicine. Not easily recognized in cats and dogs, causes of cobalamin deficiency ranges from hereditary diseases to gastrointestinal diseases. Measurement of just cobalamin concentrations does not necessarily reflect the body cobalamin status, but with the combinations of folate, homocysteine, and methylmalonic acid, can be essential in determining the cellular cobalamin status in these animals and be markers for small animal intestinal diseases in cats and dogs.

**Research Grant:** None  
**Student Support:** None
Study of T-cell infiltration into the esophagus in food allergic pigs via fluorescent immunohistochemistry

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Food allergy is a progressively serious problem that affects ~8% of the world’s population and consumes billions of dollars in government healthcare resources. One food allergy with a highly increasing prevalence is eosinophilic esophagitis (EoE), which causes difficulties with swallowing, food obstructions, and, in children, a failure to thrive. Our group is trying to develop the pig as a large animal model to study EoE and to better understand its immune mechanisms. EoE is a T-cell driven disease, so we hypothesized that pigs with EoE would have an increased presence of T cells in the esophagus. To test this, we determined the T-cell infiltration into the esophagus via fluorescent immunohistochemistry (fIHC). Due to COVID-19 lab restrictions, we started with a remote study of the theoretic aspects of the major components of the project - the immunology of EoE, and the chemistry, biology and procedures associated with fIHC and microscopy. Next, fixed and frozen esophageal tissue samples were collected from control pigs and food allergic pigs that were sensitized intraperitoneal and challenged orally with the food allergen hen egg white protein (HEWP). These frozen tissues were sectioned with a cryostat onto slides, which were then stained with the DNA stain DAPI to visualize the cell nucleus and an antibody against the T-cell marker CD3. Slides were visualized with CellSens software, and the T cells were quantified manually. Our preliminary data show that T cells can be stained and visualized via fIHC and this method can be used to enumerate tissue-infiltrating T cells. Upon completion of project, we will be able to use fIHC to determine if EoE pigs have a higher number of infiltrated T cells in the esophagus.

Research Grant: UNC and NC State Center for Gastrointestinal Biology and Disease (CGIBD, NIH Grant P30 DK 034987)
Student Support: Boehringer Ingelheim Veterinary Scholars Program, BD

Proteoglycan 4/lubricin and implications for osteoarthritis therapy: a review

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Proteoglycan 4 (PRG4) encodes for the mucinous glycoprotein lubricin best known for its lubricating role in synovial joints. A growing body of evidence suggests PRG4 has anti-inflammatory, pro-regenerative and chondroprotective effects as well. Some publications report loss of lubricin in response to joint injury, but accumulating evidence suggests lubricin may actually be increased. In naturally occurring equine carpal OA, PRG4 gene expression increases in synovial membrane tissue, and lubricin concentrations increase in the synovial fluid. In an experimental model of osteochondral fragment-induced carpal OA, lubricin synovial fluid concentrations also increased. A separate study reported increased synovial fluid lubricin concentrations of three distinct post-traumatic OA models in horses. These findings indicate that PRG4/lubricin increases in OA, yet this repair response is seemingly insufficient to quell inflammation and prevent the progression of this disease. PRG4 is a known ligand of the hyaluronic acid receptor, CD44. Studies show that PRG4’s interaction with the CD44 receptor of murine and human fibroblasts prevent the cells’ transition to a myofibroblast phenotype. Other studies have shown that PRG4 binds to toll-like receptors 2 and 4 to dampen the pro-inflammatory effects of lipopolysaccharide on human embryonic kidney (HEK) cells. Further studies should elucidate the mechanism of how PRG4 elicits these beneficial effects and explore other mechanisms responsible for the anti-inflammatory effects of PRG4/lubricin. We hypothesize that targeting PRG4 or the receptor(s) lubricin interacts with to facilitate its anti-inflammatory effects may lead to the development of novel therapeutics for OA.

Research Grant: None
Student Support: Veterinary Investigator Program T35 ODO010941
Histological and ultrastructure analysis of dark spot disease in *Orbicella* corals in the waters of St. Kitts

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Coral reefs are one of the most biodiverse ecosystems on the planet, supporting approximately 25 percent of global marine life yet only making up 1 percent of the ocean floor. The waters of St. Kitts are home primarily to the reef-building coral *Orbicella* spp, a cornerstone species found all over the world that is vital for its ability to draw calcium from seawater and construct rigid skeletons. These skeletons in turn provide durable anchors for other corals, as well as habitats for multiple species of fish. Unfortunately, reefs all over the world have been devastated by disease for decades, with *Orbicella* spp and other corals in the Caribbean withering from what is known as Dark Spot Disease. Discovered in 1990, Dark Spot Disease remains unknown to researchers. Its origins and epidemiology are still unknown, even as the disease itself has spread to every single reef around St. Kitts, afflicting over 30% of total colonies. Histological analysis has not been performed on Dark Spot Disease at this point, therefore a major portion of this project will be dedicated to histological analysis. Microscopic analysis will be performed looking for indications of disease such as a decreasing thickness of the epithelium when comparing healthy and lesioned samples. The ultrastructure portion of this project will focus on the zooxanthallae density as well as health, utilizing electron microscopy. Measurements of endolithic organisms will be taken utilizing dry weight after they are removed from the corals utilizing bleach. The goals of these methods are to develop a microscopic case definition for Dark Spot Disease, determine pathogenesis for the brown pigmentation observed with this disease, and identify potential etiologies.

**Research Grant:** Unknown
**Student Support:** Boehringer Ingelheim veterinary scholarship

Unmanned aerial vehicles (UAVs) for counting wildlife: a review of their efficacy compared to other methods

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Population counts of free-roaming wildlife are essential for scientists to monitor dynamics of a species and provide information for improved management and conservation practices. Unmanned Aerial Vehicles (UAVs) are a novel technology for scientists to conduct research on free-roaming wildlife, including to evaluate population density, monitor health and behavior, collect remote images, and identify threats to their environments. The purpose of this literature review was to evaluate the existing literature that compares the efficacy of UAVs to other methods for counting free-roaming wildlife. We hypothesized that UAVs are more efficient and welfare-conscious for evaluating free-roaming wildlife compared to traditional methods. Eighty relevant publications were retrieved from Thomson Reuters Web of Science that utilized UAVs to evaluate free-roaming wildlife: 9 literature reviews, 42 studies that evaluated terrestrial wildlife, 24 studies that evaluated marine animals, 6 studies that evaluated aquatic animals, and 2 studies that evaluated livestock. Publications that discussed behavior offered important insights to the welfare implications of UAVs. Published studies from 2006-2020 indicated researchers are using UAVs to study wildlife with increased frequency. Preliminary analyses indicated that UAVs have a shorter flight distance than manned flights, and offer overall improved accuracy, increased safety, and reduced costs, compared to traditional wildlife counting methods. Important future frontiers in the use of this technology include standardization of methodology and development of artificial intelligence technologies for processing large volumes of wildlife imagery.

**Research Grant:** None
**Student Support:** Office of the Director, NIH, T35 OD011145
A proper place for DREADD: verification of chemogenetic surgery placement in rats

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A behavioral pilot study was completed to examine the role of the prelimbic region (PL) in rodents that had previously received a time-based intervention. The experimental group (n = 6) received an inhibitory DREADD (Designer Receptors Exclusively Activated by a Designer Drug) virus to decrease activity in the region while the control group (n = 6) received a sham control virus that did not affect activity. The experimental group made more impulsive choices, suggesting that the PL may be involved in behavioral impulsivity following intervention. However, the surgical placement and expression of the viruses must be evaluated to confirm these results. This project aimed to verify and evaluate the viral expression of both viruses in the PL. Rats were euthanized and perfused, after which the brains were immediately extracted and soaked in paraformaldehyde for 24 hours followed by sucrose for 48 hours. Brains were then frozen with dry ice. Later, the brains were sectioned using the Rat Brain in Stereotaxic Coordinates Atlas (Paxinos & Watson) at 40 μm thickness. Slices were stained with DAPI (4′,6-diamidino-2-phenylindole) as a counterstain and viewed under fluorescence (Green Fluorescent Protein for sham group, mCherry for the active DREADD). The accuracy of placements and percentage of expression in the PL was then assessed. While the data show promising trends, the placements were somewhat mixed in accuracy and expressions. This suggests the need for replication and refinement of the current study.

Research Grant: NIH grants MH085739 and GM113109
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Multimedia case study development for timely on-farm euthanasia of cattle

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In instances when cattle become ill or injured to the extent that recovery is unlikely, euthanasia should be used as a tool to reduce the incidence of poor welfare outcomes. However, making euthanasia decisions on-farm is a complex, multi-step process that involves competing factors. Although standards for performing euthanasia are available and widely used, comprehensive guidelines that outline treatment and euthanasia timelines are currently deficient within the US dairy industry. Therefore, the objective of this project aimed to create an interactive training program targeting producers and caretakers on timely euthanasia in dairy cattle. Health conditions considered within case studies were selected based on primary mortality reasons identified in a 2014 report from the United States Department of Agriculture. Five case studies were developed for the production stage weaned heifers. Each study covered clinical sign recognition, pertinent animal history and relevant visual tools throughout to incorporate what would be observed on-farm. Each case study provided participants with three choices regarding their decision to manage a given condition (e.g. do not intervene and continue to monitor, treat the heifer, or euthanize immediately). Feedback was then provided (i.e. correct or incorrect) on their choice and included additional information either supporting (if correct) or guiding towards (if incorrect) the correct decision. Case studies will be integrated into a larger training program and be disseminated on a national level to improve the timeliness of humane, on-farm euthanasia across the US dairy industry.

Research Grant: United States Department of Agriculture (USDA) Agriculture and Food Research Initiative (AFRI) Animal Well-Being Grant
Student Support: NCSU Veterinary Scholars Program and Office of Associate Dean for Research and Graduate Studies
Eucoleus-associated verminous pneumonia in red foxes (Vulpes vulpes) in Atlantic Canada

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Eucoleus aerophilus is a nematode parasite found in the respiratory tract of foxes and other carnivorous mammals. There are no published reports of clinically significant E. aerophilus-associated disease in wildlife from Atlantic Canada or elsewhere in the world; however, sporadic cases of severe Eucoleus-associated pneumonia have been diagnosed in red foxes in the region through the Canadian Wildlife Health Cooperative (CWHC) diagnostic service. Therefore, the aim of this descriptive case series was to systematically review, characterize, and compare these cases to better understand the clinical disease that may be caused by E. aerophilus in free-roaming red foxes in Atlantic Canada. A search of the CWHC database from 2005-2020 was completed to establish a list of red foxes with verminous pneumonia caused by E. aerophilus, which resulted in 24 potential cases. The histopathologic description for 20 cases with available data was reviewed to compare lesions. Preliminary results indicate that the majority of affected foxes had granulomatous/pyogranulomatous pneumonia (67%), primarily located in the bronchioles (75%) and/or parenchyma (67%). The remaining cases had pneumonia that was necrotizing/necrohemorrhagic (13%), suppurative/mucopurulent (13%), or both (7%). Lesions were also identified within the bronchus (62%), and trachea (25%). Additionally, nine of the 20 cases presented with epithelial and/or glandular hyperplasia, and two had concurrent encephalitis. Of the 20 cases with histopathologic data, E. aerophilus either contributed to death or was fatal in eight cases. Final results will follow once histopathology slides are reviewed for all 24 cases and etiology and lesions are confirmed.

Research Grant: Canadian Wildlife Health Cooperative—Atlantic Region
Student Support: Canadian Wildlife Health Cooperative—Atlantic Region

Producer antimicrobial use decisions across diverse cattle farms in Ohio

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There is growing concern about antimicrobial resistance on cattle farms and its implication for agriculture and human health. However, very little is known about how different kinds of cattle producers make decisions about antibiotic use on their farm, as well as how landscape features may affect antimicrobial-resistant profiles. In our study, we use semi-structured interviews and sampled manure on representative cattle farms in three counties with contrasting farm structural profiles and landscape features in Ohio. We find that producers use diverse levels of antibiotics with varying degrees of interactions with veterinarians. Future analysis will determine and describe antibiotic use profiles and explore possible connections with producer decisions and landscape features with certain antimicrobial-resistant strains of bacteria found on the farms.

Research Grant: Initiative for Food and Agricultural Transformation (InFACT) Linkage and Leverage
Student Support: 2020 ENGIE-Axium Scholarship
Establishment of a cell culture model using whole lung to study effects of cannabinoids

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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-\(\alpha\) in response to lipopolysaccharide (LPS). We therefore hypothesized that cannabinoids would inhibit TNF-\(\alpha\) 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-\(\alpha\) production in response to different concentrations of LPS and cannabinoids. There was no difference in TNF-\(\alpha\) production by 1 or 10 \(\mu\)g/ml LPS. CBD (0.5-10 \(\mu\)M) progressively inhibited the production of LPS-stimulated TNF-\(\alpha\). 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-\(\alpha\) 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.

Research Grant: Summer Research Experience, National Institute of Health award number 5T35OD010432
Student Support: Summer Research Experience, National Institute of Health award number 5T35OD010432

Treatment of ocular feline herpesvirus using a novel topical immunotherapy

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Ocumune, a liposomal toll-like receptor ligand complex, is a novel topical immunotherapy designed to stimulate non-specific immune responses against ocular bacterial and viral pathogens and superficial cancers of the eye with potential for use in veterinary and human medicine. In a pilot study, Ocumune showed promise in treating ocular feline herpesvirus-1 (FHV-1) infection in shelter cats. The current study expands upon prior research investigating the in vitro efficacy of Ocumune to treat FHV-1 infection in cats. This study hypothesizes that pretreatment of cells with Ocumune at ideal concentrations will increase cell viability after inoculation with FHV-1 and decrease size and quantity of viral plaques in plaque assays. Ocumune was applied to Crandall Reese Feline Kidney (CRFK) cells in varying concentrations 24 hours in advance of FHV-1 inoculation, and both cell viability and plaque formation were quantified 48 hours later. Concurrent in vivo studies are assessing Ocumune as a treatment for both cats with experimentally induced FHV-1 infection and shelter cats with naturally occurring conjunctivitis, conjunctival hyperemia, chemosis or mild keratitis. If the results of these studies indicate efficacy of this treatment for FHV-1, Ocumune has the potential to be applied in trials for the treatment of human ocular herpesvirus infections, specifically herpes zoster ophthalmicus and herpesvirus keratitis.

Research Grant: Colorado’s Advanced Industry Accelerator Proof-of-Concept (AI-POC) Grant Program and Colorado State University’s Translational Medicine Institute (TMI) Translation Acceleration Program Grant
Student Support: NIH Fellowship Grant Trainee
Viability of equine embryos produced in vitro assessed with zona pellucida measurements

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With the development of procedures for intracytoplasmic sperm injection (ICSI) and embryo culture, in vitro embryo production has become prevalent in the equine industry. Therefore, there is a growing need to assess and assign quality parameters to in vitro-produced embryos, to determine early indications of viability. While quality scores are often assigned based on embryo morphology, more objective parameters would be beneficial. We hypothesized that measurements can be used to assess early embryo development and be indicative of further developmental potential. For this retrospective study, images of embryos were used that were captured at 200x magnification on an inverted microscope. The measurements were done using an image-assessment program (NIH ImageJ) for (1) inner zona pellucida area and (2) zona pellucida thickness at Days 0, 1, 4/5 and then daily or every other day until degeneration or transfer into a recipient (Day 0 = day of ICSI). ICSI-produced early embryos that do or do not develop into a blastocyst are then compared by t-test by Day and embryo stage (morula, early blastocyst and blastocyst) to determine when significant ($P < 0.05$) changes in measurements occur. From this research, we will learn more about equine embryo growth in culture and determine if objective measurements can predict embryo developmental potential, which will allow the equine veterinarian and embryologist to determine which embryos are viable and most suitable for transfer into a recipient mare.

Research Grant: The Cecil and Irene Hylton Foundation
Student Support: USDA Animal Health & Disease Scholar

Effect of Edwardsiella ictaluri live attenuated vaccines on phagocytic uptake & apoptosis in catfish B cells

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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, EiΔevpB 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 EiΔevpB 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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Student Support: National Institute of Health, 5T35OD010432
Time to failure of prophylactic topical timolol therapy in dogs with primary angle closure glaucoma

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Primary glaucoma is a heritable ocular condition in dogs manifesting as a spike in intraocular pressure (IOP), resulting in blindness. Because of its genetic and bilateral nature, after one eye develops primary glaucoma the fellow eye will also develop glaucoma. The fellow (pre-glaucoma) eye should be treated prophylactically with topical medication to delay the onset of glaucoma. Selective topical beta blockers (betaxolol) are effective for such prophylaxis, but there has been minimal research conducted on the efficacy of nonselective beta blockers. This retrospective study evaluated the efficacy of timolol maleate 0.5% ophthalmic solution in delaying the onset of glaucoma when used prophylactically in the healthy eye of dogs with primary glaucoma. This is to provide veterinarians with information about how long timolol can be expected to delay the onset of glaucoma, as timolol is a readily available, affordable medication in veterinary practice. Using patient records from the KSU Veterinary Health Center (2009-2020), the time to failure of prophylactic treatment with timolol in the healthy eye was recorded and analyzed for 34 client-owned dogs diagnosed with primary glaucoma. The number of monitoring IOP checks each patient received was also recorded, though this is not expected to significantly influence time to failure. Preliminary results reveal a mean of 7.2 months until failure of timolol therapy with time to failure ranging from 0.5-37.6 months. However, 11 patients have not experienced a spike in IOP based on KSU records (mean time since diagnosis: 30.1 months, range: 8.2-65.3 months). Follow-up continues. Of the 18 patients that experienced a spike in IOP, the mean number of rechecks was 3.7, ranging from 0-37.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

Perceived incidence, diagnosis, and treatment of anaphylaxis in veterinary medicine—a practitioner survey

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Anaphylaxis is a rapidly occurring allergic reaction that may be life threatening and requires immediate treatment. Anaphylaxis is a systemic, type I hypersensitivity reaction to an antigen such as drugs, food, and vaccines. Unfortunately, clinical signs of anaphylaxis are nonspecific in dogs and cats, which may result in delays of appropriate treatment. The most well known first-line treatment for anaphylaxis is the administration of epinephrine which counteracts the pathologic cardiovascular changes induced by anaphylaxis. These treatments are largely extrapolated from humans, which may have very different presenting signs and organ system involvement compared to veterinary species. Additionally, there are discordant results as to the utility of epinephrine in veterinary patients. A broad survey of practitioners will be performed to assess the perceived frequency of anaphylaxis cases, the approach to disease diagnosis, and the understanding of current therapeutic options for treatment of anaphylaxis. This study aims to provide valuable information on current veterinary practices that could contribute to a consensus on diagnosis and treatment protocols of anaphylaxis. Results are being analyzed as they are received.

Research Grant: Louisiana State University School of Veterinary Medicine
Student Support: Boehringer Ingelheim
Evaluating the severity of macrolide resistant *Rhodococcus equi* in foals

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*Rhodococcus equi* is a gram positive facultative intracellular pathogen and is a major cause of pneumonia in foals. Many foals are sub-clinical and will spontaneously recover without treatment, however some will acquire a severe acute respiratory distress that will cause death if left untreated. The purpose of this retrospective study is to better understand the differences in the severity of the disease when comparing macrolide resistant and macrolide sensitive strains. The hypotheses were that foals with macrolide resistant strains of *R. equi* would have more severe clinical signs, and would also have a greater number of concurrent bacterial infections. Data on foals testing positive for *R. equi* at a horse breeding farm in Louisiana over the span of 2 years was evaluated. The following was recorded for each foal with *R. equi*: Age, gender, location on farm, ultrasonographic exam findings, transtracheal wash results, and clinical signs such as cough, nasal secretions, and lymph node enlargement. Each foal was given a clinical score out of 3 based on how many clinical signs they manifested, and the number of concurrent bacterial infections was recorded. Overall there were 29 foals that tested positive, 13 of which showed macrolide resistance. The clinical scores and number of bacterial infections among the different strains will be statistically analyzed to show if there is a significant difference and if the macrolide resistant strains are more likely to have higher numbers in both categories.

**Research Grant:** None  
**Student Support:** NIH B819

Investigation of Powassan virus infection in Wisconsin dogs using a retrospective case study

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Powassan virus (POWV), a tick-borne flavivirus, is endemic in the upper Midwest and Northeastern United States and can cause severe meningoencephalitis in humans, which is commonly fatal. Our preliminary data from a 2017 prospective study of canine patients presenting to the University of Wisconsin-Madison Veterinary Care (UWVC) suggests that dogs are susceptible to infection with POWV. Of these dogs, 2 out of 193 healthy patients and 6 out of 266 systemically ill patients had detectable antibodies to POWV, including one clinically neurologic dog that was diagnosed with meningoencephalitis of unknown etiology at necropsy. These preliminary data confirm seroconversion to POWV in this population of dogs; however, this alone does not confirm POWV as a cause for neurologic disease. The goal of this retrospective study is to identify POWV infection in central nervous tissue from dogs with neurologic disease. To identify neurologic cases, we performed a retrospective case study of dogs necropsied at UWVC between the years 2015-2020. A neuro-specific terminology search of the UWVC database yielded 474 cases that were diagnosed with neurologic disease clinically or at postmortem examination. The search criteria were defined by selecting terminology frequently used by veterinary clinicians and pathologists when diagnosing diseases of the central nervous system. Dogs with clinical or histologic evidence of encephalomyelitis, especially those with underlying suspicion for infectious disease, will be selected for testing by immunohistochemistry for POWV antigen and RT-PCR for POWV genome. If positive cases are identified, this suggests that infection of central nervous tissue with POWV can cause encephalomyelitis in domestic dogs.

**Research Grant:** Companion Animal Fund, School of Veterinary Medicine, UW-Madison, PI Bernard  
**Student Support:** NIH T35OD011078, PI Bjorling
Analysis of the clinical course of Coccidioides spp. infection in dogs treated with fluconazole

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Coccidioidomycosis is a fungal infection prevalent in the Southwestern United States. A combination of clinical presentation and serology is commonly employed to establish a diagnosis. Once diagnosed, the mainstay of treatment is therapy with anazole antifungal agent; treatment duration is determined by resolving clinical signs and serial assessment of antibody titers. Our study aimed to better characterize the efficacy of fluconazole therapy for treating coccidioidomycosis. Medical records of dogs diagnosed with coccidioidomycosis from January 2015 through May 2020 at the Midwestern University Companion Animal Clinic were retrospectively reviewed. Coccidioidomycosis was diagnosed in 140 dogs during this period. 48/140 (34.3%) dogs had positive IgG titers and a minimum of two follow-up titers during fluconazole therapy. Median IgG titer at diagnosis was 1:16 and 39/48 dogs (81.3%) had a negative IgM titer. Median fluconazole dosage, interval to the first serology re-check, and IgG titer at first re-check was 19.5 mg/kg/day, 95 days, and 1:8, respectively. 43/48 (89.6%) dogs had a negative IgM titer at first re-check. The median duration until second serology re-check and associated IgG titer were 223 days and 1:4, respectively, and 46/48 (95.8%) dogs had a negative IgM titer. 24/48 (50.0%) dogs were treated until fluconazole discontinuation and the median duration of fluconazole treatment was 294 days. 3/20 (15.0%) dogs assessed after discontinuation restarted fluconazole due to recrudescence. The duration until recrudescence was 74, 174, and 233 days. This data characterizes the duration of Coccidioides spp. treatment using fluconazole and suggests that fluconazole effectively lowers IgG titers throughout treatment.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

A systematic review of current equine models of post-traumatic osteoarthritis

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Athletic injuries in children and young adults are on the rise, with post-traumatic osteoarthritis (PTOA) now representing an increased proportion of patients with osteoarthritis. Animal models of PTOA mimic these traumatic injuries in order to better characterize the disease and test novel therapeutics. Rodent joint destabilization models are most common; however, equine models provide distinct advantages over rodent models including: greater articular cartilage thickness, prevalence of naturally occurring PTOA, volume and ease of joint fluid collection and tissue sampling, and the ability to perform advanced imaging and implement exercise protocols. The majority of equine experimental models mimic intraarticular fracture with acute cartilage injury, however equine models of cartilage surface damage, traumatic impact, and ligament transection with resulting joint destabilization have also been described. The objective of this manuscript was to systematically review the current body of literature documenting equine models of PTOA induced by physical trauma and to determine the most widely utilized model. We performed a systematic review of the Web of Science, PubMed, and Google Scholar databases to gather peer-reviewed publications that performed in vivo controlled studies that assessed the impact of physical trauma on joint morphology, clinical lameness, synovial fluid composition, blood biomarker composition, and tissue histology or in vivo controlled studies that assessed the ability of an intervention to alleviate symptoms of PTOA. We hypothesized that the middle carpal joint osteochondral fragment model would be the most commonly utilized equine model of PTOA. Further investigation is underway.

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Student Support: North Carolina State University College of Veterinary Medicine, Department of Clinical Sciences
Efficacy and safety of suprachoroidal triamcinolone injection for poorly responsive equine recurrent uveitis

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Equine recurrent uveitis (ERU), a chronic, immune-mediated intraocular inflammatory disease, is the most common cause of blindness in horses. For many horses, the severity and recurrent nature of the inflammation can make the disease difficult to treat with current therapeutics leading to a poor visual prognosis (53.1% long-term vision retention). The suprachoroidal space (SCS), a potential space between the choroid and sclera surrounding the posterior segment of the eye, offers a promising alternative site for drug application to the eye. Steroid administration within this space is hypothesized to be safe and effective at controlling intraocular inflammation, especially in horses with chronic, poorly responsive ERU. A retrospective study was performed with 29 horses (36 total eyes) that received SCS injection of triamcinolone acetonide (TA) with ERU not well controlled with standard uveitis treatment. Standardized ophthalmic examination (OE) scores revealed a significant decrease in ocular inflammation over time after SCS TA administration ($P < 0.004$). Possible adverse effects of the injections occurred in $< 20\%$ of the horses at follow up, but some of these effects were attributed to chronic inflammation prior to effective treatment, long-term topical steroid use, or complications from hospitalization rather than the SCS injections. Most horses (86.7\%) in this study remained visual 3 months after SCS injection. Based on these results, SCS TA injections appear to be a safe and effective treatment modality for managing poorly responsive ERU; further clinical study is warranted.

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Student Support: North Carolina State University Veterinary Scholars Program and Equine Health Program

Impact of early life stress on the activation of gastrointestinal mast cells

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Early weaning (EW) is a common practice in pig production, which causes high levels of stress during a critical period of gastrointestinal (GI) immune development. We previously showed that EW causes long-term deleterious changes in GI barrier and immune development and increased disease risk compared to piglets weaned at a later age (LW). How EW triggers long-term developmental alterations in GI function and disease is poorly understood. Our lab showed that in comparison to LW, EW piglets exhibit higher plasma levels of histamine, a major product of mast cells (MC), and increased intestinal histamine receptor expression. MC activation and histamine may play an important role in initiating intestinal inflammatory responses that can deleteriously impact GI development. The objective of this research is to understand why EW piglets exhibit higher plasma histamine levels and intestinal histamine receptor expression. We hypothesize that the EW piglets that have increased numbers of intestinal MCs which will correlate with higher plasma histamine levels. Ileum was harvested from 15d old (EW) and 26d old (LW) piglets at 0hr and 24hr post-weaning, and then stained with Toluidine blue to identify MC; they were then quantified and categorized. Learning how wean age influences early intestinal MC activation could lead to management, nutritional or therapeutic interventions to mitigate the long-term deleterious impacts of EW to improve animals’ well-being, growth performance and disease resilience. Further, given the biological similarities between pigs and humans, this research could increase our understanding of the link between early life stress and susceptibility to chronic GI-related diseases, such as IBS, in humans.

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Histopathologic analysis of cryoablation of 6 subcutaneous canine tumors using a novel carbon dioxide device

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Cryoablation is a technique which uses freezing temperatures followed by thawing to kill tumor cells deep in the body. In veterinary medicine, cryoablation is typically only used for superficial lesions as human-grade devices capable of freezing large tissue volumes are prohibitively expensive. Kubanda Cryotherapy has developed a device that uses carbon dioxide, a low-cost, readily available gas, to perform a veterinary clinical trial as proof of principle in pets with subcutaneous masses for future translation to human breast cancer treatment. This device has been shown to form an iceball that reaches -40°C and causes necrosis in induced tumors in rats and normal pig livers. Six client-owned dogs with subcutaneous masses (>2 cm diameter) were treated with cryoablation in blocks of two dogs with escalating length of freeze-thaw cycles followed by surgical resection at 7 to 13 days. Histopathology was then performed on paraffin-embedded tissue. H&E stained slides taken primarily parallel to cryoprobe insertion were digitally scanned (Zeiss) and evaluated by manually tracing areas of necrosis, fibrosis, and granulation tissue (Proscia) by two blinded observers. The maximal length of affected tissue was compared to the maximal cryoprobe insertion length. The maximal width of tissue ablated was compared to the freeze-thaw time with longer freeze-thaw times anticipated to show larger widths of cryoablation. A Mann-Whitney test was used to compare the area/width of cryoablation for different freeze-thaw cycle lengths. This study validates a new, low-cost alternative to surgery for a variety of subcutaneous masses in pet dogs and will provide guidelines for appropriate selection of tumors for the next phase of clinical trials.

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Student Support: Boehringer Ingelheim

Evaluating health effects of microplastics in marine mammals: a scoping review

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The ubiquitous presence of plastics in the environment presents an emerging health risk to marine organisms and ecosystems due to potential physical effects, associated toxic plasticizers or additives that may leach from them, and due to their ability to interact with other environmental pollutants and pathogens. There is currently little information and no consensus opinion on the health effects of microplastics in marine mammals. This scoping review uses PRISMA(Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to provide an evidence-based evaluation and consolidation of methodologies used, risk factors, exposure pathways, and health effects for marine mammals related to environmental microplastic pollution. A systematized search of the literature was conducted and results across 4 databases were de-duplicated, imported into a review software, and screened using inclusion and exclusion criteria by 2 independent reviewers and an arbiter. All articles related to the evaluation of microplastics, macroplastics, plasticizers, and common plastic additives in marine mammal tissue were included yielding around 70 relevant articles. The quality of each study was rated based on a list of criteria including use of procedural blanks, contamination risk, sample size, and analysis methods. Results from this scoping review suggest that benthic and deep diving cetaceans may be at highest risk of plastic exposure. Additionally, prey predilection, feeding strategy and geographic location appear to play a role in exposure to plastics. The results of this review highlight key areas and avenues of future research necessary to assess the health risks of microplastics to marine mammals.

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MALDI-TOF assay for detecting β-lactam resistance in Staphylococcus aureus associated with bovine mastitis

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Mastitis has important economic implications for the Canadian dairy industry. Although several pathogens can be implicated, Staphylococcus aureus is one of the most prevalent and most frequently isolated mastitis pathogens. Mastitis is the most frequent reason for antimicrobial treatment of dairy cattle and β-lactam antimicrobial resistance is a major factor driving treatment failure. MALDI-TOF mass spectrometry is an emerging method of β-lactamase-based antimicrobial resistance detection that is both rapid and cost-effective. The aim of this study was to research the effect of four types of incubation media and three penicillin concentrations on the β-lactamase activity of Staphylococcus aureus and to measure this effect using MALDI-TOF mass spectrometry, in order to work towards developing a MALDI-TOF β-lactamase assay for detecting penicillin resistance in Staphylococcus aureus associated with bovine mastitis. Isolates developed from two strains of Staphylococcus aureus were used for this study. One strain was previously defined as penicillin resistant and the other as penicillin susceptible. Isolates were first cultured on blood agar and then subcultured on tryptic soy agar (TSA). Colonies were suspended at 0.5 McFarland in 4 types of incubation media (brain-heart infusion, phosphate buffered saline, tryptic soy broth and Mueller-Hinton broth) and at 3 penicillin concentrations (0.25 g/L, 0.125 g/L and 0.5 g/L). Results of the β-lactamase resistance test are not yet available, as the first trial using only brain-heart infusion as an incubation medium resulted in a lack of spectra on the MALDI-TOF instrument which required that the protocol be redesigned. Results from the current trial will be presented and discussed.

Research Grant: Unknown
Student Support: Atlantic Veterinary College (VetSRA), NSERC (USRA) and the BIVS program

The anatomy and histology of the copulatory organs of the male Argentine tegu

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Over the past 10 years, the Argentine tegu (Salvator merianae) has become a highly successful invasive species in southern Florida. Much of this success is attributed to the tegu’s efficiency in reproduction. Published report of the microscopic anatomy of the copulatory system in these animals is incomplete. The purpose of this project was to study the anatomy of the copulatory organs of the Argentine tegu and their relation to the mechanism of erection and sperm transport. Previously, we determined the segments of the male reproductive tract that secrete mucus. In the present study, histochemistry was used to determine the composition of this mucus. The glands at the base of the hemipenis were positive with alcian blue pH 2.5, indicating sialomucins were present. The sexual segment of the kidney, and the proctodeal and urodeal portions of the cloaca were positive with PAS and negative with alcian blue. This demonstrates that the mucins secreted were not acidic, containing neither carboxylated nor sulfated residues. Sperm morphology was analyzed and found to be similar to other species in the genus Salvator. For the mechanism of erection, a series of sinuses surrounded by elastic tissue were found to correspond with the transverse ridges on the asulcate surface. This information coupled with findings on gross dissection suggest that these sinuses are involved in the mechanism of erection when engorged causing an increase in pressure and forcing the hemipenis to evert.

Research Grant: Western University of Health Sciences intramural grant
Student Support: College of Veterinary Medicine, Western University of Health Sciences
Analyzing the effects of kisspeptin-10 on in vitro models of equine placental development

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Adequate trophoblast cell invasion and remodeling of the maternal uterine tissue is a prerequisite for placenta formation and pregnancy success. Dysregulation of these processes is known to cause complications, including early pregnancy loss, infertility, and preeclampsia. In vitro models are utilized to study trophoblast cell invasion and migration, and angiogenesis, scratch and tube formation assay, respectively. Kisspeptins (KP), a family of small peptides, have been shown to regulate both cellular migration and angiogenesis in several cell types, including trophoblast. The hypothesis of this study was that kisspeptin-10 (kp-10) would inhibit the in vitro migration of invasive equine trophoblast and fibroblast cells over time. The migration rate for each treatment group (0, 1, 10 or 100 μM exogenous kp-10) and cell type was analyzed for statistical significance using JMP. The migration rate of trophoblast cells was significantly enhanced by 100 μM kp-10 by 24 hours as assessed using MRI Wound Healing macro (ImageJ) by a single blinded operator. Preliminary analysis of the equine fibroblast cells suggests that 100 μM kp-10 has an inhibitory effect compared to the control group. These results show that kp-10 has a differential function in equine trophoblast cells. Further studies are warranted to investigate the differential function of kp-10 in various cells in the equine placenta.

Research Grant: VCS CORP Grant
Student Support: National Institute of Health T35 Grant

Immunohistochemical detection of lymphocytes and plasma cells in tissues from multiple avian species

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Immunohistochemistry (IHC) in formalin-fixed paraffin embedded tissues is commonly used in veterinary pathology. However, most commercially available antibodies are raised against mammalian epitopes, limiting applications in distant taxa (e.g., avian species). In order to assess the cross-reactivity in avian tissues of three commercially available antibodies raised against human Pax5, CD3, and MUM-1 (B-lymphocytes, T-lymphocytes, and plasma cell markers, respectively), lymphoid tissues (i.e., thymus, bursa, spleen, Harderian gland) from a variety of avian species were collected from archived postmortem cases submitted to the Ontario Veterinary College. Birds with obvious lesions in these organs or systemic immunosuppressive disease were excluded. The IHC protocol was conducted using commercially available antibodies [monoclonal: clone 24/Pax5, MUM-1p, and polyclonal: CD3] initially optimized on chicken tissues. Reaction specificity was assessed based on the known distribution of the target cells in these tissues (i.e., microanatomy). In total, lymphoid tissues from 50 avian genera in 13 families were included in the study, and the expected IHC reactivity for the three markers was observed across all genera, with few exceptions. CD3 was reactive in all genera. Pax5 was reactive in all genera except four of five species of falcons (Falco spp.). MUM-1 was reactive in all genera except canaries (Serinus sp.). Surprisingly, Pax5 reactivity was observed in T-cells in addition to B-cells in domestic geese (Anser anser domesticus). These results suggest that the Pax5, CD3, and MUM-1 are cross-reactive markers in many avian species, but discrepancies indicate that validation in control tissues is necessary prior to use.

Research Grant: Ontario Veterinary College Pet Trust
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Predictors of retrovirus transmission in an endangered carnivore, the Florida panther (Puma concolor coryi)

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Infectious disease can have major impacts on both individual and population health of free-ranging wildlife, especially species of conservation concern. The Florida panther (Puma concolor coryi) is an endangered subspecies of mountain lion found only in southern Florida which was severely affected by an outbreak of feline leukemia virus (FeLV) in 2002. FeLV remains an ongoing concern for this subspecies, and there is a need for improved preventative management to ensure panthers’ ongoing conservation. Key to defining preventative protocols, however, is a detailed understanding of how FeLV transmits among panther hosts, knowledge of which is limited by the species’ cryptic, solitary nature. In an effort to determine drivers of transmission in panthers, we studied natural transmission of a closely related, analogously transmitted feline retrovirus: feline immunodeficiency virus (FIV). We used next generation sequencing to sequence FIV isolates from free ranging panthers, and then used the program Phylloscanner to infer a who-transmitted-to-whom transmission network from these sequences. The transmission network was then analyzed using exponential random graph models (ERGM) to determine factors describing FIV transmission events (e.g. panther age, sex, relatedness) while controlling for network structure. ERGM analysis identified panther age class and pairwise geographic distance as the most important variables describing the FIV transmission network. Next, we are using these results to simulate FeLV transmission in simulated panther populations to determine how well drivers of FIV transmission can predict FeLV transmission. These results will then guide subsequent efforts to improve FeLV preventative measures in panthers.

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A description of local, severe infectious bovine keratoconjunctivitis outbreaks

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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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Identification and validation of mouse substrain variants and correlation with adenoma development resistance

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Colorectal cancer is the 3rd most common cancer diagnosed in humans, and many animal models have been designed with varying degrees of translatability. Reproducibility of studies can be uncertain due to both environmental influences and genetic drift of isolated colonies resulting in variable phenotypes. Genomes of two substrains of mice, C57BL/6JD-Apc\textsuperscript{Min} and C57BL/6J-Apc\textsuperscript{Min}, that each possess the same mutation that model human familial colon cancer were recently sequenced with the observation that Min/D mice are more susceptible to developing colon cancer. Of the sequenced genomes, several homozygous variants of Min/D and Min/J mice were identified with limited knowledge of their potential regarding adenoma development. The goal of this study is to genotype a narrowed down list of these variants in order to better understand their influence on colon cancer susceptibility. Of the thousands of variants identified we selected variants using online resources through the Mouse Genome Informatics website provided by The Jackson Laboratory, the National Center for Biotechnology Information and the UCSC Genome Browser. Broadly, we focused on variants near or within genes involving inflammatory processes, membrane transport, signaling proteins, and gene regulatory proteins. From these categories we chose 11 variants based on their expression in the alimentary system and phenotypes. 3 variants specifically from chromosome 11 from the Min/D line and 8 from different chromosomes of the Min/J line. The variants shall be validated with PCR and traditional Sanger Sequencing using the DNA from the originally sequenced mice.

Research Grant: NIH U42OD010918 Mutant Mouse Resource and Research Center. University of Missouri College of Veterinary Medicine Faculty Development grant.

Student Support: University of Missouri Veterinary Research Scholars Program and IDEXX-BioAnalytics

Postnatal metabolic effects on offspring of advanced maternal age mothers in Vervet monkeys

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Over the past several decades there has been an increase in the number of women giving birth at an advance maternal age (AMA). However, little information is known about the related risks of child health as a consequence of having an AMA mother. Previous studies have demonstrated the potential use of non-human primate (NHP) models as human pregnancy models. Our lab has previously demonstrated the African Green Monkey (Vervet) disruption of hormonal and immunological responses in AMA mothers and the postnatal growth retardation in offspring from AMA mothers. Therefore, the goal of this study was to investigate the postnatal metabolic effects on 2-4 years old Vervet monkeys offspring of AMA mothers. Our central hypothesis is that AMA impairs maternal hormonal production leading to aberrant immune programming disrupting offspring metabolism. To evaluate this, a comprehensive systemic search of the online data base CARS at Wake Forest was conducted and surviving infants born between 2011 - 2016 were identified. The surviving infants were classified in two different groups; infants born to an AMA mother (> 10 years) or to a young mother (< 10 years) at the time of delivery. The health outcomes of these surviving infants were compared, to evaluate whether there is a relationship between the mother’s age and the negative health outcomes. Preliminary results suggest that there is a kidney phenotype within the AMA offspring. Within this group, there was a negative correlation in BUN values and maternal age of the Vervet monkeys studied. Further analysis has to be done to better define how AMA can affect the offspring metabolism.

Research Grant: Unknown

Student Support: NIH-T35 Interdisciplinary Biomedical Research Training Program
Phylogenetic relationships and antibiotic resistance in *Campylobacter upsaliensis* isolates from kennels

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*Campylobacter upsaliensis* is increasingly recognized as a cause of campylobacteriosis in humans and is estimated to be responsible for 6% of these cases. Household pets, particularly canines, are thought to be the primary reservoir of *C. upsaliensis* and little is known about its molecular epidemiology in the canine host. The goal of this project is to analyze the antibiotic resistance profiles and genetic relationship of *C. upsaliensis* derived from a recent survey of seven dog breeders in three states. These isolates were found generally susceptible to clinically used antibiotics but reduced susceptibility to ciprofloxacin was detected in some of the isolates. Non-duplicate *C. upsaliensis* isolates were cultured and were subject to whole genome sequencing (WGS) analysis by Illumina MiSeq. These samples were compared with the WGS data of human *C. upsaliensis* isolates in an NCBI database to determine antibiotic resistance determinants and the phylogenetic relationship between the isolates. Based on the whole genome SNP analysis, the isolates from dams and puppies of the same breeder tend to cluster together on the phylogenetic tree and the canine isolates are generally divergent from the human isolates in the NCBI database. Several isolates with an elevated ciprofloxacin MIC harbored the known mutation for fluoroquinolone resistance. One canine isolate was determined to harbor a known genetic mutation for streptomycin resistance and was most closely related to a human isolate. These results reveal the high prevalence of *C. upsaliensis* in dogs and suggest zoonotic transmission of this organism between canine and human.

**Research Grant:** CDC Grant 75D30118C02907  
**Student Support:** NIH T35 Training Grant T335OD012199

Exploration of *Leptospira interrogans* and parasitic co-infections in Townsend’s chipmunks (*Tamias townsendii*)

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Leptospirosis is one of the most geographically widespread zoonotic diseases in the world. Human activities have altered the epidemiology of leptospirosis leading to re-emergence of disease. Many wild rodents act as reservoirs for *Leptospira* spp. and bacterial shedding into the environment can lead to infection of other animals and humans. Complex interactions between environmental conditions, wildlife, and human health underline the importance of evaluating the dynamics of co-infecting pathogens in wild rodent reservoirs affected by anthropogenic activity. The aim of this study was to examine associations between *Leptospira interrogans* and co-infecting parasites in Townsend’s chipmunks (*Tamias townsendii*) along a gradient of forest management. We hypothesized that *Leptospira* titers would be associated with the presence and burden of co-infecting parasites, and that these associations would differ along the disturbance gradient. Previously collected plasma Microagglutination Test (MAT) titers for six serovars of *L. interrogans* and intestinal parasite counts from chipmunks captured across three levels of forest management were used to compare *L. interrogans* prevalence and parasite prevalence and burden at each of the three forest management levels. Results and associated findings will be presented and discussed in the context of a One Health approach to disease dynamics and the need for understanding *L. interrogans* dynamics in natural systems. This knowledge will aid in dissecting the interactions between environmental conditions, wildlife health, and zoonotic disease transmission.

**Research Grant:** Environmental Protection Agency Science To Achieve Results (FP-91730601-3 to Hanselmann); Oregon State University College of Veterinary Medicine 2012 One Health Initiative (to Jolles and Hanselmann)  
**Student Support:** Boehringer Ingelheim
Probiotics as antibiotic alternatives for control of necrotic enteritis in poultry: a review

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Necrotic enteritis (NE) is an economically important enteric disease of poultry caused by *Clostridium perfringens* type A bacteria. Recent regulatory ban on antibiotic feed supplements in food animals has caused a spike in NE incidences in poultry, which has sped up the need for finding effective alternatives to antibiotics for NE control. Many means of NE control strategies have been tested, of which, probiotics seem to offer a better antibiotic alternative strategy. Probiotics are live microbial supplements that benefit gut health by maintaining a healthy microbial balance necessary for mucosal integrity and intestinal immune health. The current review focuses on studies that evaluated the effects of probiotics on *C. perfringens* growth and development of NE in poultry. Several probiotic species, including those belonging to the genera of *Lactobacillus, Enterococcus, Bacillus* and *Bacteroides* have been successfully tested in chickens and turkeys against *C. perfringens* and NE to show improved bird performance, reduced enteric burden of *C. perfringens* and associated pathology. Research invested in understanding the probiotic mechanisms of NE control suggest that probiotics can help maintain a healthy gut microbial balance by changing its composition, improve mucosal integrity by upregulating expression of tight-junction proteins and modulate the inflammatory response by downregulating expression of interleukin (IL)-1β, IL-17, IL-8 and other cytokines. Collectively, these studies indicate that probiotics can offer a promising degree of NE control strategy and that more investigations are needed to study if these research-developed probiotics can effectively prevent NE infection in a commercial poultry operational setting.

**Research Grant:** None

**Student Support:** Unknown

Case study design for timely on-farm dairy cattle euthanasia training

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Euthanasia decisions are a critical component of animal welfare to eliminate undue pain and suffering of an animal who is unlikely to recover. The US dairy cattle industry currently lacks clear guidelines for timely euthanasia decisions, and previous work suggests producers varied regarding health condition management and demonstrated a strong proclivity to treat compromised cattle even if treatment response was unlikely. To empower producers and caretakers to make effective decisions specific to on-farm euthanasia, an interactive, multimedia case study training program was developed for common diseases of dairy cows. For each case study, users were provided basic information and background of an individual animal in conjunction with realistic pictures, videos, and narratives. From there, users were able to practice their observational skills and apply their knowledge to select one of three treatment options for an animal (e.g. monitor, treat, or euthanize). Based on the decision chosen, feedback was provided to help learners understand if their decision was correct or incorrect. Incorrect responses provided additional information detailing a more appropriate response. These case studies will be implemented into a larger training program and disseminated on a national basis to US Dairy producers to improve timely euthanasia on dairy cattle farms.

**Research Grant:** United States Department of Agriculture (USDA) Agriculture and Food Research Initiative (AFRI) Animal Well-Being Grant

**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Evaluation of tumor infiltrating macrophages, MCP-1 and TGF-β expression on canine appendicular osteosarcoma

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Osteosarcoma (OSA) is the most common form of bone cancer in dogs and is most frequently diagnosed in the appendicular skeleton. Amputation of the affected limb improves quality and length of life. However, canine OSA is particularly dangerous because of its tendency to metastasize to the lungs. Therefore, amputation is often used in conjunction with additional treatment modalities. Of the additional treatments available targeting canine OSA, immunotherapy has shown promise. One of the understood ways that the cancer cells sustain life is by immunosuppression of the body. However, the mechanisms are not completely understood. Identifying and targeting the mechanisms of this complex could ultimately prevent metastasis and improve immune responses against cancer. Many cell types and molecules are involved in this process, and typically cause phenotypic, chemotactic, and environmental changes to benefit the tumor. However, the impact of immunosuppression on canine OSA is still incompletely defined. The objective of this study was to evaluate the presence of MCP-1, TGF-β, and tumor infiltrating macrophages in a clinical canine OSA sample. It was hypothesized that these biological factors would be present. RT-PCR was first performed on two cultured OSA cell lines to determine if the mRNA was transcribed for MCP-1 and TGF-β. Upon validation that they were actively being transcribed, western blots were performed on the cell lines to confirm translation of MCP-1 and TGF-β. Finally, RNA in situ hybridization was performed using RNAscope and revealed presence of MCP-1, TGF-β, and IBA-1 (a protein expressed in macrophages). Overall, this study supported the hypothesis that MCP-1, TGF-β, and macrophages are present in canine OSA.

Research Grant: None
Student Support: Blue Buffalo, Florida Veterinary Scholars Program

The Alouatta pigra diet: the influence of age on diets

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Zoo nutritionists must design diets that complement the needs of animals in all age groups. Different species require different nutrients to remain healthy; however, there is less knowledge on dietary needs within a species depending on age. Therefore, age-based studies that observe the dietary patterns of wild populations can help to better formulate diets for captive animals. This study aims to examine the dietary patterns of Yucatan/Guatemalan black howler monkeys in the region of Tabasco, Mexico; specifically, how age influences their diets. The alternative hypothesis is that there is a statistically significant difference between the eating patterns of adult versus juvenile Alouatta pigra. Data for this project was collected in 2015 through a series of focal studies/observations. Individuals were organized into two age groups: juvenile (4 total) and adults (9 total). Consumption of each plant was recorded as a percentage of that individual’s total diet. Preliminary results indicate that age does not have an influence on Alouatta pigra diets; meaning there is no difference between the plant choice of adults versus juveniles. Further statistical testing will include unpaired t-tests on the percent-ages of each plant. Results from age-based studies, such as this one, would target nutrition for each age group of each species. Further intraspecies studies based on age are encouraged to ensure that animals in captivity are being provided the nutrients they need at each life stage.

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Identification of NPDHN4OMe, a novel small molecule inhibitor with antifungal activity

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*Candida albicans* one of the most common fungal infections in immunocompromised or critically ill patients. *Candida* is a major cause of bloodstream infection that often results in sepsis or shock and, ultimately, a mortality rate ranging from 40-60%. Current treatment options are limited to only a few antifungal drugs, which often display cytotoxicity. In this study, our goal is to identify novel compounds with antifungal activity without causing host cell toxicity. We screened several series of novel compounds against *C. albicans* with an aim to identify and develop new antifungal drugs. Surprisingly, our screening identified that NPDHN4OMe, a novel small molecule inhibitor with potent antifungal activity without causing mammalian cell cytotoxicity. The effect of this compound on fungal virulence is currently being investigated. Collectively, the result from study identified a novel compound with potent antifungal activity that can be potentially developed as a novel antifungal drug to treat drug-resistant fungal infections.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Assessing the risk factors associated with presence of influenza A virus in exhibition swine at county fairs

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Influenza A virus (IAV) is commonly detected in exhibition swine populations at county fairs where many pigs are brought together to be shown. Commingling of many IAV infected swine and subsequent contact with humans present at the fairs presents a public health risk of the commonly reported zoonotic transmission of IAV from exhibition swine to humans. We investigated risk factors that may contribute to the presence and transmission of IAV in swine at county fairs from 2013-2019. The presence of IAV at a fair was determined by the PCR testing using nasal swab and snout wipes samples at 633 individual fair events in the United States. Chi square tests were used to test the association of potential risk factors with IAV status of fairs. We found that whether the fair had a breeding show in addition to a market swine show (*P* = 0.000), if the fair held an open show in addition to the junior show (*P* = 0.004), if pigs were removed due to illness prior to sampling (*P* = 0.002), if pigs showed any signs of respiratory disease at the time of sampling (*P* = 0.000), and if the fair had a primary tagin prior to the show (*P* = 0.031) were significantly associated with the presence of IAV. Furthermore, it was found using Kruskal-Wallis tests that whether pigs were removed prior to show due to illness (*P* = 0.0195) and if pigs showed signs of respiratory disease (*P* = 0.0001) were significantly associated with the prevalence of IAV among positive fairs. These findings can inform protocols in an effort to decrease the presence and prevalence of IAV in exhibition swine at county fairs. In turn, this can also help improve the public health risk associated with zoonotic IAV cases from agricultural fairs.

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Student Support: The Ohio State Veterinary College Summer Research Program
Oxygen tension and macrophages mediate proliferation of osteosarcoma cells in 3-dimensional culture

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Osteosarcoma (OS) is the most common primary malignant tumor of bone in children, young adults, and canines. Unfortunately, 5-year survival rates in patients with metastatic disease have remained at 20% for the last 40 years, motivating the urgent need to understand what triggers the migration and metastasis of OS. Current approaches to interrogate key signaling pathways in OS are pursued in monolayer culture using homotypic cell populations, each of which lacks physiological and clinical relevance and represents a likely stumbling block in effectively discovering druggable targets to inhibit metastasis. Since current models are insufficient to model this cancer, we hypothesized that OS would exhibit differences in MMP production, metabolic activity, and proliferation as a function of local oxygen concentration and entrapped in a clinically-relevant engineered hydrogel. We entrapped a homotypic culture of highly metastatic murine K7M2 osteosarcoma cells (HOM) or a heterotypic combination of K7M2 and RAW 264.7 murine macrophages (HET) in fibrin-alginate interpenetrating network hydrogels. In HOM gels, K7M2 proliferation correlated with increasing oxygen tension, and we observed similar trends in proliferation as a function of oxygen tension with heterotypic (HET) cultures, though DNA content was significantly greater in HET gels compared to HOM gels after 6 days at both 5% and 21% O₂. These data demonstrate the importance of oxygen tension on OS proliferation, as well as the effect of the addition of macrophages, a common cell type present within tumors.

Research Grant: UC Davis Health
Student Support: Lodric Maddox Graduate Fellowship

Investigating the prevalence of equine limb edema via video education and survey

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The lymphatic system is an important aspect of equine health. The lymphatic system is responsible for delivering fluid from horse’s tissues, filtering it through their lymph nodes, and allowing their immune system to clear harmful substances. When this system is disrupted, horses can suffer from poor circulation and an inadequate immune response. Horses are prone to lymphatic issues due to their lack of lower limb musculature. Lower limb edema, called “stocking up” is anecdotally a very common lymphatic drainage issue in horses, with limited treatment options available. Currently, there is very little data to document the extent that lymphatic drainage issues affect horses. This research will provide a better understanding of the prevalence of limb edema in horses and current treatment methods. An educational video was distributed in order to give the equine-oriented public a basic understanding of the lymphatic system and lower limb edema. A survey was designed to collect feedback from equine professionals and horse owners about their experience with lower limb edema, lymphangitis, and other pathologies. The data collected from this survey will contribute to public knowledge of various equine lymphatic drainage issues, as well as the impact of those issues on horse owners and veterinarians. In addition, the data collected will focus future research efforts towards developing efficacious therapy for equine lymphatic concerns that are most significant.

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How do early home range attributes influence neonatal moose survival?

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In northeastern Minnesota, USA, the moose (Alces americanus) population has steadily declined from 2006, but it has begun to stabilize. Recruitment of calves, or survival to one year of age, is low (30-40%). Understanding factors that can contribute to neonatal moose survivorship can provide insight on Minnesota’s entire moose population, and this is particularly vital in a population experiencing low survival rates. In northeastern Minnesota, global positioning system (GPS) collars have been deployed on moose neonates and monitored during 2013-2019. Using hourly GPS location data and various packages in RStudio, 95% minimum convex polygons (MCPs), 95% kernel density estimators (KDEs), and Brownian bridge movement models (BBMMs) were created to estimate the home ranges of neonatal moose. Each method increased in complexity and detail. Attributes of home ranges that influence survival may change as calves become weaned and incorporate more forage into their diets. We calculated home ranges for age classes of birth to 1 week, birth to 2 weeks, birth to 1 month, and birth to 3 months. As expected, home range area increased as neonates aged and became more mobile, from 0.1 km² at 1 week to 11.3 km² at 3 months for MCPs. Preliminary analyses do not indicate differences in home range area between calves that survived summer and those that did not, but we will next examine potential differences in land cover composition and wolf predation risk within home ranges. Moose calves are vulnerable to predation at early ages. This project aims to understand moose calf home ranges because the landscape in which they dwell may influence their health and survival.

Research Grant: Van Sloun Foundation
Student Support: University of Minnesota, College of Veterinary Medicine, Office of Graduate Programs

The genetics of paternal behavior in unique stickleback fish populations

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Parental care is crucial for offspring survival but can vary widely across populations of the same species. Stickleback fish (Gasterosteus aculeatus) provide a unique opportunity for study in this area. Stickleback parental care is male-exclusive: males build a nest, court a female, fertilize her eggs, then chase her away. However, different populations (called commons and whites) exhibit highly divergent parenting strategies. Commons care for eggs with frequent nest fanning and poking until they hatch, but whites immediately disperse eggs into surrounding algae before building a new nest. Behavior of pure common or white fish was compared to common/white F2 progeny. Assessing F2 survival, willingness to mate, and presentation of characterizable behaviors was a major goal of this study as they had never been produced. Nesting behaviors post-fertilization were recorded in 15-minute intervals twice daily for 4 days, at which time patterns were clearly identifiable. Parenting data was designated as time 0 (immediately post-fertilization) and long-term parenting (days 0.5-4). Data analysis, including principal component analysis, was performed using R. F2s exhibited high survival rates and readily mated with females. There was extensive variation in paternal behavior among F2 males in nesting time, fanning time, fanning bouts, and long-term nest attendance. Most F2 males provided care instead of dispersing eggs. These data provide a foundation for the long-term goal of this project, which is to use quantitative trait locus (QTL) mapping to identify genes that underlie differences in paternal care.

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Student Support: Office of the Director, NIH, T35 OD011145
Effects of increased temperature on zoonotic *Salmonella* spp. in chelonians

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Many unanswered questions remain to the true effects of climate change as Earth’s overall temperature continues to rise due to current anthropogenic causes. Our questions focus on how host-associated microbiomes may be affected by a changing climate. One group that is particularly sensitive to increasing temperatures are ectotherms, mainly reptiles. Reptiles are one of the most popular exotic pets to keep in captivity and are also known carriers of *Salmonella enterica*, the species responsible for human salmonellosis. Interestingly, a strong correlation between temperature and salmonellosis has been shown repeatedly. In this project, we experimentally manipulated temperature and quantified *Salmonella* spp. found in the microbiomes of captive reptiles to better understand how external temperatures influence *Salmonella* spp. shedding and thus zoonotic risk. Three-toed box turtles (*Terrapene carolina triunguis*) were exposed to increasing levels of ambient temperature for a period of 2 weeks in order to allow the microbiome to equalize. At the end of the 2-week period cloacal swabs were taken from the turtles followed immediately by another increase in ambient temperature. These cloacal swabs will be analyzed using fluorescence in-situ hybridization (FISH) to quantify *Salmonella* spp. Given the constraints of the FISH probe, Salm63, being only able to target highly conserved ribosomal RNA, further diagnostic culturing methods will be used to identify and confirm presence of *Salmonella enterica*. We hypothesize that there will be an increase in the number of *Salmonella* spp. as the temperature increases. This experiment could be a good model for changes in zoonotic threats as the planet continues to warm at an abnormal rate.

**Research Grant:** WesternU CVM Office of Research  
**Student Support:** Boehringer Ingelheim

Susceptibility of peridomestic rodents to infection with SARS-CoV-2 & their role in interspecies transmission

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On December 31, 2019, the World Health Organization was alerted to a novel outbreak of severe respiratory disease of unknown origin, now referred to as COVID-19. While the causative virus, SARS-CoV-2, is known to be transmissible among humans, an exhaustive host range is not known. Understanding which species are susceptible to the virus as well as how it is transmitted within and between species is important to developing a strategy to control virus transmission. Laboratory mice are not susceptible to infection, but Syrian hamsters have proven to be a valuable model for infection and pathology. Considering the often close contact of humans and wild rodents, we hypothesized that some peridomestic rodents may be susceptible to infection, shedding, and transmission of SARS-CoV-2, and thereby serve as potential reservoir hosts. To date, we have inoculated deer mice, wood rats, and house mice with SARS-CoV-2. Oropharyngeal swabs were collected on days 1-5 post-inoculation and groups of animals were euthanized on days 3, 7 and 28 post-inoculation to assess viral burden in organs and antibody responses. Thus far, we have demonstrated oral shedding of virus in deer mice and wood rats and high titers of virus in turbinates of deer mice 3 days after infection. Additional species soon to be evaluated include fox squirrels and prairie dogs, and we are planning to study inter and intra-specific transmission among susceptible rodents in a mixed species ecosystem. These studies will be helpful in classifying the host range of SARS-CoV-2 and enhance our understanding of potential sources of zoonotic origins to SARS-CoV-2.

**Research Grant:** Animal Models Core at Colorado State University  
**Student Support:** U.S. Department of Agriculture—Animal Health and Disease Scholar
RNA-seq of liver to examine the effect of maternal opioid exposure on female offspring fed different diets

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As the use of opioids worldwide continues to increase, the long-term implications of the opioid epidemic will be felt in the medical community for years to come. Indeed, a growing body of evidence supports the possibility that there will be effects of preconception opioid use on future offspring of opioid dependent women. Thus, clinical effects could be observed in many future generations. Concurrently, the diets of many individuals have contributed to a growing obesity epidemic. We hypothesize that the interaction between opioid systems and feeding circuits may create vulnerabilities in the offspring of opioid exposed mother. In this study, female rats were exposed to morphine for 10 days during adolescence. Following at least 3 weeks of abstinence, they were mated to drug naive males. The offspring were maintained on three distinct diets for 6 weeks, control diet (CD), high sugar diet (SD) or high fat and sugar diet (FSD). Livers were then extracted, and the RNA was isolated and sequenced. The male offspring of the same cohort were analyzed previously, and it was found that rats fed FSD diets were associated with upregulation of genes associated with neuronal plasticity and downregulation of genes associated with immunity, inflammation, and metabolism. This analysis will provide insight into the sex differences associated with a history of maternal opioid exposure as well as the interaction between that history and different diet conditions in the rearing environment.

Research Grant: NIDA 025674 R01, Tufts Provost
Student Support: Cummings Summer Scholars Research Program

Systematic review: comparative efficacy of regenerative therapies in horses with tendinitis or desmitis

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The aim of the systematic review will be to evaluate the literature for the comparative efficacy of mesenchymal stem cells (MSC) and platelet rich plasma (PRP) therapy for equine tendinitis or desmitis, focusing on return to performance and reinjury rate in studies that have at least a 6 month follow up period. Ligament (desmitis) and tendon (tendinitis) injuries are common musculoskeletal injuries in sport horses. Unresolved ligament and tendon tears in horses can prevent return to athletic performance resulting loss of use and potential economic loss. Intra-lesion injections of PRP and MSC have been assessed in experimental and clinical settings for treatment of tendinitis and desmitis. A proper systematic review evaluating MSCs vs. PRP vs. no treatment has not been conducted. Knowledge of the comparative efficacy would enable informed decisions about treatment options. It is also important to assess adverse events associated with these interventions, such as local or systemic inflammation. All current studies published in PubMed, CABI, and equine and orthopedic conference proceedings will be evaluated for relevance to the review. The eligible population is previously active horses diagnosed with tendinitis or desmitis who are expected to return to normal performance following treatment. Eligible studies include parallel and historical cohort studies and controlled trials. We will extract the effect size for days to return to function and reinjury risk and use meta-analysis to estimate the pooled effect size and report the 95% confidence interval as a measure of our uncertainty about pooled effect size.

Research Grant: NIH T-35 grant (5T35OD016477-19)
Student Support: Vilma Yuzbasiyan-Gurkan
Evaluating ELTD1 antibody therapies for glioblastoma multiforme

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Glioblastoma multiforme (GBM) is a malignant grade IV glioma with a poor prognosis. ELTD1 (EGF, latrophilin, and 7 transmembrane domain containing protein 1) is an angiogenic marker highly expressed in human high-grade gliomas. Polyclonal, monoclonal (mAb), and single chain variable fragment (scFv) anti-ELTD1 antibody (Ab) treatments have been evaluated in xenograft orthotopic glioma models and successfully decreased tumor volume, increased survival, and normalized the vasculature, by decreasing microvessel density (MVD) and increasing regional cerebral blood flow (rCBF). Additionally, OKN-007, a nitrone based agent, showed similar results to the ELTD1 Ab treatments for GBM’s. The purpose of this study is to test the efficacy of a humanized anti-ELTD1 Ab in an orthotopic xenograft glioma model and to further analyze the anti-ELTD1 mAb and scFv antibodies, and OKN-007 treatments in terms of cell proliferation, apoptosis, and migration in GBM’s. In vivo humanized anti-ELTD1 Ab treatment was assessed via magnetic resonance imaging (MRI) to determine animal survival, tumor volumes, and rCBF. In vitro mAb and scFv anti-ELTD1 antibodies and OKN-007 treated cell migration speeds were determined by use of microfluidic chambers. Immunohistochemistry (IHC) was used to assess cell proliferation and apoptosis. Prior treatment results using mAb and scFv anti-ELTD1 antibodies and OKN-007 in an orthoptic xenograft glioma model suggest that the humanized Ab treatment result in comparable efficacy. Initial results from the analysis will be presented. As a single therapy or in conjunction with other therapies, anti-ELTD1 antibodies may be a valuable vascular promoting and tissue normalizing clinical therapeutic for high-grade gliomas.

Research Grant: Oklahoma Medical Research Foundation
Student Support: Oklahoma State College of Veterinary Medicine

Clinical aspects, epidemiology, virology and pathology of coronavirus infections in companion animals

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Coronaviruses are spherical, enveloped RNA viruses capable of causing respiratory, enteric, and systemic disease in a variety of mammalian hosts, including dogs, cats, ferrets and horses. Coronavirus infections range in clinical severity from asymptomatic infection to fatal disease. Host range and tissue tropism are largely determined by the spike protein, which initiates cellular infection by promoting fusion of viral and host membranes. Companion animal coronaviruses responsible for causing enteric infection include feline enteric coronavirus, ferret enteric coronavirus, canine enteric coronavirus and equine coronavirus, while canine respiratory coronavirus results in respiratory infection. Ferret systemic coronavirus and feline infectious peritonitis virus, a mutated feline enteric coronavirus, can lead to lethal immuno-inflammatory systemic disease. Human viral pandemics, including severe acute respiratory syndrome (SARS) virus, middle east respiratory syndrome (MERS) virus, and most recently, SARS-CoV-2, all thought to originate from bat coronaviruses, demonstrate the high zoonotic potential of coronaviruses and their capacity to have devastating impacts on both human and animal health. A better understanding of the major coronaviruses of companion animals and their capacity for cross-species transmission has the potential to improve prevention and control measures for future emerging zoonotic coronaviruses.

Research Grant: WINN Feline Research Foundation grant
Student Support: UC Davis, School of Veterinary Medicine STAR program through the NIH T35 OD010956 grant
The impact of osteosarcoma tumor secreted factors and standard of care therapy on human macrophage phenotype

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Osteosarcoma (OS) is the most common bone cancer in children. Following resection of the primary tumor, one third of all OS patients develop metastasis despite treatment with standard of care adjuvant MAP (methotrexate, doxorubicin, cisplatin) chemotherapy. This resistant, metastatic stage of OS is associated with a mortality rate of over 70%. OS metastasis is preceded by the formation of a pre-metastatic niche that has been “primed” for tumor cell seeding by factors released from the primary tumor. M2 polarized macrophages are known to be pro-tumorigenic and play a role in the formation of this pre-metastatic niche. Methotrexate treatment has been suggested to alter macrophage phenotype and cytokine production. Thus, we aimed to evaluate the effect of OS tumor-secreted factors on the polarization state of macrophages and how this state may be altered following methotrexate therapy. To investigate this, we experimentally differentiated human blood-derived monocytes as well as the human monocyte cell line, THP-1, into macrophages. We then treated these macrophages with OS cell secreted factors over a 24-hour period. Subsequently, we treated the macrophages with physiological doses of methotrexate and evaluated cell phenotype, viability, and the resultant cytokine profile using ELISA. In addition to evaluating macrophage phenotype in vitro, we evaluated the biodistribution and cellular uptake of OS tumor-secreted exosomes (extracellular vesicles ~150 nm in diameter) in a mouse model and show that macrophages can indeed take up and respond to OS exosomes in vivo. Our findings suggest that OS primary tumors may alter the phenotypic state of macrophages in distal sites via secreted factors to promote metastasis.

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Student Support: NIH T32 Training Grant T32GM136628

Risk factors associated with veterinary anesthesia-related deaths in the state of Arizona

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A seminal case-control study on anesthetic risk factors from the early 2000s determined the mortality rate of dogs to be 0.17% (95% CI [0.14%, 0.19%]) and cats to be 0.24% (95% CI [0.20%, 0.27%]). The aim of our study was to perform a similar investigation of the mortality rate and risk factors for dogs and cats undergoing anesthesia between 2016-2019 in the state of Arizona, which uniquely requires a log documenting anesthetic recoveries. A list of licensed clinics was acquired from the Arizona State Veterinary Medical Board. Clinics that serve dogs and cats were invited via email and print advertisements to provide anonymized anesthetic logs and medical records for cases, along with four randomly selected same-species controls per case. The number of anesthetic events and deaths for dogs and cats were determined for each clinic and the medical records coded for variables determined to be relevant from prior research. Logistic regression was used to calculate odds ratios (OR) for potential anesthetic risk factors. Five of the 395 (1%) clinics provided anesthesia logs and the medical records for 10 cases and 40 controls. There were 10,284 log entries and 13 deaths for dogs, resulting in a mortality rate of 0.13% (95% CI [0.07%, 0.22%]). There were 5,806 log entries and five deaths for cats, resulting in a mortality rate of 0.09% (95% CI [0.03%, 0.20%]). Seven patients had an ASA status greater than I and three procedures were considered urgent. Preliminary logistic regression determined an OR of 5.0 for female sex (P = 0.064), and 2.6 for each increase in ASA status above I (P = 0.149). Overall, anesthetic-related mortality was relatively low and cat mortality rates appear improved in comparison to historical data.

Research Grant: Midwestern University College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program Fellowship
Does misoprostol reduce inflammatory cytokine release caused by circulating endotoxin in equine patients?

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Inflammation mediated by pro-inflammatory cytokines can have a profound negative effect on diseased equine patients. These cytokines are produced in response to circulating endotoxin and can lead to a severe inflammatory state known as Systemic Inflammatory Response Syndrome (SIRS) which can lead to further systemic, possibly fatal disease. NSAIDs are a common choice to treat inflammation but are often contraindicated. Thus, it is vital to identify other pharmaceuticals that can combat increased cytokine production. Misoprostol, a prostaglandin E1 analog, has anti-inflammatory effects in multiple species and may provide an alternative to conventional therapies. In vitro, misoprostol decreases the release of inflammatory cytokines by peripheral blood equine leukocytes. The aim of this study is to determine the effect of misoprostol on inflammatory cytokine release in vivo. Using a crossover design, six healthy horses were given a saline control or oral misoprostol and then challenged with lipopolysaccharide (LPS). Blood was then collected serially over 24 hours. Neutrophilia in all horses confirmed the expected inflammatory response to the LPS challenge and was not different between groups. RNA was extracted from frozen peripheral blood leukocyte pellets. Inflammatory cytokine production (TNFα, IL-1β, IL-10, and IL-6) will be measured using quantitative PCR and results compared between the saline control and misoprostol treatment samples. This study will hopefully provide clinicians with evidence to support this novel therapy for equine patients with endotoxemia and systemic inflammation.

Research Grant: American Quarter Horse Foundation, Auburn University Animal Health and Disease Research Program
Student Support: USDA Multistate Research Funds

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

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*Tritrichomonas foetus* (TF) is a 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, vaginitis, abortions, etc. 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 on bovine reproductive epithelium, to help cattle resist or clear TF infection. 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 induce expression 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 concentration TF1.17-vaccinated heifer 1276 serum at 1:1000, negative control ultra-low IgG FBS 1:100, secondary anti-body anti-bovine IgG conjugated to HRP at 1:7500, based off titration & 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.

Research Grant: None
Student Support: Summer Research Experience, Mississippi State, MS, NIH: 5T350D010432
Human neuron infection with Zika virus and hybrid IgG4 antibody response to *Aedes aegypti* salivary proteins

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Zika virus (ZIKV) and Dengue virus (DENV) are flaviviruses carried by the mosquito vector *Aedes aegypti*. ZIKV has been linked to the development of microcephaly in fetuses of infected pregnant women, along with other serious neurological complications. DENV is endemic in many of the regions affected by ZIKV, and it has been suggested that prior immunity against DENV may have an impact on ZIKV infection. The aim of this study is to determine whether pre-existing DENV antibodies bispecific for viral particles and *Ae. aegypti* salivary proteins act to decrease ZIKV infection of human neuron cells by activating anti-inflammatory pathways or increase infection through antibody-dependent enhancement. Our results have shown that IgG antibodies against total *Ae. aegypti* salivary gland extract are much higher in exposed individuals living in DENV endemic areas than in non-exposed samples. In addition, there were fewer antibodies expressing \( \lambda \) light chain in exposed patients, indicating a bias in the immune response that is different than that seen in non-exposed individuals. Neuroblastoma cells will be infected with ZIKV strain MR-766 (original African strain) in the presence or absence of hybrid IgG4 antibodies in order to determine antibody effect on viral entry and replication inside the cells. It is anticipated that this study will suggest a vital role of pre-existing DENV immunity on ZIKV neuronal infection through hybrid antibodies and establish a better understanding of the neurological pathology of this disease.

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**Student Support:** Boehringer Ingelheim Veterinary Scholars Program

Characterizing the association of proteinuria with dental disease in dogs and cats

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Periodontal disease is a frequently diagnosed condition in small animal medicine, impacting millions of dogs and cats each year. Periodontal inflammation is often considered as a differential for proteinuria in these patients; however, periodontal disease in humans has been linked to chronic kidney disease, which may also lead to proteinuria. The goal of our study was to retrospectively determine the prevalence of proteinuria in dogs and cats with periodontal disease and to prospectively evaluate urine protein patterns in a subset of patients. Between February 2015 and June 2020, 1,522 dogs and cats presented to Midwestern University’s Companion Animal Clinic for dental prophylaxis. Preliminary data suggests that > 60% of canines and > 80% of felines with periodontal disease are proteinuric. We also collected leftover urine supernatant from proteinuric dental patients that presented between June and July 2020. Urine protein electrophoresis is currently being performed on the collected supernatants and protein patterns characterized. The results of our study will help determine the association of proteinuria with dental disease in dogs and cats and may shed light on whether this proteinuria is related to inflammation or underlying renal disease.

**Research Grant:** None
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Investigating the role of equine platelet lysate on wound healing in vitro

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Aberrant wound healing is a significant issue in equine medicine and we hypothesize equine platelet lysate (ePL) will promote timely and organized healing. The aim of this study was to determine if using ePL in cell culture could enhance wound healing as determined by an in vitro scratch assay on equine primary dermal fibroblasts. Fibroblasts were isolated from skin biopsies obtained from the cannon bone region of 6 healthy horses. Fibroblasts were passaged to P2 before seeding at a density of 100,000 cells per well onto glass coverslips within 24-well plates. After incubating and reaching 80% confluence, a scratch assay was performed and media was replaced with treatment groups containing: 10% FBS, 10% ePL, 20% ePL, 10% DHS, and 20% DHS with and without 2ng/mL TNF-α. Stimulation of cells via TNF-α was utilized to represent the presence of inflammatory mediators naturally found at the site of injury. Cell supernatant was obtained before the scratch assay as well as 24, 48, and 72h afterwards. Supernatants were frozen at -80°C until further analysis via ELISA for TGF-β1 and IL-6. At the same time points, coverslips were formalin fixed and immunohistochemically stained for the presence of a-SMA, a marker for myofibroblast differentiation. Once a-SMA was evaluated from a subset of samples, all samples were stained via the Diff-Quik method before being digitally scanned. Images generated from the digital scanner were used to measure the rate of scratch closure expressed as the area of the scratch over time using the software program Image J. Preliminary data suggests ePL allows for more organized and faster healing as compared to DHS. This study is ongoing and results are anticipated within the coming weeks.

Research Grant: Morris Animal Foundation
Student Support: Morris Animal Foundation

Quantifying epidermal changes of naive and subsequent Amblyomma americanum bite lesions of dogs and cats

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Amblyomma americanum, lone star tick, readily bite on a variety of animals. Inflammatory responses in the integument in response to tick bites are not grossly appreciated. Our aim was to quantify epidermal changes caused by A. americanum attachment on dogs and cats. Punch biopsies (5 mm) were collected from 8 dogs and 8 cats during naive and subsequent infestations. Samples were processed by standard histological procedures and stained with H&E. Image software was used to scan, view, and measure stained cross-sections. Thickness of the epidermis was measured every 0.25 mm from the center of the tick bite to the edge of the tissue and compared using two-way ANOVA. Radii of the ulcer and neutrophilic inflammation surrounding the tick bite were measured and compared with linear regression. Results were compared to the control (no tick infestation) between day and sex for dogs and cats separately. For cats, the epidermis was thicker at 0.25 mm (F = 7.348, P = 0.030), 0.5 mm (F = 7.348, P = < 0.001), 0.75 mm (F = 5.986, P = < 0.001), 1 mm (F = 3.183, P = 0.012), 1.25 mm (F = 5.757, P = < 0.001), 1.5 mm (F = 4.908, P = < 0.001), 1.75 mm (F = 5.722, P = < 0.001), 2 mm (F = 2.985, P = 0.018), and 2.25 mm (F = 6.203, P = < 0.001). For dogs, the epidermis was thicker at 0.25 mm (F = 5.427, P = < 0.001), 1 mm (F = 5.427, P = < 0.001), 1.25 mm (F = 9.416, P = < 0.001), 1.5 mm (F = 4.327, P = 0.001), 1.75 (F = 7.430, P = < 0.001), 2 mm (F = 2.743, P = 0.026), 2.50 mm (F = 8.593, P = < 0.001), and 2.75 (F = 3.911, P = 0.007). Radii of ulcers were larger (F = 6.287, P value = 0.016) in subsequent infestations on dogs. Tick attachment leads to a vigorous inflammatory response in the epidermis of dogs and cats supporting the use of tick preventives.

Research Grant: Bayer
Student Support: OSU, College of Veterinary Medicine
Quantitative and qualitative assessment of mitochondrial dynamics of bison oocytes during in vitro maturation

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Oocyte competence is influenced by the mitochondrial number and distribution inside the cell. The objectives of this study were: 1) to assess the 3-dimensional distribution pattern of mitochondria in bison oocytes before and after in vitro maturation (IVM) by using a computer-assisted quantitative method and 2) to validate a rapid visual classification method (qualitative/subjective scoring) using the quantitative data. Ultrasound-guided follicle aspirations were performed on wood bison (n = 24) to obtain cumulus-oocyte-complexes (COC; n = 157). A total of 67 COC underwent IVM for 25-28 h, while the remaining (n = 93) were processed within 2 h (immature). All COC were denuded and oocytes were incubated in Mitotracker™ Deep Red FM for 2 h. Stained oocytes were mounted on slides, and 10 μm-thick 3D image stacks were obtained using a Leica TCS SP5 confocal microscope. Each confocal stack was converted into a 2D image and the pattern of mitochondrial distribution was classified as scattered or clustered by visual examination. Data is modeled through logistic regression. Initial visual scoring detected a greater proportion of clustered mitochondria pattern in the IVM group than the immature group (42/52 [80.8%] vs 34/52 [65.4%]; \( P = 0.07 \)). A subset of the oocytes (n = 27 immature, 26 matured) are currently undergoing 3D image analyses using Autoquant (deconvolution) and Imaris software (thresholding and segmentation) wherein identities and classification are not known to the researcher (JH). Imaris data will provide the number, size/volume, and localization (peripheral vs. central) of individual and clustered mitochondria within the oocyte. Analyses are in progress and we anticipate final results by mid-August.

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Student Support: Boehringer Ingelheim Veterinary Scholarship and the Western College of Veterinary Medicine

Canine cutaneous mast cell tumor identification by artificial intelligence and machine learning algorithms

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Cutaneous mast cell tumors are one of the common skin tumors of the dogs. The prognosis prediction is based on a Kiupel 2-tier grading system that accounts for mitotic figures, multinucleated giant cells, bizarre nuclei and karyomegaly in the tumor cells. Machine learning tools can assist the pathologist in grading these tumors, therefore, we aimed to develop algorithms for grading canine cutaneous mast cell tumors. Our short-term goal for this project was to identify the mast cells and infiltrating eosinophils. Previously diagnosed cases of mast cell tumors (50 cases) at the Oklahoma Animal Disease Diagnostic Laboratory were retrieved and scanned using the Aperio slide scanner. Random H&E stained images of the tumors were captured from 12 cases to train the algorithms. Images were annotated using LabelMe for different cellular components such as eosinophils, mast cells, mitotic figures, bizarre nuclei and multinucleated giant cells, etc. We developed a machine learning-based detection framework for localizing and recognizing mast cell tumors and eosinophils. We trained two state-of-the-art object detection models (Faster-RCNN model and YOLOv4) in an ensemble approach. We quantified the precision and recall rates at different localization thresholds. We were able to identify at least 1 correct mast cell in 54.9% and 1 correct eosinophil in 43.9% of the cases respectively. We correctly localize 27% of all annotated mast cells and eosinophils. At 10% localization, we achieve a precision rate of 2.35% at 19.9% recall. At 50%, we obtained a precision rate of 1.35% at 10.1% recall. Given that current deep learning models require thousands of samples for training, our results are an encouraging first step in this direction.

Research Grant: None
Student Support: College of Veterinary Medicine, Oklahoma State University
Effects of radiation on skeletal development in rhesus macaques

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Irradiation is a diagnostic tool and at high doses can be used to treat cancer or to induce immunosuppression before bone marrow transplants. Despite the benefits, there has been little research on long-term effects of irradiation on bone growth during adolescence. We hypothesize that long bone growth and vertebral body length will be significantly impaired by irradiation exposure. Measurements of long bones and vertebral bodies will be taken from 277 annual computed tomography (CT) scans of 40 rhesus macaques who were exposed to a single, whole body dose of irradiation (avg = 6.5 ± 1.7 Gy range: 1.14 - 8.5 Gy) (avg. age at time of scan = 9.4 ± 3.2 yrs, range: 3.1 to 18.1 yrs) (avg. age at irradiation = 3.6 ± 0.6 yrs, range: 2.3 to 5.5 yrs) and from 10 macaques who were never irradiated (avg age at time of scan = 13.5 ± 2.7 yrs, range: 9.0 to 19.1 yrs). Based on preliminary analysis of high dose animals (7.2-8.5 Gy) compared to controls, there is not a significant effect of age on bone length in our mostly mature adult population. There is a 2-3% reduction in length of the humerus (P = 0.0002) and femur (P = 0.0164) in irradiated macaques. The tibia did not show a significant difference in length (P = 0.1491). In all vertebral bodies measured, T1, T8, L6, and L7, irradiation reduced vertebral length, by 5-10%. Understanding how irradiation affects bone growth over time allows for better evaluation of the risks of exposing pre-adolescent and adolescent humans to irradiation in clinical settings. This study will further develop the knowledge base investigating what happens to bone after irradiation exposure and will support pursuing further studies examining why bone growth is stalled after irradiation.

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Student Support: T35 OD010946, U01 AI150578

Molecular and serologic surveys of shelter dogs for tick-borne disease in Ohio

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Tick-borne disease (TBD) in domestic dogs is on the rise in the United States. Determining the prevalence of canine infection, exposure, or coexposure, to Anaplasma spp., Ehrlichia spp., spotted fever group rickettsiae, and Borrelia burgdorferi is of great importance to veterinary medicine and public health. Due to their increased environmental exposure risks, shelter dogs can be effective sentinel animals for TBD risk and geographic distribution. This study assessed infection prevalence among 26 shelter dogs from southern Ohio using molecular and/or serological assays. A total of 65 ticks representing four tick species were collected (range 1-16) per dog. Serology was performed on 26 dogs, 14 of which were seropositive (53%), including three that did not have an active tick infestation. Eight dogs were seropositive for multiple TBD (32%). Only one seropositive dog had related clinical symptoms and one additional dog had hematological results suggestive of illness. Real-time PCR was performed on blood from 24 dogs and 64 ticks to determine the prevalence of active infection. No dogs were PCR-positive, but ticks were positive for B. burgdorferi, A. phagocytophilum, and spotted fever group rickettsiae. Our results underscore the emerging threat of TBD in Ohio and highlight the need for routine screening of dogs even in absence of observed ticks. With the relatively recent invasion of the blacklegged tick (Ixodes scapularis) in Ohio, the prevalence and distribution of TBD in canines in Ohio is an important step in mitigating health complications for canines as well as humans.

Research Grant: Canine Grant No. 2019-30 “Molecular and serologic surveys of shelter dogs and their ticks as sentinels for tick-borne disease risk in Ohio”
Student Support: PetSmart Charities
Hematologic findings in cats associated with Bartonella

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Little is known about hematologic changes associated with Bartonella spp. serological positivity or PCR positivity in cats. There has been speculation that neutropenia may be associated with Bartonella in felines; however, this has not been consistently reported. The goal of our retrospective study was to characterize hematologic findings in cats with Bartonella. Thirty-eight feline patients tested for Bartonella spp. over a 3-year period were categorized into positive ill (N-6), positive healthy (N = 10), negative ill (N = 14), and negative healthy (N = 8). There were 27 neutered males and 11 spayed females and the age range was 1-6 years. All seropositive cats (N = 16) had test reactivity for Bartonella henselae, and 8 cats had 2 species and 6 cats had 3 species. All patients (N = 38) tested negative for Bartonella PCR. The CBCs documented that 5/16 positive felines had lymphopenia, 4/16 had neutropenia, and 4/16 had leukopenia. Out of the negative cats (N = 22), 8/22 had lymphopenia, 5/22 had neutropenia and 2/22 had leukopenia. Both the healthy positive and healthy negative chemistries were unremarkable; whereas, the chemistries for the negative ill and positive ill felines were inconclusive since it could not be determined if the changes were related to Bartonella or their presenting complaint. This first phase of our study did not document specific hematologic changes related to Bartonella in cats. Additional studies with larger case numbers are needed to further investigate the relationship between specific Bartonella species seropositivity and PCR positivity and hematologic changes in cats.

Research Grant: Boehringer Ingelheim Vetmedica Inc. (BIVI)
Student Support: Merial Veterinary Scholar and CVM Summer Scholar Program

Efficacy of antimicrobials to protect against clinical anaplasmosis in immunosuppressed cattle

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Bovine anaplasmosis is a tick-transmitted disease that costs the United States cattle industry an estimated $300 million annually. The agent of anaplasmosis is Anaplasma marginale, a pathogen transmitted to cattle by ticks or contaminated fomites. The only FDA approved drug to control active anaplasmosis and to help limit economic loss is chlortetracycline (CTC). The effectiveness of CTC treatment to control anaplasmosis has been documented in immunocompetent animals, but the usefulness of CTC during periods of transient immunosuppression is unclear. Due to intensive tetracycline usage, the efficacy of CTC to control active anaplasmosis caused by contemporary A. marginale strains may be reduced, such that upon immunosuppression, cattle are not protected from recrudescence clinical anaplasmosis. The objective of this study was to determine the efficacy of CTC to control active anaplasmosis in transiently immunosuppressed cattle infected with a historic or contemporary A. marginale strain. We first generated infected animals by inoculating 18 Holstein calves with the historic Virginia or the contemporary KS2 strain. Animals were monitored for signs of clinical anaplasmosis (packed cell volume, temperature, and bacteremia) during acute disease. Upon transition to persistent infection, calves will be immunosuppressed using dexamethasone. The efficacy of CTC to protect transiently immunosuppressed calves will be determined by evaluating groups of calves: immunosuppressed with CTC treatment, immunosuppressed without CTC treatment, and not immunosuppressed with CTC treatment. The results of this study will provide evidence of the effectiveness of CTC to control active anaplasmosis in cattle during transient immunosuppression.

Research Grant: USDA-NIFA (2018-68003-27463); Foundation for Food and Agriculture Research
Student Support: Foundation for Food and Agriculture Research Fellow
Pharmacokinetics of thiamine hydrochloride (Vitamin B1) in adult horses

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Sepsis is a major cause of morbidity and mortality in neonatal foals and adult horses. Effective treatment of sepsis requires that the excessive inflammatory response, known as Systemic Inflammatory Response Syndrome (SIRS), is interrupted. Thiamine is a vital co-factor in many metabolic processes, including those that are critical in mitigating inflammation. Previous studies in humans have shown that metabolic resuscitation, a term used to reference the use of thiamine hydrochloride (TH), ascorbic acid, and hydrocortisone, can be performed in horses, pharmacokinetic (PK) analysis is necessary. We hypothesized that intravenous (IV) TH at increasing doses results in corresponding increases in plasma TH concentrations without causing adverse effects. A PK analysis of 3 doses of TH was performed and any adverse effects were documented. A randomized cross-over study included 9 healthy adult horses treated with IV TH at 5, 10, and 20 mg/kg. For each treatment, blood was collected immediately prior to drug administration (T0) and 5, 10, 15, 20, 30, 45, 60, 90 minutes and 2, 4, 6, 8, 10, 12, 24, and 48 hours after drug administration. A physical examination was completed at T0, 6, 12, 24 and 48 hours. No clinical signs of adverse effects were observed. Results of high-performance liquid chromatography with mass spectrometry to quantify plasma TH concentrations are pending. Understanding the PK profile of TH in horses is the first step in evaluating the potential benefits of exogenous TH in equine patients.

Research Grant: Pilot Grants in Equine Clinical and Translational Research, College of Veterinary Medicine, Purdue University
Student Support: Boehringer Ingelheim and College of Veterinary Medicine, Purdue University

Prevalence of unsuspected thyroid lesions: a histologic study of thyroid gland in equine necropsy

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Goiter in aged horses is a common occurrence typically seen in horses older than age 16 but can be present in younger populations, these changes are either hyperplastic or neoplastic in nature but often produce no significant symptoms. The etiology of these thyroid changes is not currently understood, and little research historically has been directed at gaining a greater understanding of these changes or any resulting sequela. In this study we attempt to evaluate the incidences and nature of these changes in horses presented to TUCVM between the years 2013 to 2018 for necropsy to better elucidate the hypothalamus-pituitary-thyroid axis in the hopes of providing knowledge to owners and veterinarians.

Material and Methods: In this retrospective study thyroid samples from horses presented for necropsy were evaluated for the presence of lesions that would indicate hyperplastic or neoplastic changes. Samples were first examined using gross assessments for any consistent macroscopic trends in thyroid tissue that could be used to quickly differentiate desired samples from normal tissue. Once identified, tissue samples were prepared for microscopic evaluation using standard sectioning and H&E staining methods.

Results: With a sample consisting of 7 males (intact:3; castrated:4), 10 intact females, from ages 10 to 30 years old. The overall prevalence of thyroid lesions in this sample set was found to be roughly 80% of all assessed samples. Follicular adenoma was the most common lesion with a prevalence of 42.8% (9/21), followed by follicular hyperplasia 28.57% (6/21). Thyroid malignancy observed was C-cell carcinoma (9.5%) and Follicular adenocarcinoma (4.7%). The severe lesions were more often observed in the older age group.

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Student Support: HRSA COE #D34HP00001
Systematic review of research on access to medical and veterinary care for Deaf and hard of hearing persons

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The Deaf and hard of hearing population has been found to suffer disproportionately from barriers to healthcare access. Progress has been made towards improving access to medical care for this population; however, the veterinary field has fallen behind in this regard. More research in this area is necessary in order to improve the disability accessibility of the veterinary field. This systematic review aimed to evaluate all primary research articles pertaining to medical and veterinary healthcare access for deaf and hard of hearing adults in the United States of America. Our review included 40 articles related to Deaf or hard of hearing access to medical care as well as 8 articles related to access to veterinary care in general. We found no articles related to Deaf or hard of hearing access to veterinary care nor any articles on disability accessibility to veterinary care that met our inclusion criteria. Our results outline several significant barriers to Deaf and hard of hearing persons’ access to healthcare, the identified needs of this population as patients, and improvements that must be made to increase access to medical care for this population. Our results also suggest that research must be done to investigate the barriers to veterinary care experienced by Deaf and hard of hearing pet owners, the unique needs of this population, and how the veterinary field could better accommodate them.

Research Grant: PetSmart Charities
Student Support: PetSmart Charities

Aggregation propensity of serum amyloid A beta-sheet misfolding in exotic, wildlife and zoo animals

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AA Amyloidosis is characterized by the deposition of fibrils in the liver, spleen, and kidneys. These deposits are comprised of fragmented serum AA (SAA), a protein that is synthesized by the liver during states of chronic inflammation. SAA is highly prone to misfold and aggregate due to high density of beta-sheet. Fibrils are generated through a seeded-nucleation process, inducing neighboring healthy SAA peptides to elongate into mature fibrils and aggregate in the tissue. The accumulation of insoluble fibrils results in subsequent tissue damage and loss of function (organ failure). AA amyloidosis has been reported in humans, domestic and wild animals, with prevalence, clinical presentation, and pathogenesis of the disease varying between species. Transmission of AA amyloidosis is an investigative topic that has particular importance in the zoo industry and captive management of exotic animals. This project aims to evaluate the propensity of amyloid formation in zoo and wildlife animals by designing serum AA peptide fragments and in-silico aggregation profiling to determine species-specific risk of disease. Bioinformatic programs such as TANGO and Aggrescan were used to predict the aggregation probability and locality in the peptide. Protein sequence alignment of SAA was assembled via BioEdit to identify variance, and a phylogenetic analysis was performed in MEGA to determine potential amyloidogenic lineages. Future steps include a combination of biophysical assays (i.e. thioflavin T fluorescence assays, circular dichroism, and transmission electron microscopy) of the designed peptide library to pinpoint region(s) or residues that contribute to SAA misfolding.

Research Grant: None
Student Support: NIH T35 OD016477
Analysis of the test-retest reliability of an owner performed sustained gaze test in senior dogs

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Canine Cognitive Dysfunction (CCD) is a syndrome of progressive degeneration in cognitive function and often referred to as the canine analog to Alzheimer’s Disease in humans. The sustained gaze test can capture this decline since loss of attention and social interaction are important components of CCD. Duration of sustained gaze correlates with degree of cognitive dysfunction in pet dogs when performed in laboratory settings. However, the ability to quantify neurological dysfunction by owners at home would greatly increase case recruitment to longitudinal studies of canine aging. Currently, the validity and reliability of owner performed sustained gaze testing in the home is unknown. We hypothesize that owners can perform the sustained gaze test at home with their dogs and produce reliable data. The aim of our study is to evaluate test-retest reliability of owner performed testing. We created a training video to teach owners to perform the test while recording their dog’s gaze. The test involves getting the dog’s attention, holding a high value treat at the owner’s face and recording until the dog breaks eye contact. A successful video showed the dog’s face and the entirety of their gaze. In each session, owners recorded 3 sustained gaze tests of their dog, repeating every 7 days for 3 weeks. Researchers recorded gaze duration of owner submitted videos. Test-retest reliability was assessed by calculating intraclass correlation. Currently, 5 people are actively testing, 16 have completed 3 weeks of testing and 93.3% of owner videos were successful tests. With preliminary data only, it appears owners can perform valid and reliable sustained gaze tests of their dogs at home.

Research Grant: None
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070

Imaging filovirus VP40-mediated VLP egress from the plasma membrane via TIRF-SIM

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Total internal reflection fluorescence (TIRF) microscopy provides a clear view of cellular processes that occur at or close to the membrane by limiting fluorescence excitation to a narrow region of approximately 100 nm from the coverglass on which the cells are grown. By combining TIRF with structured illumination microscopy (TIRF-SIM), imaging is further enhanced by increasing lateral resolution to approximately 120 nm. This technique is a valuable tool when observing interactions of filovirus VP40 with the plasma membrane and the surrounding cytoskeleton scaffold to form budding VLPs. TIRF-SIM can therefore reveal changes in these interactions when the function of host proteins involved in VP40 trafficking to, insertion into, or budding from the plasma membrane is perturbed.

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Student Support: NIH 5T35OD010919-23
Broad spectrum of spinal muscular atrophy revealed by animal and human studies

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Spinal muscular atrophy (SMA) is the second most prevalent genetic disease of children and infants after cystic fibrosis. SMA is primarily caused by low levels of Survival Motor Neuron (SMN) protein due to deletions or mutations of the SMN1 gene. The SMN2 gene, nearly identical to the SMN1 gene, produces very low levels of SMN due to predominant skipping of SMN2 exon 7. Therefore, the SMN2 gene is unable to fully compensate for the loss of SMN1 in SMA. High SMN2 copy number and several other factors are known to modify the severity of disease. SMN is involved in multiple cellular processes, including DNA repair, transcription, pre-mRNA splicing, translation, stress-granule formation, macromolecular trafficking, microtubule dynamics and cell signaling. Low levels of SMN affects development and functioning of all tissues, including heart, liver, lung, muscle, bone, kidney, pancreas, gastrointestinal system, testis, ovary, spinal cord and brain. This report provides an analysis of recent findings that challenge the old concept of SMA being a disease of only central nervous system and motor neurons. The spectrum of SMA is broad, ranging from embryonic lethality to near normal life expectancy with limited disability. The tissue-specific phenotypes of SMA appear to depend upon expression levels of SMN as well as several modifying factors. Lessons learned from SMN functions employing in vitro and in vivo models underscore how relative expressions of SMN impact survival and maintenance of different tissues of animals and humans.

Research Grant: NIH R01 NS055925
Student Support: NIH T35 Training Grant

A veterinary perspective on the most significant emerging viral pathogens

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There is an interconnected relationship between animal health, human health, and the prosperity of the environment; the acknowledgement of this coexistence is termed One Health. As the status of the environment changes, emerging and re-emerging pathogens follow suit- leading to diseases that impact both humans and animals. (1) The World Health Organization (WHO), composed a list of the most severe emerging pathogens likely to cause “a public health emergency,” that lack concrete prevention and restorative care. (2, 3) The pathogens listed include: Crimean Congo hemorrhagic fever (Nairovirus), Ebola, Marburg, Lassa fever, MERS, SARS, Nipah, and Rift Valley fever viruses; all zoonotic, and all enveloped viruses. (2, 3) Given their pathogenicity, these and/or very similar viruses have a significant impact within the field of veterinary medicine. In an attempt to improve preparedness and response tactics for those whose lives and livelihood are closely linked with animals, we have categorized the significance of these viruses from a veterinary perspective. We looked at factors such as the species of animals infected, known treatment and prevention, mortality rates, geographic region and their economical significance to analyze the impact of these viruses within the field of veterinary medicine. These data were then used to quantify and rank the significance of these viruses globally. The ranking of viruses deemed most significant should aid the dedication of resources for research and development, to properly combat these pathogens on a global One Health scale.

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Student Support: Veterinary Investigator Program NIHT35 Training Grant T35 ODO010941
GLP1 receptor signaling stimulates GLP1 production & increases bihormonal insulin+/glucagon+ cells in islets

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Dept of Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY (Holter, Saikia, Donahue, Lee, Zheng, Todero, Weikert, Garibay, Danko, Cummings). Diabetes and Complications, Lilly Research Labs, Eli Lilly and Co, Indianapolis, IN (Sloop), Icahn School of Medicine at Mount Sinai, New York, NY (Ocana)

Glucagon-like peptide-1 (GLP1) regulates islet function by enhancing glucose-stimulated insulin secretion (GSIS). We reported that the β-cell GLP1 receptor (GLP1R) contributes to improved islet function in mice that had vertical sleeve gastrectomy (VSG); and that VSG increases α-cell GLP1 expression by activating prohormone convertase 1/3 (Pcsk1) expression in a β-cell GLP1R-dependent manner. We hypothesize that β-cell GLP1R signaling induces α-cell GLP1 expression to amplify GSIS in a paracrine positive feedback loop. To test this, we first studied the impact of conditioned media from β-cell GLP1R WT and KO mouse islets treated with a GLP1R agonist, liraglutide (lira), on α-cell gene expression. Conditioned media from lira-treated islets increased α-cell Pcsk1 and Mafa, a β-cell-enriched factor, expression in a β-cell GLP1R-dependent manner. To assess the translational relevance of our data, we used a scRNA-seq platform, DART-seq, in human islets. Lira increased PCSK1, INS, MAFA, and decreased ARX, a driver of α-cell maturity, in a subset of α-cells. IHC of human islets revealed that lira increased α-cell GLP1 expression and bihormonal insulin+/glucagon+ cells. To assess the islet cell type through which lira signaling induces bihormonal cells, we performed IHC on pancreas of lira and saline-treated WT and KO mice. Lira increased α-cell GLP1 expression and bihormonal insulin+/glucagon+ cells in a β-cell GLP1R-dependent manner. Our data show that the effect of β-cell GLP1R signaling to increase α-cell GLP1 expression is mediated by a secreted factor, can be stimulated by pharmacological activation of the GLP1R, is translationally relevant, and increases bihormonal insulin+/glucagon+ cells in mouse and human islets.

Student Support: NIH F30 DK126538

A modern approach to planarian learning and memory

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Learning and memory are crucial functions needed for animals to adapt and survive in their environment. Most vertebrates store memory through a mechanism called long term potentiation (LTP). LTP is a process by which synaptic connections become stronger with frequent activation. LTP induces changes in gene transcription and protein distribution to increase key players like NMDA receptors to synapses, strengthening connections between neurons to form memories. However, old evidence suggests that unlike other animals, planarians, most well known for their regenerative properties, store their memories outside of the central nervous system. Planarians can learn to avoid undesirable stimuli such as light, vibration, or electric shock. After training, planarians were cut into pieces, and fragments were allowed to regenerate. The head fragment retained the learned behavior. Surprisingly, the tail fragment, which had regenerated a completely new central ganglion, also retained the learned behavior. To study the role of LTP in planarian learning, we will establish a robust behavioral paradigm that planarians will retain after regeneration, and determine important mechanisms responsible for it by knocking down molecules such as NMDA receptors. In addition to behavioral tests, we will locate planarian neurons and use electrophysiology to monitor electrical currents between neurons. If the old hypotheses of decentralized memory were to be true, it could mean that memories are stored across vertebrate bodies and not just centralized in the brain.

Research Grant: Unknown
Student Support: Cornell University (Boehringer-Ingelheim match)
Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* from dogs in Grenada

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Antimicrobial resistance has become a major health threat for animal and human population worldwide. Clinical infections caused by the multidrug-resistant pathogenic bacteria are associated with increased morbidity and mortality imposing severe economic burden. *Pseudomonas aeruginosa* is a common gram-negative, encapsulated bacterium that could cause severe respiratory, skin and soft tissue, urinary tract and systemic infections. This organism exhibits a high level of acquired antimicrobial resistance to its selective antimicrobial drugs, which makes the treatment of clinical infections more challenging. Considering these facts, the present study was designed to determine the Antimicrobial Susceptibility Test pattern of *P. aeruginosa*. In this retrospective study, the diagnostic laboratory reports of canine patients for a period from 2015 through 2020 from Small Animal Clinic of St. George’s University in Grenada, West Indies were analyzed. The clinical isolates were collected from various clinical conditions such as otitis, abscess, infected wounds, and urinary tract infections. Out of total positive 52 samples, *P. aeruginosa* are found to be 100% susceptible to gentamicin, amikacin (Aminoglycosides), ciprofloxacin (Fluoroquinolones), imipenem (Carbapenems) and 98% susceptible to ceftazidime (Cephalosporins), but showed resistance to Enrofloxacin (16%), Ceftriaxone (38.8%) and Cefotaxime (34.7%). The results of the present study were in accordance with the susceptibility study of *P. aeruginosa* conducted in 2009 using the clinical isolates from dogs in Grenada. Therefore, it became evident from this retrospective study that *P. aeruginosa* found to be susceptible to the recommended antimicrobial drugs in Grenada.

Research Grant: None
Student Support: Boehringer Ingelheim & St. George’s University Island Veterinary Scholar Program (IVSP)

Use of neuroprotective therapies by veterinary ophthalmologists for canine retina and optic nerve diseases

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In veterinary medicine, there are numerous neurodegenerative conditions that cause blindness in canine by damaging the retina and or optic nerve head. These conditions include glaucoma, sudden acquired retinal degenerative syndrome (SARDS), progressive retinal atrophy (PRA), and retinal detachment. Various treatments that have been developed for the sole purpose of neuroprotection, and some already developed treatments have been found to provide neuroprotection as a secondary effect. However, no drug has passed clinical trials to earn approval from the FDA, both in the veterinary and human field, for neuroprotective purposes. Despite lack of FDA approval, some veterinarians prescribe treatments for neuroprotection as a last resort effort since many neurodegenerative conditions will, at some point, cause loss of vision. Thus, we developed a survey to gauge whether veterinary ophthalmologists on which treatments they use and the efficacy of the treatment in their experience. By evaluating these responses, our goal is to determine the efficacy of these treatments as neuroprotectants in order to stimulate future research and neuroprotective drug development for ophthalmic treatments.

Research Grant: R01-EY025752
Student Support: NIH Grants 5T35OD016477-19
Analysis of milk for ceftiofur and its metabolites following intramammary and parenteral administration

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Ensuring there are no antibiotic residues in milk has been a central focus of veterinarians and producers for many years. With growing consumer concerns regarding antimicrobial resistance and the presence of antimicrobials in milk, this has become an increasingly important area of research. Prior to leaving the farm, each truck load of milk is tested for β-lactam antimicrobials utilizing ELISA technology. These tests have sensitivity levels that are able to detect residues at or below the allowance of U.S. tolerances, however they do not identify the specific antimicrobial and are not quantitative measurements (Gorden 2017). The main goal of this study was to create milk samples that contain ceftiofur and its metabolites following intramammary and parenteral administration. The objective is to characterize how the parent drug is metabolized and how it and the metabolites are cleared from milk after administration. There were two treatment groups, with three Holstein cows (2+ years old) in each treatment (n = 6). For the first treatment, three cows were injected subcutaneously with 2.2mg/kg of ceftiofur HCl (Excenel, Zoetis). In the second treatment group, three cows underwent intramammary infusion in one quarter with one tube (125mg) of ceftiofur HCl (Spectramast LC, Zoetis) once at the beginning of the trial and again 24 hours later according to the label. Milk samples were collected every 12 hours for 72 hours after the final treatment in both treatment groups. All milk samples were frozen and are currently undergoing analysis via liquid chromatography and mass spectrometry to determine drug residues. Results of this work will guide violative residue investigations on dairy farms following ceftiofur administration.

Research Grant: Boehringer Ingelheim Vetmedica Inc. & Merial
Student Support: CVM Summer Scholars

Prevalence of Rickettsia spp. in Lone Star ticks (Amblyomma americanum) from horses in northeastern Oklahoma

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Ticks are important vectors of pathogens across a range of hosts, but minimal research attention is given to ticks infesting horses in the United States. In Oklahoma, the Lone Star tick (Amblyomma americanum) was previously found as a prevalent tick infesting horses, and a few tick surveys, along with a single case report, suggest spotted fever group Rickettsia (SFGR) may be clinically significant in horses. To better understand the prevalence of SFGR in ticks infesting horses in Oklahoma, A. americanum (n = 250) collected from 40 horses at 6 ranches in September 2019-May 2020 across northeastern Oklahoma were evaluated for Rickettsia spp. using PCR (polymerase chain reaction) targeting a 17kDa partial gene fragment followed by sequencing for species identification. In addition, serum samples (n = 51) obtained from infested and uninfested horses from the 6 ranches were evaluated for antibodies to Rickettsia rickettsii using indirect fluorescence antibody testing. Rickettsia spp. were detected in 27.6% (69/250) of the ticks tested, with the majority of gene sequences matching R. amblyommatis (n = 61) and the remainder identified as unspecified Rickettsia spp. (n = 8). Of the 40 horses with A. americanum, 27 (67.5%) hosted at least one tick testing positive for a Rickettsia species, and 9 horses had positive ticks at multiple tick examination visits. Serologic screening revealed no individuals with detectable antibodies to R. rickettsii. Together, these results demonstrate a high prevalence of SFGR among A. americanum of this region, although further research is needed to explore the relationship of SFGR in horses and their potential implications for equine health.

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Student Support: Summer Research Training Program, College of Veterinary Medicine, Oklahoma State University
Environmental and genetic effects on embryologic development of the American alligator

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“A is for alligator.” a phrase taught in grade schools across the nation, and although many people could recognize an alligator, information about the growth and development of \textit{A. mississippiensis} has yet to be discovered. As a multi-million dollar enterprise, alligator farms aim to rear large alligators with blemish free hides as these animals are highly sought after in the luxury leather market. Many methods of environmental control are currently used during alligator incubation to increase animal size and decrease unwanted scarring, with genetics now under consideration too. Interestingly, alligators in Louisiana (LA) are known for their large size, whereas alligators in Florida (FL) are smaller but known for their quality. Whether these differences are related to environmental factors or genetics has yet to be studied. It is concluded from past studies that environmental exposures during embryonic development influence the presence of umbilical scaring and potentially growth rates. The aim of this study is to evaluate genetic and environmental effects on the growth rates of embryos from both LA and FL, and to determine if growth rates predict umbilical scarring. To test this, 12 incubators were set up with 6 containing eggs from LA and 6 containing eggs from FL. Three incubators from each 6 were provided supplemental oxygen increasing atmospheric oxygen concentration from 20.9% to 27% to determine the effects of oxygen on embryo growth rate and umbilical scarring prevalence. Embryonic growth rates were collected via candling during egg incubation. Umbilical scarring were scored by an experienced assessor at 2, 12, and 26 weeks post hatch. Results are not yet reportable as the study is still ongoing at this time.

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Microglial activation following nose-to-brain transport of silver silicate nanoparticles in the rodent

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Engineered nanoparticles are in a plethora of applications and approximately 50% of all nanoparticles in commercial use today are composed of silver. Inhaled nanoparticles have been found to translocate to the brain via the olfactory epithelium (OE), a direct connection between the nose and the brain. Such nanoparticles in the brain could possibly trigger an immune response mediated by microglia, the resident macrophage of the central nervous system (CNS). However, little is known if such activation is present due to the uptake of nanoparticles into microglia. This study evaluated whether inhaled silver silicate (AgSiO$_2$) could be translocated to the brain via deposition in the nasal cavity olfactory region. Visualization of nanoparticles in the OE, nerve fascicles and microglia were done using autometallography. Activation of microglia in the olfactory bulb (OB) was assessed by cell morphology as a further measure of nanoparticle transport to the CNS. Sprague-Dawley rats were exposed to filtered air or to 1\% AgSiO$_2$ for 6 hours at a concentration of 1 mg/ml in a nose-only inhalation exposure system. Aerosol characterization was made by collecting air samples during the six-hour exposure period to measure gravimetrically nanomaterial concentration, silver concentration by X-ray fluorescence, and particle size and morphology by transmission electron microscopy. AgSiO$_2$ nanoparticles were visualized in the nose, nasal epithelium and nerve fascicles using autometallography, while the site and state of activation of OB microglia were determined using immunohistochemistry with Iba-1. These measures are ongoing for presence of silver and activated microglia in the OB using a systematic approach of morphometric analysis.

Research Grant: NIEHS U01 ES027288
Student Support: University of California, Davis, School of Veterinary Medicine
Exploring state-level risks for introducing chronic wasting disease into wild cervid populations

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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 virginianus*) 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 the 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 every 1,000 cervids/h). Understanding risk factors for CWD is essential for continuing efforts to prevent state cervid populations from becoming infected.

**Research Grant:** Unknown
**Student Support:** National Institute of Health

Impact and prevalence of cranial cruciate ligament disease in field trial Labrador Retrievers

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Cranial cruciate ligament disease (CCLD) is the most prevalent orthopedic problem in canines today, causing stifle mobility dysfunction and pain. The aim of this study is to look at the prevalence of CCLD in field trial retrievers and assess owners and handlers’ perceptions surrounding this topic. Given the evidence that CCLD is genetic in Labradors it is likely that because the field trial community is mostly Labradors, this disease may have a large impact on this population. A survey instrument was created, validated, and emailed to a population of retriever field trial participants to collect information on perceptions, prevalence, and exposure to canine cruciate disease. Inclusion criteria for data on the specific dogs was limited to AKC registered field trial retrievers currently owned by the respondent. Questions were split into three groups; group one included questions about the handler and their experience with field trials, group two focused on their experience with CCLD, such as how CCLD may affect their breeding and purchasing decisions and group three consisted of dog specific questions, such as breed, and if they were diagnosed with CCLD. The statistical analysis includes ANOVAs, Chi square, Student’s t test and thematic analyses with the significance set at \( P < 0.05 \). We expect an increased prevalence of CCLD in this population, affecting somewhere around 11% of dogs. In addition, we predict that owners will selectively avoid buying or breeding dogs that have an association with CCLD but will not recognize the scope of this problem in the community. CCLD is predicted to have a large impact on the sport of retriever field trials.

**Research Grant:** None
**Student Support:** Boehringer Ingelheim and the Graduate School at Michigan State University
A zebrafish model of mild traumatic brain injury

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The effects of underwater anthropogenic sound (sound produced from human activity) on aquatic life and the efforts to limit such sounds have been debated for decades. Studies on anthropogenic sounds have proposed these sounds may impact the behavior and health of aquatic life and humans, including the potential for sonically induced mild traumatic brain injury (mTBI). The purpose of this research study is to test whether molecular markers of mTBI can be induced in zebrafish by exposure to a single high intensity sonic impulse. The molecular indicators that will be evaluated include the inflammatory cytokine IL-6, reactive microglia using L-plastin antibody, and cleaved-caspase-3 as a marker of cell death. We will induce a sonic impact by developing an experimental apparatus in which an anesthetized zebrafish will be placed securely on a pedestal inside a 40-gallon injury-induction tank. The zebrafish will be exposed to a half-second 157 decibel pulse at 500 hertz from an underwater speaker, which should generate a four microjoule impact head injury. Control fish will be anesthetized and placed on the pedestal but will not receive a sonic pulse. Once the fish recover, their behavior and swimming patterns will be recorded, and they will be monitored for 24 hours prior to undergoing euthanasia. Brain tissue will then be extracted, and western blot and immunohistochemistry will be used to compare the levels of the mTBI markers between the experimental and the control groups.

Research Grant: Midwestern University Intramural Funding and Grants
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Novel approaches to elephant endotheliotropic herpesvirus 1A hemorrhagic disease in a juvenile Asian elephant

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Elephant endotheliotropic herpesvirus (EEHV) is the causal agent of an acute fatal hemorrhagic disease (EEHV-HD) affecting captive Asian elephants (*Elephas maximus*). This clinical report documents the disease progression and successful treatment of EEHV1A hemorrhagic disease in a 1.9-year-old female Asian elephant, outlining the use of novel therapies (intravenous stem cells, famciclovir fortified whole blood and plasma, and aminocaproic acid) and diagnostic tests (thromboelastography and serology). High levels of EEHV1A viremia were detected for 12 days with severe clinical signs. Treatment was initiated with famciclovir (15 mg/kg), rectal fluids, and supportive therapies. A single intravenous dose of 21.28 x 10^6 allogeneic mesenchymal stem cells grown from elephant umbilical tissue was administered to promote tissue regeneration. Fortified whole blood and plasma, obtained from donor elephants that received a single dose of famciclovir, provided colloidal support, coagulation factors, and therapeutic concentrations of penciclovir. Thromboelastography was used to monitor the effects of the antifibrinolytic aminocaproic acid and to better understand the impact of EEHV infection on *in vivo* hemostasis in Asian elephants. Serology assays measuring anti-EEHV antibodies before and after viremia showed a lack of seroconversion in a previously established EEHV1A-specific antibody marker (ORF-Q). Post-viremia, oral and rectal detomidine gel were used for standing sedation to facilitate weekly blood collection without adverse effects. Though the efficacy of individual therapies cannot be proven in this case, this study documents the successful management of EEHV1A hemorrhagic disease using a novel diagnostic and treatment plan.

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Student Support: University of California Davis School of Veterinary Medicine endowment funds
Clinical utility of scoring canine feces and evaluation of daily fecal scoring fluctuations in healthy dogs

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Various fecal scoring systems characterize canine bowel movements in clinical settings. The most common have been developed by Nestle Purina PetCare Company and The Waltham Petcare Science Institute. Both systems categorize feces based on characteristics such as shape, color, consistency and visual features. The data from these systems assist in monitoring gastrointestinal disease and therapeutic responses. Currently, the use of these scoring systems requires in-person scoring, limiting the potential for use in clinical trials. Moreover, little is known about the daily variation of scores in healthy dogs. This study aims to: 1. Determine if alternate scoring methods can be used in place of an in-person fecal score; 2. Evaluate daily variability of fecal scores in healthy dogs. For Aim 1, 113 canine bowel movements were scored in person by two veterinarians using both the Purina and Waltham scoring systems. Alternate methods were also used for scoring by three veterinarians. Details on the alternate scoring methods are included in the poster presentation. Data was analyzed using Cohen’s and Fleiss’ kappa statistics and Bland-Altman agreement plots. For Aim 2, 21 client-owned, healthy dogs were enrolled in a 4-week study where clients were trained on using the Purina and Waltham scoring systems to score daily defecations and prepare for alternate scoring of feces. Results for Aim 1 demonstrate agreement between in-person scoring and alternate methods using Bland-Altman plots and moderate to substantial agreement between raters among both scoring systems based on kappa statistics. Aim 2 is still in progress. Overall, these findings support the use of alternative scoring methods in lieu of in-person fecal scoring in dogs.

Research Grant: None
Student Support: OSU CVM Summer Research Program

Anatomical investigation of the giant Aldabra tortoise (Aldabrachelys gigantea)

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The giant Aldabra tortoise, Aldabrachelys gigantea, is an exotic pet and zoo animal found across the globe. The literature currently available does not provide a comprehensive anatomical guide or is presented on a case-by-case basis. Anatomical descriptions of exotic species are vital to proper husbandry and veterinary care. To research Aldabra tortoise anatomy, a 10-year-old male specimen was dissected, anatomical structures were described and photographed, and organ and muscle quantitative data collected. CT scans of various organs were performed, and muscle maps of forelimb, hindlimb, and head/neck musculature were created. A male Hermann’s tortoise, Testudo hermanni, was also dissected for comparison. Presented here is the first comprehensive anatomical guide of musculature, vasculature, and viscera for A. gigantea. Dissection revealed that general anatomy was typically consistent with other tortoise species, however some interesting differences were observed. The pancreas, which is otherwise located close to the stomach, was tightly adhered to the dorsal duodenum. Clinically relevant neurovascular structures were documented. The testoscapularis and claviculobrachialis were observed adjacent to the latissimus dorsi. These pectoral girdle muscles are typically found in chelonioids and not testudinids. Several forelimb muscles were enlarged and had more bony attachments. This morphology is likely an adaptation to the constraints of gravity on locomotion in giant tortoises. The information gathered provides basic knowledge on larger tortoise species with comparisons to smaller species. Further research can involve locomotion, normal versus abnormal findings, and physiological differentiation.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Impact of *Borrelia burgdorferi* on apoptotic neutrophil regulation of macrophage cytokine production

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Acute inflammation is a physiological response to infection or tissue injury during which neutrophils infiltrate the affected tissue. Failure to resolve acute inflammation can lead to various chronic inflammatory diseases. Resolution of inflammation is still not fully understood, but clearance of apoptotic neutrophils by macrophages (efferocytosis) is now seen as an important process in the shift from a pro-inflammatory response to one that is pro-resolving. The objective of this study is to test whether efferocytosis of neutrophils previously activated by *Borrelia burgdorferi* (*Bb*, a bacterial species which causes Lyme disease) alters macrophage production of cytokines. We hypothesize that efferocytosis of *Bb*-infected neutrophils will promote pro-inflammatory cytokine release and suppress anti-inflammatory cytokine release from macrophages when compared to macrophage efferocytosis of unstimulated neutrophils. Monocytes and neutrophils will be isolated from murine bone marrow and cultured separately, then co-cultured once cells are mature. Macrophages will be cultured alone, with *Bb*, with unstimulated apoptotic neutrophils, and with activated apoptotic neutrophils. Production of pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and KC (CXCL1) and anti-inflammatory cytokine interleukin-10 (IL-10) will be measured from supernatants. We expect that efferocytosis of *Bb*-cocultured neutrophils will promote release of TNF-α and KC and suppress release of IL-10 from macrophages. These results would indicate that macrophages respond to apoptotic neutrophils differently depending on the continued presence of microbes. This may be a key control step in initiating resolution of inflammation or allowing it to progress.

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**Student Support:** University of Missouri College of Veterinary Medicine Office of Research

Prevalence and geographic distribution of *Babesia conradae* in California coyotes (*Canis latrans*)

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*Babesia conradae* is a re-emerging intraerythrocytic piroplasm causing disease characterized by severe hemolytic anemia and thrombocytopenia in domestic dogs and can be fatal without treatment. Although the mechanism of transmission is not known, coyotes (*Canis latrans*) have been a proposed reservoir of disease, and previous work has shown that dogs with known aggressive interactions with coyotes are at greater risk for infection than those without these interactions. This study aimed to determine the prevalence of *B. conradae* in wild coyote populations in California in order to assess the viability of coyotes as a potential source of infection for domestic dogs. 465 splenic samples were obtained during post-mortem examination of coyote carcasses collected as part of a convenience sample or from previous epidemiological studies from Southern California, Fresno, and Hopland. DNA was extracted from samples and amplified using real-time PCR with primers specific for the *B. conradae* ITS-2 gene. In total, 22 coyotes tested positive in Fresno (n = 15), Orange (n = 4), San Bernardino (n = 1), and Los Angeles counties (n = 1) with an overall prevalence of 4.7%. Coyotes from Fresno (P < 0.0001) and rural coyotes (P < 0.0001) were significantly more likely to be infected with *B. conradae*. This study demonstrates that coyotes can become infected and harbor *B. conradae* and should be investigated as a possible source of infection in domestic dogs. Further avenues for research include investigating antibody seroprevalence and mechanism of transmission in coyotes.

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**Student Support:** Students in Advanced Research (STAR) Program through Boehringer-Ingelheim Animal Health
Immunogenicity of parainfluenza virus (PIV) vectored SARS-CoV-2 vaccines in the ferret animal model

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SARS-CoV-2 emerged in late 2019, quickly spread across the globe and has infected more than eleven million individuals resulting in more than 500 thousand deaths. While rapid strides have been made towards the development of diagnostic tests, treatments and experimental vaccines to prevent SARS-CoV-2 infection, there remains a critical need to develop an approved, safe and effective vaccine candidate. In collaboration with the laboratory of Dr. Biao He, we assessed the antibody response to ferrets vaccinated with a novel parainfluenza virus 5 (PIV5) vectored vaccine expressing the S and/or N protein of SARS-CoV-2 (PIV5-S and PIV5-N). Groups of ferrets were vaccinated or mock-vaccinated intranasally and blood samples were collected every 7 days. About 40 days post-vaccination, ferrets were infected with SARS-CoV-2, monitored for clinical disease, and sampled for infection. The ferrets were humanely euthanized and necropsied 7 days post-challenge. Blood samples were processed for serum and analyzed by enzyme-linked immunosorbent assays (ELISAs). The objective of this study was to determine the immunogenicity of the PIV5 vaccines by assessment of the serum antibody responses to the SARS-CoV-2 S protein. Ferrets had a robust antibody response to vaccines. While the PIV5-S had low antibody responses post-vaccination, the day seven post-challenge responses suggest the vaccine primed S-specific immune responses as mock-vaccinated ferrets had undetectable antibody responses seven days after challenge. While analysis of infection is pending, these data suggest that the PIV5-based COVID-19 vaccine-elicited immune responses against SARS-CoV-2 and likely protected against infection and disease.

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Survival impact of cervical lymphadenectomy combined with surgical excision of oral malignant melanoma in dogs

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Given the local invasiveness and high metastatic potential of oral malignant melanoma (OMM), surgical excision of the primary tumor combined with lymphadenectomy and adjuvant therapy is considered the standard of care. Currently, there is a lack of information concerning the therapeutic and survival benefit that cervical lymphadenectomy provides for dogs with OMM. This multi-institutional retrospective cohort study aims to compare the outcome of dogs that have received surgery as treatment for OMM with or without lymphadenectomy. The criteria for inclusion were: (1) diagnosis of OMM was confirmed with histopathology; (2) surgical excision of the primary tumor was performed with curative intent. The criteria for inclusion for cases with cervical lymphadenectomy in addition to surgical excision was that both procedures were performed concurrently. Cases where surgery of the primary tumor was palliative were excluded. Data reviewed included information on signalment, staging, local disease therapy, adjuvant therapy, histopathology, and disease progression. The current study group consists of 90 confirmed OMM patients retrospectively identified from The Ohio State University Veterinary Medical Center medical records. Of these 90 cases collected, 22 had lymphadenectomy performed concurrently with surgery of their primary tumor. This study is currently ongoing with cases from other contributing institutions still pending.

Research Grant: Pelotonia Grant
Student Support: Busey Endowment
COVID-19 pandemic: economic and social influences on the food industry

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The pandemic of coronavirus disease (COVID-19) presents an unprecedented global challenge and multifaceted socio-economic consequences comprising the food industry. The purpose of this study is to appraise the selected integrative reviews related to the direct and indirect influence of COVID-19 on food safety and security. A comprehensive systematic literature search sources were mainly from Medline, and The Cochrane Library. An additional search for grey literature was conducted on the websites of food industry, food associations and networks. Within this review, we analyzed how the COVID-19 pandemic has affected the food industry and its related subsectors. The review also emphasized on the importance of guidelines placed on workers/consumers and certain actions of retailers that should be taken to prevail through the COVID-19 emergency. Food safety and security are at risk and the appropriate actions must be taken to ensure the most vulnerable populations. Unless a sound control and prevention of COVID-19 pandemic is tailored and rigorously executed nationally and globally, the impact of the pandemic can be severe and complicated, including the food industry and associated sectors. Assessment and improvement of the existing work guidelines and ethics in the agriculture workforce sectors is needed. Further study is inevitable to update the epidemiological dynamics of the COVID-19 pandemic, which is critical to develop evidence-based assessments, control, and prevention of the pandemics.

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Student Support: Grant # HRSA #D34HP00001

Transmission and control interventions for Shiga toxin-producing Escherichia coli in cattle

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Shiga toxin-producing Escherichia coli (STEC) can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome, which is a major cause of acute renal failure in children. As cattle serve as the primary reservoirs for STEC, pre-harvest interventions aimed at decreasing prevalence are being investigated. However, previous intervention studies provide conflicting results on the effectiveness of the interventions. The objective of this study is to determine which intervention strategies are proven most effective at reducing the prevalence and incidence of STEC O157 in steers. Transmission experiments were conducted on a 70-head feedlot by inoculating 5 steers with a commensal E. coli strain. We quantified the interactions of 70 steers by using real-time location data collected during the transmission experiments. The empirical data were then used to develop and calibrate an agent-based model for E. coli transmission in Netlogo 6.1.1-a specialized software for creating agent-based models. We evaluate the impact of several literature-supported intervention strategies on O157 prevalence and incidence within the model: use of vaccination, use of probiotics, cohorting of animals, alteration of water volume available in troughs, alteration of feed bunk spacing, sanitization of water troughs, and sanitization of feed bunks. Preliminary simulations indicate that vaccination and probiotics are the most likely to decrease O157 prevalence, while alteration of water volume and feed bunk spacing, and sanitization of water troughs and feed bunks have minimal effects. Additional simulation experiments will be conducted to assess the overall effectiveness of such intervention strategies.

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Comparison of intra-/inter-observer variability for measuring femoral varus in radiography and CT in the dog

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The medial angulation of the distal femur in the frontal plane is called the femoral varus, which can be evaluated by measuring the anatomic lateral distal femoral angle (aLDFA). The femoral varus is a contributing factor for medial patellar luxation (MPL). The treatment of MPL in dogs associated with femoral varus by distal femoral osteotomy (DFO) is well recognized. Reliable quantification of femoral varus, such as aLDFA, is a prerequisite for the identification of potential DFO candidates. Traditional radiographic measurement of aLDFA is sensitive to malpositioning of the femur. Positioning is influenced by normal and/or pathologic variations in position and size of the patella, fabellae, and lesser trochanter. Computed tomography (CT) has also been used to measure aLDFA in dogs. When isotropic spatial resolution is achieved, CT permits reorientation of the femoral image without loss of resolution. This renders patient positioning irrelevant and facilitates definition of landmarks for rotational and sagittal plane orientation which would be impossible when using conventional radiography. Radiographic and CT images have already been obtained of both femurs for five large breed canine cadavers. Six blinded examiners will independently measure radiographic aLDFA (R-aLDFA) and computed tomographic aLDFA (CT-aLDFA), on 2 separate occasions, one week apart. The CT-aLDFA will be measured on two versions of the same CT image, an opaque bone model and a transparent model. Data will be analyzed to compare intra and inter-observer variability for R-aLDFA and CT-aLDFA. Our research will help us determine which imaging modality has high (or low) repeatability and/or reproducibility for aLDFA measurements.

Research Grant: Louisiana State University School of Veterinary Medicine
Student Support: National Institute of Health T35 Grant

A system dynamics model of shelter capacity for care

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Shelter animal welfare concerns lead to the development of capacity for care calculators, which produce an optimal animal inventory based on average 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.

Research Grant: None
Student Support: Mississippi State University College of Veterinary Medicine
The effect of microglia-derived olfactomedin-like 3 on mouse brain endothelial cell angiogenesis

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Glioblastoma multiforme (GBM) is a uniformly fatal brain cancer in humans and canines. Within GBM, microglia comprise up to 50% of the tumor mass and promote new blood vessel formation. Currently, there is no effective anti-angiogenic therapy targeting GBM. Preliminary data from our laboratory indicated that 1ng recombinant olfactomedin-like 3 (rOLFML3) increased multiple parameters of EC differentiation, including total tube formation and branching points. Therefore, we extended this analysis into primary mouse brain ECs. While no difference was observed in EC differentiation following exposure to 1ng rOLFML3, we observed an increase in EC total tube formation (12%; \( P = 0.009 \)), a decrease in mean tube length (6.5%; \( P = 0.018 \)) and an upward trend in the number of branching points (11.3%; \( P = 0.107 \)) following exposure to 10 ng rOLFML3 compared to mock treated cells. To elucidate the contribution of microglia-derived OLFML3, ECs were exposed to conditioned media (CM) from immortalized isogenic control microglial cells and \( Olfml3 \) knockout microglia. CM from isogenic control microglia increased EC total tube formation (31.1%; \( P = 0.024 \)) relative to mock treated cells. Strikingly, CM from \( Olfml3 \) knockout microglia increased EC branching (39.7%; \( P = 0.029 \)), total tube formation (31.2%; \( P = 0.016 \)) and decreased average tube length (6.5%; \( P = 0.028 \)), despite the lack of OLFML3 in this media. This data suggests that OLFML3, as well as other soluble factors secreted by microglia, promote the differentiation of mouse brain ECs into numerous, short, highly branched vessels within brain tissue.

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Student Support: NIH T-35 Training Grant OD010956-21

Clinical biomarkers of cancer cachexia in cats

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Cancer cachexia (CC) is a multifactorial syndrome that occurs in human cancer patients, and is associated with involuntary weight loss, anorexia, sarcopenia, and increased serum levels of inflammatory cytokines and acute phase proteins (APP). No effective interventional treatments currently exist. Some cats with cancer present with clinical signs similar to humans with CC; however, specific clinical biomarkers in cachectic cats have not been investigated. In this study, we aim to characterize abnormalities indicative of CC in cats by comparing body weight, body condition, complete blood count, serum chemistry and inflammatory cytokine concentrations in cachectic and non-cachectic cats with cancer, and healthy cats. Additionally, we aim to evaluate clinical follow up data to determine prognostic value of measured biomarkers. Initial analysis reveals mean body weight and BCS were not significantly different, demonstrating the importance of measuring muscle mass in clinical evaluation of CC. Cachectic cats had significantly lower hematocrit and albumin compared to both non-cachectic and healthy cats. Cancer-bearing cats had significantly higher white blood cell counts compared to healthy cats. Cachectic cancer-bearing cats had significantly higher serum amyloid A compared to both non-cachectic and healthy cats. Analysis of \( \alpha \)-acid glycoprotein is in progress; we hypothesize cachectic cancer-bearing cats will have elevated \( \alpha \)-acid glycoprotein, and cats with elevated APP will have shorter overall survival. Results thus far indicate the clinical and inflammatory profiles of feline CC is similar to humans signifying potential for a feline model to further research of CC in human cancer patients.

Research Grant: Mary Lowe Center for Comparative Oncology Seed Grant University of Pennsylvania, School of Veterinary Medicine
Student Support: NIH/BI Summer Veterinary Scholars Program
**EphA4 limits pial collateral remodeling following ischemic stroke**

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Stroke is the fourth leading cause of death and the most common cause of serious long-term disability in the United States. The majority of strokes are caused by a vascular obstruction resulting in reduced cerebral blood flow (CBF). These ischemic events ultimately lead to permanent cell death and neurological deficits. Research has implicated the density of leptomeningeal anastomoses, or pial collateral vessels, and their ability to remodel into large arteries as a major determinant of neuronal damage. Pial collateral vessels are formed during embryonic development and bridge distal arterioles in the pia mater of the brain. Under normal conditions, these vessels are small and inactive. Following ischemic stroke, these vessels remodel and enlarge through a process termed arteriogenesis, thereby allowing increased CBF. Recent evidence suggests the EphA4 receptor, a member of the largest family of tyrosine kinase receptors, plays a major role in various neurological insults. We hypothesize that EphA4 negatively regulates pial collateral remodeling following ischemic stroke. After a surgically induced stroke, EphA4 knockout (KO) mice had a significantly reduced infarct volume and significantly higher CBF compared to wild-type (WT) mice at 1-day post stroke. Additionally, 4.5 hours post stroke, KO mice had increased pial collateral remodeling, as demonstrated by an increase in diameter and length, compared to WT mice. These findings suggest EphA4 limits collateral remodeling and represents a novel therapeutic target for ischemic stroke patients.

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**Student Support:** Virginia-Maryland Regional College of Veterinary Medicine dual degree program

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**Parasites of the wild red fox (Vulpes vulpes) in North America**

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The red fox (Vulpes vulpes) is the most widely distributed of all the carnivores and can be found in urban, suburban, rural and wild regions of the world. The parasites of the wild red fox have been documented frequently within the scientific literature. To date, there has been no published comprehensive review of the parasite fauna of red foxes from North America. As part of a larger review of the parasites of wild canids and felids in North America, we searched PubMed, CAB Abstracts and Google Scholar for papers reporting parasite species recovered from wild foxes. It was the aim of this work to consolidate information from all published papers recording parasites in wild North American red foxes in order to determine what parasite species are present in this host and the geographic areas in which parasites have been found. There were 121 papers that fit our inclusion criteria. These papers cited 89 distinct parasite species (58 helminth, 12 protozoan, 19 arthropod) from many locations within the United States and Canada. Reports included the zoonotic parasites Trichinella spiralis, Echinococcus multilocularis, Toxoplasma gondii, and Toxocara canis, which were frequently recorded from foxes. Zoonotic parasites pose a threat to humans in contact with foxes or their feces, as they are transmissible to and can cause disease in man. Therefore, parasites of wild foxes are significant because they present potential threats to human and animal health through parasite transmission.

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2020 Survey of non-human primate enrichment practices in the United States

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Environmental-enrichment activities are vital for the well-being and welfare of laboratory-housed non-human primates (NHPs). However, NHP enrichment practices have not been thoroughly categorized since a survey circulated in 2014 (Baker 2016). A widespread understanding of common practice is beneficial in aiding facilities to evaluate and forecast necessary improvements in their own programs. It can also inform the need to continue improving standards for NHP behavioral management programs as a whole. This paper describes the feedback from a 2020 survey distributed to university, private, and government facilities around the country that house NHPs. We will present our survey findings that include questions that explore the development, implementation, structure, and management of NHP enrichment that are used in both behavioral and non-behavioral research. The areas of enrichment that are queried include social housing, positive reinforcement and human interaction, structural enrichment including housing amenities and exercise enclosures, and an array of different types of enrichment devices. We also analyze facilities’ rationale for increasing enrichment management and when limitations on enrichment may be needed. Because in recent years, technology has become more pervasive in all areas of our society, the research world included, an exploration of how technology has been incorporated into enrichment programs is necessary to establish standard practices. Thus, we will also present findings that explore the utility of digital enrichment through the use of music, television, or tablets.

Research Grant: None
Student Support: NIH/Boehringer Ingelheim Veterinary Scholars Program (NIH T35 Training Grant 5T35OD010919-23)

Microglial morphological changes in areas associated with breathing following respiratory motor neuron loss

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Respiratory distress due to motor neuron death (e.g., phrenic and intercostal) is a clinical sign seen in multiple neurodegenerative diseases. Increased microglia correspond with motor neuron death, but whether this increase is beneficial or harmful remains unknown. Microglia can exist in multiple states including resting (ramified) and activated (amoeboid) morphology. In a novel rat model of respiratory motor neuron death induced by intrapleural injections of cholera toxin B conjugated to saporin (CTB-SAP), increased microglial density has been observed in the phrenic and intercostal motor nuclei. We hypothesize that microglia in both the phrenic and intercostal motor nuclei of CTB-SAP treated rats will have amoeboid morphology and corresponding changes in microglial characteristics that are consistent with activated microglia. Immunohistochemistry staining and confocal microscopy techniques were used to visualize microglia in the C4 and T2-7 spinal cord sections in controls and CTB-SAP rats (N = 8/group), and we are now analyzing these sections using IMARIS software and a Scholl analysis. We expect that the following microglial morphological characteristics will be affected in the phrenic and intercostal motor nuclei: decreased branch number, decreased filament and branch length, and increased filament and branch volume in CTB-SAP rats vs. controls. These results would indicate increased microglial activation in areas controlling breathing. Future studies will be focused on understanding whether these activated microglia and subsequent cytokines that they release are beneficial or detrimental following respiratory motor neuron death, and whether these could be targets for preventing respiratory distress.

Research Grant: Spinal Cord Injury and Disease Research Program (SCIDRP), and University of Missouri College of Veterinary Medicine Committee on Research (COR)
Student Support: University of Missouri College of Veterinary Medicine Office of Research
Prospective evaluation of the fecal microbiome of dogs treated with doxorubicin for naturally occurring cancer

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Specific characteristics of the fecal microbiome in humans have been associated with a positive response to chemotherapeutic agents. Dogs spontaneously develop cancer and are exposed to many of the same environmental factors and carcinogenic compounds as their human counterparts making them ideal for fecal microbiome studies. Having an understanding of the relationship between the fecal microbiome, antibiotics, and chemotherapy can help to improve clinical outcomes for patients. The effects of chemotherapy on the fecal microbiome in dogs with cancer has not been studied. The aim of this study was to evaluate the changes that occur to the fecal microbiome of dogs after receiving doxorubicin chemotherapy and to assess whether those changes are predictive of clinical outcomes. Fecal samples were collected from healthy controls and cancer dogs on days 0, 4, 7, and 21 after doxorubicin administration. The following clinical data were collected for each patient: age, breed, weight, specific diagnosis, diet, medications, appetite, gastrointestinal signs, hematology, and response to treatment. Fecal DNA was extracted and 16s rRNA amplicon libraries were sequenced and annotated at the MU DNA and Informatics Research Cores. The richness, alpha and beta diversity will be compared via permutational multivariate ANOVA (PERMANOVA) to identify the influence of doxorubicin on the canine fecal microbiota, and features of the microbiota associated with clinical outcomes following treatment. We hypothesize that administration of doxorubicin chemotherapy will result in an overall loss of microbial diversity. As a secondary aim, we will evaluate if changes in microbial diversity are associated with risk of chemotherapy related side effects.

Research Grant: Veterinary Cancer Society Resident Research Grant
Student Support: Supported by an endowment established by IDEXX-BioAnalytics

A computer-aided detection (CAD) program for lung nodules in computed tomography scans of dogs and cats

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The role of computers has expanded from acquisition of images and transferring data, to aiding in the detection of abnormalities and forming diagnoses for patients. Computer-Aided Detection (CAD) programs have been designed for various uses in human medicine, including detection of lung nodules, but very few have been developed for use in veterinary medicine. The overall goal of this experiment is to develop a computer-aided detection program, requiring minimal user input, to identify lung nodules (such as pulmonary metastases) in thoracic Computed Tomography scans of cats and dogs. Pulmonary nodules in CT are recognized visually by their generally finite spherical shape and soft tissue attenuation of X-rays, which separates them from low (gas) attenuation of the surrounding lung. This program uses attenuation differences to focus explicitly on the lung field, and utilizes Watershed Segmentation to detect potential nodules and analyze them individually. Nodules are selected based on criteria including shape and attenuation, using empirically derived boundaries. The program utilizes Maximum Intensity Projections (MIP), slice differences, and orthogonal reconstructions to mitigate the quantity of false-positive detections, while ensuring a high sensitivity. While in development, our program has been able to successfully identify nodules with a high false-positive rate. Therefore, future areas of development should aim at decreasing the false positive rate to allow veterinarians to make more accurate diagnoses and prognoses, which can heavily influence the treatment for patients.

Research Grant: None
Student Support: NIH-Boehringer Ingelheim Veterinary Research Scholars Program

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The Marine Mammal Center (Sausalito, CA) rescues hundreds of ill and injured marine mammals throughout Northern and Central California each year. Stranded pinnipeds commonly present with trauma from human activity and predators. The severity of these wounds often necessitates rescue for further assessment and characterization of the wound to plan for treatment and rehabilitation. Radiography is integral in the diagnostic work up to screen for osseous trauma. Records from 48 stranded pinnipeds (42 California sea lions (*Zalophus californianus*), three Pacific harbor seals (*Phoca vitulina richardii*), two Northern elephant seals (*Mirounga angustirostris*), one Guadalupe fur seal (*Arctocephalus townsendi*)) were reviewed for this retrospective study. Radiography was included if an abnormality of the appendicular skeleton or bone involvement was suspected. Of the 48 individuals, 47 (97.9%) within the subset of rescued pinnipeds that received radiographs were immature. Forty-five (93.75%) had some version of osseous pathology (acute fracture or osteomyelitis); 3 (6.25%) were only soft tissue pathology. Soft tissue and/or bony injuries of multiple limbs were common, affecting 87.5% (42/48). Polystotic lesions were identified in 70.8% of individuals (34/48). Most common sites of bony pathology were phalanges (35.4%), radius (29.2%), and carpal bones (18.75%). Radiographic findings consistent with erosive arthropathy was found in 31.25% of individuals (15/48). Five individuals had a sequestrum (10.4%) associated with lytic bone disease. Bony pathology is common in young, stranded pinnipeds. Radiography can be incorporated in health assessments to identify injuries and guide treatment/rehabilitation plans.

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**Student Support:** National Institutes of Health T35 Training Grant

Etiological factors of migrating plant awn-associated disease in dogs

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Migrating plant awns (MPAs) are a common cause of a spectrum of diseases and significant morbidity in dogs. Despite the frequency of MPA-related pathology, there is sparse information on the specifics of awn taxonomy and associated disease. To fill this void, we initiated a retrospective cohort study of canine patients at the UC Davis Veterinary Medical Teaching Hospital. The goal of the study was to determine the taxonomy of surgically recovered awns and examine possible association with clinical disease. As a first step, 40 MPAs were collected from 32 canine patients over the span of 1 year, and data were obtained from medical records. The site of patient exposure to MPA was determined from client home address and patient travel history, and mapped to display the geographical distribution of MPAs. Surgical reports indicate that 17/40 (42.5%) MPAs were removed from subcutaneous tissue, 12/40 (30%) from thoracic cavity, and 11/40 (27.5%) from body orifices (mostly ear/nose). Bacteria were isolated in 12/15 (80%) patients who had bacterial cultures performed, resulting in 8 aerobic and 7 anaerobic bacteria identified; *Pasteurella* sp. was the most common isolate. All bacterial isolates were susceptible to amoxicillin-clavulanic acid, which was empirically prescribed to all of the cultured patients. These data provide evidence-based support for the empirical use of amoxicillin-clavulanic acid in clinical patients with MPA-associated infections. In addition, these findings will provide the relevant geographic and clinical data (anatomical location, pathology, microbiology) to correlate with the future molecular determination of MPA taxonomy.

**Research Grant:** None  
**Student Support:** Boehringer-Ingelheim Animal Health
Development and validation of a nontraditional selective broth culture medium for *Clostridioides difficile*

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*Clostridioides difficile* is a strict anaerobic spore forming bacterium that causes disease in man and animals through toxin production (Toxin A, Toxin B and Binary toxin). The disease this bacterium causes is characterized by fever, diarrhea, and abdominal pain and, more importantly, the recurrences of the infection can progress to fatal clinical conditions and death. In research labs, when trying to grow and isolate *Clostridioides Difficile*, an anaerobic environment is required. This can quickly become expensive when considering the price of sachets, which are small sheets used to pull oxygen away from a sealed environment. The aim of this study is to not only create an alternative broth culture medium that would eliminate the need to use sachets, but also to produce a medium that can outcompete the primary commercial choice in both sensitivity and growth rate. The key ingredients in the alternative are the amino acids Glycine and Histidine, which are proven to induce spore germination and Sodium thioglycolate and agar (50%) which will quench oxygen from broth medium rendering it anaerobic medium. Another important factor to consider is that this medium is being tested by 6 different *C. Difficile* isolates, so it must produce a relatively equal amount of growth and display equal sensitivity across all 6 strains. The final composition of the broth was decided by comparing 4 treatments with varying amounts of glycine and histidine. The treatment that showed the highest sensitivity was treatment 3, it produced more spores at the lowest concentration, 5 microliters, across all 6 samples, so the final medium is CCFB with 0.2 g Glycine and 0.4 g Histidine. This medium will be tested against the commercial similar medium.

**Research Grant:** Boehringer Ingelheim Scholars Program  
**Student Support:** Boehringer Ingelheim Scholars Program

Genetic analysis of swine and human influenza A virus subtype H3N2 from 2014-2019 in the United States

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Influenza A virus (IAV) surveillance is a vital aspect of both swine and human health industries because new IAV strains can arise by inter-species transmission, antigenic mutations (drift) and genetic reassortment. These characteristics create a genetically variable virus which can result in a pandemic with significant public health and economic implications. Previous research has shown that the H3N2 subtype has a high potential for cross-species transmission, yet little is known about IAV transmission between humans and swine. H3N2 sequences of human and swine isolates from July 2014 to July 2019 were collected from NCBI’s Influenza Virus Database. All RNA segment sequences from these strains were then aligned. The best-fitting models were selected using a Bayes factor with marginal likelihoods implemented in Beast. The resulting trees were then summarized in a maximum clade credibility tree after performing a 10% burn-in using TreeAnnotator. FigTree was then used to differentiate the human and swine strains. Swine strains found in human clades were considered human-to-swine spillover while human strains in swine clades were considered swine-to-human spillover. Results showed that the current influenza strains were more adapted to move from human hosts to swine hosts which was contrary to expectations from previous studies. Continued genetic surveillance and analysis of IAV is crucial to ensure the health and safety of swine and human populations.

**Research Grant:** FFAR AAVMC Fellow 100578  
**Student Support:** Foundation for Food and Agriculture Research
Accuracy of 3D virtual surgery plans—design to execution

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Fractures of the navicular (distal sesamoid) bone in horses are relatively uncommon, but typically have a guarded prognosis and can be very difficult to treat. Avulsion fractures are more common and do not require surgical intervention, whereas parasagittal fractures can be managed via lag screw fixation. Screw placement is very critical in this procedure. Just 1mm of deviation could lead to penetration of the navicular bursa or the coffin joint, making surgical preparation a critical component of this procedure. A previous study at the University of Florida determined that there was no significant difference in screw placement using virtual surgery planning (VSP) as opposed to conventional technique. Although, there was a significant decrease in surgical time. However, these studies did not evaluate the extent to which the executed surgeries followed the virtual surgical plans. The assumption from these procedures was the surgery was planned correctly. However, the possibility exists that the surgeries were imperfectly planned, leading to the lack of significance in the previous findings. The specific aim of this project is to objectively evaluate the similarity of a virtually designed surgical plan with the executed surgery of screw placement into a navicular bone utilizing patient specific drill guides. It is important to note that all previous measures of surgical accuracy have been based on a subjective grading assessment, whereas this study will measure the differences between the design and execution objectively using the Hausdorff Distance.

Research Grant: 2019 ACVS Surgeon in Training
Student Support: Florida Veterinary Scholars Program, University of Florida College of Veterinary Medicine

The epidemiology and clinical characteristics in the nervous system of human patients with COVID-19

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Understanding the neurological complications of SARS-Coronavirus-2 is crucial for early recognition, treatment and recovery of patients. The objective of this systematic review was to summarize the current evidence to support healthcare professionals and improve the prognosis of these patients. Multiple electronic databases and gray literature were searched until June 6, 2020 with no time, or geographical restraints. A total of 126 descriptive (case reports and case series) and observational (cross-sectional, case control, and cohort) studies were included. Risk of bias in individual studies were assessed. We found that elderly patients and those with more severe SARS-Coronavirus-2 infections had a greater risk of experiencing neurological complications. Common symptoms included headache, fever, cranial nerve deficits, altered mentation, consciousness disturbance, new-onset seizure, myalgia and smell or taste disorder. Electrodiagnostic testing was performed in 30 studies with 90% being abnormal. Lumbar puncture was done in 42 studies in which 22.2% tested for evidence of SARS-Coronavirus-2, with 3.17% being positive for the virus. Imaging performed included radiographs, computed tomography, and magnetic resonance imaging. Common abnormalities on radiography included ground-glass opacities or other indications of pneumonia. Central nervous system lesions seen on imaging were most commonly found in the ventricles, temporal lobe, frontal lobe and cerebellum. The systematic review of these data was vital for informing healthcare professionals on the symptoms, objective findings and lesions seen on imaging to improve the prognosis of these patients.

Research Grant: None
**Bartonella prevalence, diversity and associated risks in Appalachia**

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Many infectious diseases, including Bartonellosis, are understudied in the Appalachian Region. Bartonella spp. are causative agents of both human and animal morbidity. Bartonella spp. are primarily vectored to humans, pets, and small mammals through fleas and other ectoparasites, but other routes of transmission, including cat scratches, are also documented. The current study had two specific aims: First, to develop a survey to assess Bartonella-associated risks in Appalachian residents and second, to determine the prevalence and diversity of Bartonella spp. in rodents from the Cumberland Gap Region of Kentucky, Virginia, and Tennessee. A cross-sectional survey is being designed to determine Bartonella-associated risks for people living in the Appalachian Region. The survey will undergo review by the LMU IRB board before deployment. DNA was extracted from 91 rodent spleens that were collected as part of a larger project on zoonotic diseases. Currently, 43.1% (22/51) of the collected rodent spleens were PCR positive for Bartonella spp. [North American Deermouse, 57.9% (11/19); House Mouse, 43.8% (7/16); Hispid Cotton Rat, 21.4% (3/14); Northern Short-Tailed Shrew, 50% (1/2)]. Data on the 40 remaining samples is pending. Sequencing of positive amplicons will occur to determine the species of Bartonella followed by phylogenetic analyses of sequences, which will be submitted to GenBank. Initial data suggests that Bartonella spp. are present in the Cumberland Gap Region. Further screening of ectoparasites, wildlife, and domestic animals is necessary to determine the risk to humans and pets in the Appalachian Region.

**Research Grant:** Lincoln Memorial University  
**Student Support:** Boehringer Ingerheim Veterinary Scholars Program

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**Evaluation of neutrophil myeloperoxidase index as a novel biomarker of canine neutrophil extracellular traps**

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Neutrophil extracellular traps (NETs) are webs of DNA and granular proteins including myeloperoxidase (MPO) that are extruded from activated neutrophils to trap and kill invading microorganisms, but NETs may cause significant host tissue damage and initiate pathologic thrombosis. Markers of NETs such as cell-free DNA (cfDNA) are increased in dogs with immune-mediated hemolytic anemia (IMHA), sepsis, and systemic inflammatory response syndrome (SIRS) and may provide useful prognostic information. However, these markers are challenging to measure in the clinical setting. Clinical hematology analyzers routinely measure MPO activity in circulating blood leukocytes, a parameter termed the myeloperoxidase index (MPXI). The purpose of this study was to investigate whether MPXI correlates with another marker of NETs, cfDNA, and whether MPXI is increased in dogs with diseases commonly associated with NETosis including sepsis and SIRS. 15 dogs with SIRS, 6 dogs with sepsis, and 8 healthy controls were prospectively enrolled. Dogs were eligible for enrollment if they met ≥ 3 SIRS criteria and had a highly suspected bacterial infection (sepsis) or had normal exams and routine laboratory tests (healthy). MPXI was measured using an Advia 2120i and plasma cfDNA was quantified based on fluorescence of an extracellular DNA binding dye. Enrollment is ongoing, but preliminary data shows that MPXI is higher in dogs with SIRS and/or sepsis (median 22.7, range 9.3-29.6) compared to healthy controls (18.0, 9.9-20.7) \((P = 0.041)\). cfDNA does not correlate with MPXI \((r = -0.09, P = 0.8;\) Spearman correlation). Our preliminary results suggest that although MPXI may not represent NETosis it may be a marker of severe inflammatory disease.

**Research Grant:** None  
**Student Support:** Morris Animal Foundation Veterinary Student Scholar
Efficacy of selenium supplementation on enhancing humoral immunity in gravid beef cattle

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Many areas in the United States are selenium (Se) deficient. Dietary Se is an essential micronutrient acting on numerous health aspects, including optimal immune responses. The goal of this randomized controlled study is to determine if gravid beef cattle supplemented with organic Se-yeast boluses have enhanced humoral immunity. Three groups of gravid beef cattle were given Se-yeast boluses: group one at the first trimester, group two at the second trimester, and group three during the third trimester. The fourth group is a control that received no Se-yeast boluses. The cattle were vaccinated at the end of the second trimester with the Vira Shield vaccine, a killed vaccine containing: IBR, BVD types 1 and 2, PI3, BRSV. They were vaccinated in the third trimester with ScourGuard 4KC vaccine, a killed vaccine containing: strains of *Escherichia coli* with the K99 pili adherence factor, bovine rotavirus (serotypes G6 and G10), bovine coronavirus, and *Clostridium perfringens* type C. Serum neutralization tests for IBR, BVD types 1 and 2, PI3, and BRSV will be performed on 80 serum samples collected from all four groups of cattle. Group three and the control will be compared with the first two groups to see if the latter have a higher titer to the viruses than the control and group three. We plan to find an ELISA kit that tests for antigens to one of the four pathogens covered by the ScourGuard 4KC vaccine to compare the four vaccinated groups. We predict that the results of the serum neutralization tests will show higher titers for groups one and two, as compared to the control and group three. Overall, we hypothesize that receiving Se-yeast boluses has a positive effect on enhancing humoral immunity to specific antigens.

**Research Grant:** USDA Animal Health and Disease Program  
**Student Support:** Boehringer-Ingelheim Veterinary Scholars Program

Smoke inhalation in dogs and cats

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Smoke inhalation, associated most commonly with exposure to house fires, is a potential cause of severe injury in dogs and cats. Despite its prevalence, there is little information available about dogs and cats exposed to smoke, with the most comprehensive retrospective review from more than 20 years ago. Additionally, measuring carboxyhemoglobin in smoke inhalation patients has only been examined in one veterinary study thus far. The specific aim of this retrospective, descriptive study is to review outcomes of dogs and cats post-smoke inhalation, with regards to pre-hospital care, severity of presentation, treatment, and progression. Factors examined include: vitals at presentation, pertinent physical exam findings, carboxyhemoglobin, PCV/TS, lactate, thoracic radiographic findings, and length of hospital stay. Descriptive statistics will be used to analyze the data compiled from reviewing the medical records. Since 2004, there has been an active drive to equip first responders with face masks for animals rescued from fires, but there has not yet been reported outcomes following their use. The percentage of pets treated by first responders will be reported and evaluated as if the number has increased using a Fisher’s exact.

**Research Grant:** National Institute of Health’s National Center for Research Resources  
**Student Support:** Tufts Cummings School of Veterinary Medicine
A pilot study of pain assessment and activity tracking in dogs undergoing radiation therapy

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Radiation therapy is an important modality for cancer treatments in dogs and is effective for localized tumors. Radiotherapy has shown to increase the lifespan and quality of life for dogs; however, monitoring health changes and treatment response to cancer therapy is challenging with subjective assessments. The aims of this pilot study were 1) to better assess radiation therapy response and monitor side effects including pain and distress, 2) to understand the changes in the radiation patients’ activity levels and sleep quality, and 3) to investigate overall quality of life throughout radiation therapy and follow-up. Eight healthy control dogs without cancer or radiotherapy, four dogs receiving palliative radiotherapy for cancer, and one dog receiving definitive radiotherapy for cancer were enrolled. In this study, two methods were used for monitoring changes: an owner questionnaire and an activity tracking device. The online questionnaire for pain assessment and quality of life was developed and sent every two days for the first two weeks and then weekly to the owners of the treatment group, and twice, two weeks apart, for the control group. An activity tracking device was attached to each dog’s collar to provide minute-by-minute data. Preliminary results revealed a slight decrease in activity, an initial decrease in the quality of sleep, and a marginal increase in overall well-being of treated dogs compared to control dogs. These results indicate that activity tracking and a novel questionnaire might provide useful remote assessment and stimulate proactive communication regarding quality of life, which has potential to improve patient care for dogs undergoing radiation therapy for cancer.

Research Grant: Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University
Student Support: Kurz Family Scholarship and KSU CVM Office of Research

Developing a protocol towards understanding chimpanzee mortality: a systematic review

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Great ape species, including the chimpanzee (Pan troglodytes), are increasingly threatened with extinction due to human activities in the age of the Anthropocene. Veterinarians play a key role in the protection of both captive and free-ranging populations through the Species Survival Plans and Taxon Advisory Groups as members of Veterinary Advisory Groups, which are sub-committees of the Association of Zoos and Aquariums Animal Health Committees. A proper understanding of causes of mortality from naturally occurring disease is vital to the preservation of these populations and can be established through systematic reviews of the existing literature. A systematic review involves a detailed and repeatable search protocol derived a priori with the aim of synthesizing all relevant knowledge about a specific topic. Systematic reviews are also an integral component in the development of evidence-based medical practices. Previous efforts exist, however, most of the peer reviewed literature is exclusive to captive populations and limited chronologically to the time period 1990-2014. Other efforts have been exclusive to reviews of database knowledge and have also been limited to records from captive populations. The aim of this project was to develop a protocol for a systemic review of the literature to answer the research question: “What are the documented causes of chimpanzee mortality from naturally occurring diseases in zoological and free-living populations?” Through synthesis of all the existing published literature an up-to-date framework can be developed, which can be used by veterinarians to implement evidence-based methods in the captive or free-ranging populations, and at all levels of conservation intervention.

Research Grant: None
Student Support: Western University of Health Sciences College of Veterinary Medicine Office of Research
Temporal changes of the blood and gastrointestinal microbiota of healthy dogs

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A microbiota is a diverse community of bacteria vital to host health. The gut microbiota, predominantly Bacteroidetes and Firmicutes in dogs, regulates numerous functions including immunity. The blood microbiota is of low biomass and has not been well studied. As blood circulates, the organs it supplies, including the lungs may affect its bacterial community composition. In healthy cats, the blood microbiota is largely composed of Proteobacteria, which mimics the respiratory microbiota. Dysbiosis, defined as deviation from healthy microbiota, occurs in many respiratory diseases including feline asthma. A blood draw is appealing in lieu of invasive respiratory sampling; further characterization of the blood microbiota may allow development of markers for respiratory dysbiosis. In healthy cats, the composition of blood and fecal microbiota change over time; similar data need to be collected in dogs. Understanding temporal changes in health is important to interpret changes in disease. We hypothesized in healthy dogs that (1) richness and diversity would be higher in fecal versus blood samples at all time points and (2) there would be some overlap in community structure between fecal and blood samples with temporal changes of key taxa noted. Thirteen healthy dogs were enrolled. Samples were collected on days 1 and 7 for each dog: AM blood, AM fecal, PM blood, PM fecal and PM control (needle stick without hitting a blood vessel). Blood samples were collected via the jugular vein and fecal samples were collected using a rectal swab. DNA was extracted from each sample and sequenced using Illumina next generation sequencing. Data analysis is ongoing.

Research Grant: Discretionary funds from the PI
Student Support: Grant from Boehringer Ingelheim

Evaluation of performance of individual custom-made 3D printed surgical guide in canine caudal maxillectomy

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A combined dorsolateral and intra-oral approach has been described to increase surgical exposure during caudal maxillectomy. However, achieving an accurate resection can be difficult even for a trained surgeon due to the complex nature of the anatomy and the necessity of performing some cuts without complete visualization behind the orbit. Using computer-aided design (CAD), our goal was to develop a 3D-printed patient-specific surgical cutting guide to improve performance during canine caudal maxillectomy procedure. We hypothesized that the cutting guide will (1) increase the accuracy of the resection margins and the efficiency of the procedure, (2) reduce intraoperative complication rate and trauma to surrounding tissue, and (3) increase the accessibility of this procedure to novice surgeons. Postoperative CT scans will be performed, and the cutting margins compared with the planned margins in the CAD program Mimics. Seven canine cadaveric heads will be used per group, left and right maxilla being used for intrinsic comparison in each group. The study will measure and compare the mean cutting deviation, procedural time, soft tissue protection and ease of use (interference saw/guide) within 2 types of groups, and compare (1) free-hand osteotomy vs. guided osteotomy performed by a board-certified surgeon, and (2) guided osteotomy performed by a board-certified surgeon vs. a novice surgeon. If deemed accurate and efficient, the surgical guide can be translated onto canine patients with caudal maxillary tumors.

Research Grant: NIH T35
Student Support: North Carolina State University Research fund
Use and applications of judgment bias tasks in veterinary species: a systematic review

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The judgment bias task has been used in various animals and humans to assess affective state and has been used in veterinary species for a variety of purposes. There has been no review of the use of this task across all its applications. In dogs, methodological questions remain, including evaluation of reliability of coding. Our first objective was to review the judgment bias task in veterinary species, focusing on the methodology and feasibility for assessing valence. Our second objective focused on evaluating the reliability of live vs. video coding of the judgment bias task in dogs. We conducted a systematic search for articles using the terms judgment bias, cognitive bias, veterinary, animal, affective state, affect, emotion, mood, and welfare. Abstracts were reviewed and full-texts were obtained for eligible articles. For reliability, videos were manually coded for latency of each judgement bias trial. A total of 61 full-text articles were reviewed. Judgment bias tasks were used in dogs, cats, horses, pigs, poultry, cattle, sheep, goats, dolphins, bears, and honeybees. These studies focused on development of a validated judgment bias task and repeatability of the task, and the effects of welfare and pharmacological treatments. Welfare included environmental and human handling manipulation, isolation, personality, stereotypy, depression- and anxiety-like conditions, sleep, and pain effects on judgment bias. Reliability coding is ongoing, but we expect to find reasonable reliability between live and video coding. Our results demonstrate the scope of applications of the judgement bias task in veterinary species. Further, we will provide information on how coding should be conducted for judgment bias tasks in dogs.

Research Grant: None
Student Support: North Carolina State University Veterinary Scholars Program Fluoroscience Endowment

Evaluating open field behavior among mice harboring differing standardized complex gut microbiota

Jessica Y. Kwan, Aaron C. Ericsson, KC Kent Lloyd, Craig L. Franklin

Veterinary Research Scholars Program (Kwan), Department of Veterinary Pathobiology, Mutant Mouse Resource & Research Center, MU Metagenomics Center (Ericsson and Franklin) University of Missouri, Columbia, MO and Mutant Mouse Resource & Research Center, Mouse Biology Program (Lloyd), University of California, Davis, Davis, CA

To study the role of complex gut microbiota (GM) in rodent model phenotypes, the Mutant Mouse Resource and Research Center at the University of Missouri created four colonies of outbred CD-1 mice that harbor different GM representing the four major mouse producers (Jackson Laboratories, Taconic, Charles River, and Envigo). Differences in activity were noticed among these colonies suggesting that differing GM may be influencing behavior. The objective of this study is to further define behavioral phenotypes among the four standardized complex GM lines. Eight female and eight male mice from each colony were put through the International Mouse Phenotyping Consortium (IMPC) phenotyping pipeline used for the Knockout Mouse Project-2 at UC Davis, which includes a standardized set of behavioral tests. One such test is the Open Field Test, which is used to assess exploratory and anxiety-related (e.g. thigmotaxis) behaviors when a mouse is introduced to a novel environment. Measurements of mouse activity were recorded in four time points of five-minute increments up to 20 minutes. This two-factor (sex and GM) design will allow us to elucidate which factor may drive phenotypic differences. We expect that there will be a difference between sexes with males being more exploratory and exhibiting less anxiety-related behavior than females. Furthermore, we expect to validate earlier observations and confirm differences in activity among the GM groups. These studies will set the stage for further mechanistic studies designed to elucidate the role of GM in rodent behavior and further our understanding of the “gut-brain” axis.

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Student Support: American Society of Laboratory Animal Practitioners Foundation; IDEXX-BioAnalytics endowment
Exploring optical coherence tomography imaging depth to differentiate tissues at surgical margins

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Optical coherence tomography (OCT) is an imaging modality that provides real-time visualization of tissue microstructure. Subjective tissue characteristics were used to train observers to evaluate OCT images of surgical margins of canine soft tissue sarcomas (STS). The goal of this study was to assess imaging depths and other objective tissue characteristics of tissue types at surgical margins in canine STS. In this study, a single observer reviewed 248 images of four predominant tissue types (sarcoma, skeletal muscle, adipose and fascia) collected from 25 dogs with STS. The observer evaluated for tissue characteristics and measured imaging depths utilizing ImageJ software. Imaging depths were measured at 5 points of equal distance from tissue surface to the last pixel identified as contiguous tissue. Images were evaluated in normal, Threshold and Binary formats. Measurements were repeated one week later to evaluate for intra-observer variability. The median imaging depth in Binary format for sarcoma, skeletal muscle, adipose and fascia are 0.356 mm, 0.470 mm, 0.493 mm and 0.355 mm, respectively. The values calculated in Threshold format for sarcoma, skeletal muscle, adipose and fascia are 0.355 mm, 0.510 mm, 0.649 mm and 0.356 mm, respectively. Neovascularization, identified as black voids surrounded by white, was observed in 66.1% sarcoma. Skeletal muscle frequently displayed linear white lines that represent fascia surrounding muscle bundles (93.5%). These observed differences between tissue types in OCT images can be utilized to improve observer evaluation and aid in development of deep learning algorithms to improve the accuracy of surgical margin assessment.

Research Grant: American Kennel Club Canine Health Foundation
Student Support: Ohio State University College of Veterinary Medicine, NIH T35 Training Grant

Gross and histological progression of bovine tuberculosis in African Buffalo (Syncerus caffer)

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The pathogenesis of bovine tuberculosis (BTB) has been under study to improve diagnosis, prevention, and explore vaccination potential. While the economic impact of BTB is associated mainly with cattle herds in areas where BTB is endemic, tuberculosis is found in an extensive range of animals, including zoonotic potential in humans. A four year cohort study on African Buffalo was completed in Kruger National Park, South Africa. BTB was introduced naturally to the population in the 1990s, allowing for a data set including infection dates and length. Furthermore, the buffalo were split into an experimental group, receiving a long-lasting anthelmintic bolus, and a control group left untreated. The African buffalo were captured every 182 days over the four year period; BTB status, reproductive status, as well as blood chemistries and fecal exams were recorded. At the end of the four years, those infected with BTB were culled, and full necropsies completed. Previous research has been conducted on this data set, exploring the relationship between anthelmintic treatment on BTB infection rate and survival post infection, risk alleles associated with the infection rate based on genotype specific variation in Interleukin-12 production, as well as the cost and benefits of resistance traits associated with tuberculosis infection. The aim of this study is to utilize the gross pathology data, as well as the histological data completed on retropharyngeal lymph nodes and lung lobes, and explore the progression timeline of BTB this wild model. Continued work is being conducted to include body condition scoring in this timeline, as well as the effect of anthelmintic treatment on the histological pathogenesis of BTB in African Buffalo.

Research Grant: National Science Foundation Ecology of Infectious Disease Grant
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Oregon State University
Comparison of parasitic helminth species in phocid seals

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The distribution, prevalence, and intensity of helminth diseases varies greatly amongst phocid seals. Phocid species include ringed seals (Pusa hispida), bearded seals (Erignathus barbatus), spotted seals (Phoca largha), ribbon seals (Histriophoca fasciata), and harbor seals (Phoca vitulina). Few studies have been done to determine the overall parasitic fauna of these seals, which can be of concern to the native population who uses them as food resources. To establish an understanding of these infections, we compared parasitic data of phocid seals in the literature while noting their geographical distribution. We examined necropsy and diagnostic findings of four parasitic helminth species: nematodes, cestodes, trematodes, and acanthocephalans. There are several factors that influence the presence of helminth populations in these seals including geographical location and environment of host tissues. We found correlations suggesting that nematodes are the most common helminth species inhabiting these seals. Anisakid nematodes, including Contracaecum, as well as Dipetalonema and Parafilaroides species are the most prevalent nematode genera infecting these seal hosts in the heart, blood vessels, liver, lungs, and gastrointestinal tract.

Research Grant: University of Florida College of Veterinary Medicine
Student Support: Florida Veterinary Scholars Program

Comparing the effectiveness of Gigli wire and an osteotome to perform femoral head and neck excision (FHNE)

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Identifying the efficacy of manual tools used in femoral head and neck excision (FHNE) will allow veterinarians to make informed clinical decisions if a powered sagittal saw is unavailable. This study compared the surgical results of FHNE using Gigli wire (GW) or an osteotome (O) against an ideal computer generated ostectomy. Three canine and three feline cadavers had bilateral FHNE performed using GW on one leg and an O contralaterally. Computed Tomography (CT) images were obtained before and after surgery. Surgical duration times and number of ostectomy cuts were recorded. Three-dimensional models were made from the CT images using dedicated software, and an ideal FHNE cut was made on the pre-surgical femurs to compare with the post-FHNE models evaluating ostectomy angle, volume, edges, and part comparison. Statistical analyses included descriptive statistics, Student’s t-tests, and Fisher’s exact tests with significance set at $P < 0.05$. No statistical differences were seen. Mean angle difference was $12.90 \pm 8.49^\circ$ (GW) and $18.59 \pm 9.46^\circ$ (O), and mean femur volume difference was $205.30 \pm 116.45$ mm$^3$ (GW) and $205.17 \pm 169.08$ mm$^3$ (O). All femurs had residual bone (mean $242.65 \pm 172.99$ mm$^3$ GW; $230.30 \pm 190.37$ mm$^3$ O), which occurred most often at the caudal edge of the femoral neck, and seven femurs (3/6 GW, 4/6 O) had excess bone removal (mean $19.15 \pm 41.66$ mm$^3$ GW; 92.51 $\pm$ 188.11 mm$^3$ O). Fragments were present in 1/6 GW and 4/6 O femurs. Part comparison analyses allowed more accurate identification of regions of residual bone. Both tools performed equally and may be used to perform FHNE if a sagittal saw is unavailable, but it is recommended to check for residual bone at the caudal edge of the neck and avoid excessive bone removal.

Research Grant: Michigan State University Institutional Funds
Student Support: NIH Grant 5T35OD016477-19 award to Michigan State University
Epidemiological investigation of *Dirofilaria immitis* prevalence in Oregon

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*Dirofilaria immitis*, commonly known as heartworm, is a parasitic roundworm that spreads through bites for infected mosquitoes. Numerous species of mosquito are known vectors for this parasite in the state of Oregon, with the primary species contained within the genera *Aedes* and *Culex*. *D. immitis* requires both an animal host, typically either domestic dogs or wild canids, such as wolves and coyotes, and a mosquito host in order to complete their life cycle. In domestic dogs, *D. immitis* infections are a serious veterinary concern as both the infection and the treatment can be life-threatening to the affected animal. For this project a one health approach will be used in order to identify trends between climate, location, and transmission dynamics in order to better evaluate prevalence and the risk of *D. immitis* infections in Oregon. Currently, only positive antigen tests are utilized in surveillance of this parasite. Our aim is to utilize PCR testing of mosquitoes collected, similarly to the techniques currently utilized for vector borne diseases of human concern, such as West Nile Virus, in order to obtain a more accurate picture of the species and locations that present the highest risk of *D. immitis* infection. We are exploring these aspects in our present study.

**Research Grant:** Carlson College of Veterinary Medicine  
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program

Standardization of antimicrobial usage in Canadian dairy cattle

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Antimicrobial resistance (AMR) is a growing threat to human and veterinary medicine that is attracting enhanced regulation of antimicrobial usage (AMU). Attention has been focused on monitoring AMU since increased AMU is linked to an increased prevalence of AMR in food animals. The aim of this study was to develop a reproducible method to quantify AMU that is consistent across dairy farms in Canada. This method was developed using data from veterinary clinic dispensing records from four Nova Scotia dairy herds. Microsoft Excel (Microsoft 365 16.0.13001.20266) and Python (3.8.3) were used to build the program. AMU data was standardized into defined daily and course doses (DDDbovCA and DCDbovCA, respectively), and antimicrobial drug use rates (ADUR$_{DDDbovCA}$ and ADUR$_{DCDbovCA}$). This work resulted in a program that reports AMU data in standardized metrics by antimicrobial product and organizes the data into distinct categories. Preliminary results from the four farms by ADUR$_{DDDbovCA}$ demonstrated that the highest proportion of antimicrobials used fell into Category IV (low importance to human medicine). The most common route of administration was the oral route. These results were driven by the long duration of action from the high use of monensin in these herds. Limitations of this study include determining accurate durations of action and long-acting antimicrobial products overshadowing other antimicrobials. This data handling will be essential for future studies, as it will allow for easily acquired AMU data and transparent comparison between datasets. Overall, having a standardized approach will help to monitor AMU and AMR in dairy farms and will assist in the development of improved antimicrobial stewardship protocols.

**Research Grant:** NSERC Undergraduate Student Research Awards  
**Student Support:** Dairy Research Cluster III Program
Alphacoronavirus 1 spike gene phylogenetics and survey for risk factors of feline infectious peritonitis

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Feline coronavirus (FCoV) is a common, fecal-orally transmitted, enteric virus in cats, and is the cause of feline infectious peritonitis (FIP), which is a frequently fatal, immune-mediated disease. Although FCoV transmission can be due to transiently infected cats, a major source of FCoV is persistently infected cats without FIP. Previous data in FCoV and other viruses suggest that shedding phenotypes and/or virulence may be associated with specific viral genotypes; thus, identification of genetic markers for FCoV is needed. The spike (S) protein of coronaviruses is responsible for receptor binding and viral entry. As such, the spike is exposed to diversifying immunological pressure and variation may result in immune evasion allowing for persistent infection and shedding. Currently, there are no accepted phylogenetic divisions of FCoV that correctly identify the relationship between strains. Currently, there are no accepted phylogenetic divisions of FCoV that correctly identify the relationship between strains. Using Geneious and the sequences available on GenBank, a meta-analysis of all available FCoV spike gene sequences was performed to evaluate current phylogenetics. Secondly, several risk factors for the development of FIP in FCoV-infected cats have been suggested, including vaccination. We hypothesized that cats that developed FIP will be more likely to have been vaccinated recently than age-matched cats that do not develop FIP. We will investigate this hypothesis by means of an online survey.

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Prevalence and antimicrobial resistance of enteric bacteria in retail meat from Iowa grocery stores

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The National Antimicrobial Resistance Monitoring System (NARMS) is a program set in place by the United States Food and Drug Administration (FDA) that is designed to combat antimicrobial resistance (AMR) by accurately identifying and tracking the sources and trends of select enteric bacteria present within raw retail meat around the country. Those monitored are (non-typhoidal) Salmonella, Campylobacter, Escherichia coli (E.coli), and Enterococcus within the four major meat products of chicken breast, ground beef, ground turkey and pork/pork chops. Our laboratory at Iowa State University (ISU) established a partnership with NARMS and became a network laboratory for AMR surveillance within the state of Iowa. Since the start of the project in January 2017, a total of 3,000 retail raw meat samples were collected through March 2020. The overall prevalence rates of all meat types for Salmonella, E. coli, and Enterococcus were 4.9%, 53.8% and 79.50% respectively and the overall prevalence for Campylobacter for chicken and turkey samples was 11.03%. E. coli and Enterococcus have both been consistently stable despite some fluctuations throughout the course of this project. The minimum inhibitory concentration (MIC) data (available for isolates from 2017/2018 only) showed that Salmonella and E. coli were highly susceptible to carbapenems. While both species of Campylobacter remained susceptible to macrolides, Campylobacter jejuni exhibited high susceptibility to phenicols and aminoglycosides while ketolides and lincosamides were more active against Campylobacter coli. We believe Iowa’s participation in this surveillance has contributed to the monitoring of sources and trends of food borne pathogens and AMR within the food chain.

Research Grant: Unknown
Student Support: NIH T-35
Bioinformatics analysis of gibbon dopamine genes to human dopamine genes

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Gibbons are the last common ancestor of humans after gorillas, chimpanzees, and orangutans. Great apes have been investigated in neurobiological and cognitive studies while gibbons have not been studied as much. The aim of this study is to compare protein coding sequences of dopamine related genes between humans and gibbons. Dopamine is important in modulation of many complex cognitive tasks which includes problem solving in gibbons, such as getting swings unstuck after use in captivity. This will allow for comparisons of dopamine neurotransmitter-related genes between species. It was noted that dopamine degradation enzymes were found to contain over 60 total amino acid changes and dopamine synthesis enzymes were found to contain over 90 total amino acid changes. There was identification of both conserved and non-conserved amino acid changes. For example, some conserved DBH changes occurred in transmembrane alpha helices from one hydrophobic amino acid to another while non-conserved COMT changes occurred primarily in amino acid binding sites. These differences can indicate how dopamine levels in the brain can influence behavioral phenotypes in the brain associated with novelty seeking, attention, working memory, and problem solving. Translation of these biochemical differences can help identify benefits necessary for enrichment of gibbons that are in captivity. Genetic diagnostics can provide clinically relevant information for identifying those prone to captivity stress and maladaptive behaviors. Future work on other neurotransmitters and genes associated with behavioral phenotypes, such as serotonin and depression, can also landmark the importance for captivity enrichment.

Research Grant: Small Grants Involving Student Projects—TEAM Gibbon
Student Support: WesternU Summer Research Fellowship Program, Advanced Laboratory Apprenticeship

Prospective evaluation of the fecal microbiome in dogs with lymphoma treated with CHOP chemotherapy

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Complex interactions exist between gut microbiota and many diseases. Certain features of the microbiota have been associated with reduced chemotherapeutic efficacy and adverse side effects in humans. The relationship between response to chemotherapy and microbiota has not been investigated in dogs. Dogs with naturally occurring lymphoma are optimal for this project, because they are treated with the same drugs as humans. In the modified CHOP chemotherapy protocol for dogs, chemotherapy agents are administered weekly instead of simultaneously, and rectal exams are part of a routine physical exam simplifying stool collection. We collected stool samples from 17 dogs undergoing chemotherapy for lymphoblastic lymphoma throughout the CHOP protocol (Weeks 1, 2, 3, 4, 5, and 6). Fecal DNA was extracted using Qiagen PowerFecal kits, and the microbiome was analyzed via 16S rRNA amplicon sequencing at the University of Missouri DNA Core. Read merging, clustering, and annotation of the DNA sequencing occurred at the MU Informatics Research Core. Treatment-induced changes in richness and alpha diversity will be determined via comparison to data from healthy controls (n = 27) using a repeated measures ANOVA. We hypothesize that drugs in the CHOP protocol will reduce microbial diversity from baseline measurements, while no change will be seen in healthy controls. We also predict that a greater reduction in microbial diversity will correlate with a greater incidence of side effects, specifically chemotherapy-induced-gastrointestinal disease (CIGD) and sepsis.

Research Grant: AVMA/AVMF 2nd Opportunity Research Scholarship
Student Support: University of Missouri Research Council
Infectious disease in the etiology of myasthenia gravis: a systematic review

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Myasthenia gravis (MG) is a debilitating autoimmune disorder of unknown etiology in the majority of patients, in which autoantibodies target and destroy neuromuscular junctions by complement-mediated lysis and antigenic modulation. We hypothesized that microbes play a pathogenic role in the initiation of MG. We searched PubMed and Web of Science for relevant primary research. Papers captured by a search algorithm (n = 415) were manually assessed, yielding a total of 40 publications meeting the inclusion and exclusion criteria. An additional 8 papers were retrieved from the reference list of relevant articles. For each pathogen identified in a paper, an integrated metric of evidence (IME) value, ranging from minus 8 to plus 8, was computed based on the study design, quality of data, confidence of infectious disease diagnosis, likelihood of a causal link between the pathogen and MG, confidence of MG diagnosis, and the number of infected patients. Negative IME values corresponded to studies providing evidence against a role for microbes as etiological triggers in MG. One hundred and thirty-seven myasthenic patients infected with 19 different pathogens were documented. Epstein-Barr virus (median = 4.88), poliovirus (median = 4.29), and human polyomavirus 7 (median = 3.96) demonstrated the highest median IME values. The median IME for the dataset was 2.64 (mean = 2.48; range -3.79 to 5.25), suggesting a general lack of evidence for a causal link. There was a notable absence of mechanistic studies designed to answer this question directly. In conclusion, while there is a sizable body of literature documenting concurrence of infectious disease and MG, the question of the pathogenic contribution of microbes to MG remains open.

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Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

A comprehensive review of p53 mutations and cancer incidence in companion animals

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In all multicellular organisms, normal growth and cell turnover must be balanced and alteration of this balance leads to abnormal cell growth. This is one of the hallmarks of tumorigenesis concurrent with proliferative advantage, neovascularization, metastasis and other mechanisms which make cancer such a relentless disorder. Tumor Protein 53 (TP53) is one of the most well studied and important tumor suppressors which detect DNA damage and implement complex pathways to correct, stop or destroy deviant cells. Both human medicine and veterinary medicine benefit from p53 research, as p53 mutations themselves revealed to be a prevalent culprit in cancer development. The aim of the current review is to assemble and compare canine and feline p53 mutations to human p53 mutations. We addressed this issue by performing extensive literature review in order to obtain known mutational data from dogs and cats; and used comparative sequencing analysis to align them with human data. We sequentially describe all germline and somatic p53 mutations in canines and felines alike and point out specific associations between these common pets and human malignancies. Finally, we describe some of the unique and relevant features of p53 function in exotic species such as elephants and chimpanzees so as to substantiate the value of oncologic studies in our animal counterparts.

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Expression of BMPs in uterine tube tissues and their implications in ovarian cancer

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Bone morphogenetic proteins (BMPs) are TGFβ family members, and alterations in their expression can be pro-tumorigenic. Epithelial ovarian cancer (EOC) is the fifth-leading cause of death in U.S. women. A large fraction of EOC originates in the distal uterine tube (aka oviduct or Fallopian tube). However, the exact tubal cell types and molecular mechanisms of their regulation are not well understood. Eighteen BMP-associated genes hypothesized to have a role in ovarian cancer were selected from the literature. Using mouse tissues, single-cell transcriptome analysis was done to identify cell types and assess whether any candidate genes are preferentially expressed. Tubal epithelial stem/progenitor cells preferentially expressed BMP receptor BMPR1B, BMP ligands BMP3 and BMP7, and BMP downstream factor SMAD6, which suggests receptor-ligand autocrine mechanisms involved in normal tubal functions, such as regeneration and control of oocyte fertilization. BMP1 was preferentially expressed in the stroma, which may indicate an important role of the microenvironment in the regulation of tubal epithelium stem/progenitor cells. BMP4 and Id1 were especially highly expressed in endothelial cells, suggesting an important role of BMP signaling in vasculature and neighboring cells. To assess if alterations in any of these genes have human ovarian cancer implications, cBioPortal data were used. Increased mRNA copies or amplification of BMP2, BMP10, Id3, or Mki67 are associated with the worst survival of EOC patients. Our findings support the important role of BMP signaling in reproductive functions and EOC development. They also provide an essential basis for the development of new prognostic markers and therapeutic modalities.

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Functional adaptations in the forelimb myology of the snow leopard (Panthera uncia) compared to other felidae

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The endangered snow leopard (Panthera uncia) is native to Central and South Asia and occupies high, rocky mountain ranges. Snow leopards are unique in comparison to other felids because they live in harsh winter climates, attack by pouncing from high distances, scale great steep slopes in the snow, and manipulate their prey instead of hunting in packs or across long distances. Therefore, we predicted well-developed muscles that help with stabilization and range of motion in the forelimb. Our study provides the first detailed anatomical descriptions of the forelimbs of two adult snow leopards and muscle maps of forelimb muscles for comparative purposes with other felids. Findings revealed notable, functional differences in the P. uncia forelimb. The m. pectoralis profundus of P. uncia was substantially larger than other felids with five bellies, and only one belly noted for m. pectoralis superficialis. This configuration may be helpful for climbing and pouncing. Enlarged scapular musculature found in the P. uncia provides extra power and stability of the forelimb for climbing steep, icy, mountainous terrain. A small, unarticulated bony clavicle was identified within the m. brachiocephalicus, providing greater stability to the forelimb. M. brachialis showed three bellies and fused with m. cleidobrachialis, and the m. biceps brachii tendon of insertion bifurcated to attach on both the ulna and radial tuberosity. All bellies of mm. brachialis, pectoralis superficialis, and cleidobrachialis attached to the lateral bifurcation of the m. biceps brachii tendon. These differences suggest a greater need for power and stability in the forelimbs of P. uncia and may help with scaling through rugged terrain and manipulation of prey.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program
Comparison of China and Europe’s approach to antimicrobial resistance stewardship in the pork industry

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Antimicrobial resistance is a known global public health concern. While the detailed interaction between antimicrobial in food animals and the increase in antimicrobial resistance in humans is unknown, the link between the two has been established and recognized globally. Antimicrobial stewardship in livestock was first implemented in Sweden in 1986 with the rest of the European countries following suit. Despite this head start, antimicrobial resistance is still an on-going challenge for Europe today. China is the largest producer and consumer of pork globally. Chinese consumers’ demand for pork has increased in line with the country’s rise in prosperity. To meet the strong demand, domestic pork producers commonly use antimicrobials during production for disease treatment, prevention, and management to increase output. Recent publicized food safety scandals, disease outbreaks in domestic livestock products and increased awareness of antimicrobial resistance have resulted in an increased willingness-to-pay and demand for organic meat by Chinese consumers. Responding to the growing concerns of antimicrobial resistance, the Chinese government introduced a national pilot program in 2016 to reduce unnecessary use of antimicrobials. Since its launch, there has been a reported decline in usage of antimicrobials in human healthcare. Similar surveillance information on livestock antimicrobial usage is currently not available. Compared with China, Europe is the next largest pork producer and is at a different stage of industrialized agriculture. Antimicrobial stewardship is a global challenge with no definite solution. Comparing and understanding the different approaches may provide useful insights for the global community.

Research Grant: None
Student Support: None

Mechanisms of demyelination in prion disease

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Prion diseases are progressive neurodegenerative disorders that pose risks to human health and wildlife conservation. Key features of these disorders include spongiform encephalopathy, gliosis, and neuronal death. The infectious agent, PrPSc, is a misfolded, aggregated form of normal cellular prion protein (PrPC). Currently, the function of PrPC is unclear and how it is subverted in prion-induced neurotoxicity remains unknown. Utilizing a knock-in mouse model with a single amino acid substitution in PrPC that exhibits a rapidly progressive neurodegenerative phenotype in the absence of PrPSc, we aim to determine how an aberrant PrPC structure can cause neurotoxicity. The neurotoxicity in the Prnp93N mice is characterized by neuronal vacuolation and demyelination. Since Prnp93N homozygous mice can survive up to 250 days, we hypothesize that oligodendrocytes, the cells that myelinate neurons in the CNS, develop normally and then degenerate. WT, Prnp93N homozygous, and Prnp93N heterozygous mice were euthanized every five days, from birth to 25 days of age (n = 5 mice for each genotype). Brain samples were collected for histologic and biochemical analyses and ultrastructural imaging to characterize and quantify myelin and precursor and mature oligodendrocyte populations in the brain. Olig2, NG2, and PDGFrα were used to label oligodendrocytes and myelin in hippocampus sections. ImageJ will be used to quantify immunostaining, and Multi Gauge will be utilized to analyze western blot data of myelin associated glycoprotein (MAG) and myelin basic protein (MBP) levels. Sciatic nerves will be analyzed for axon diameter and myelin thickness using Marvoquant. Future studies will focus on understanding the mechanism of myelin loss.

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Student Support: National Institutes of Health T35 OD010956
Detecting digital dermatitis with computer vision and LED indication for beef cattle

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Digital dermatitis (DD) is a superficial infectious claw disease localized to the plantar/palmar interdigital space of cattle feet (Dopfer, 2009). Although commonly associated with dairy cattle, beef cattle also suffer from DD as an infectious cause of bovine lameness. Safe, efficient, and early detection of DD upon entry of beef cattle to farms is critical for effective treatment and reduced transmission within the herd. We hypothesized that computer vision (CV) models can accurately detect DD and signal the presence of DD lesions by flashing an LED light. Video footage of cattle feet was obtained from feedlots at hoof level by a trained veterinarian. Each video was divided into individual images and labeled in YOLOv3 format (Redmon et. al) with the M-Stage scoring system as defined by Dopfer, et al. (1997). These images served as the dataset for training a real time object detection model with CV in the Darknet framework (Redmon et. al). The output of our custom model can be adapted to a signal that turns an LED light on as indication of a DD lesion. Validation of the model’s predictions for detection of DD lesions will be accomplished by quantifying prediction accuracy. Digital dermatitis is a global well-being concern in cattle industries. Detection of DD takes time, training, equipment, and can be dangerous for producers and veterinarians. This CV system will be especially useful for detecting lesions on cattle in a chute setting in real time. Automated detection of DD lesions in cattle represents a powerful tool for early detection, prompt treatment, and reduced transmission of the disease. This will improve health and quality of life for the cattle while saving time, labor, and human risk.

Research Grant: Program Revenue Funds from Doepfer Lab
Student Support: Boehringer-Ingelheim Summers Scholars Program

Custom 3D printed splints for fracture repair in birds

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Avian limb fractures create unique challenges due to the anatomy of the bones, loads applied and postoperative compliance. Fractures most commonly involve humeri, which are not well suited for holding corrective implants, and the implants available often pose a significant risk to the bird throughout recovery. Humeral fractures in birds are often repaired with internal splints made of polymethylmethacrylate (PMMA), which is not resorbable nor practical and increases the risk of infection. The purpose of this project is to create an alternative internal splint that is resorbable and better tailored to the bone anatomy. Preliminary data were generated from last year’s project. Peak load values were measured using 3-point bending and compared between 8 groups of polylactic acid (PLA) splints and 1 group of PMMA splints. As anticipated, the PLA splints were significantly less strong and less stiff than PMMA splints. Despite the difference in strength, a 2mm thick x 20mm long PLA splint supported a load of 29.68 N. This splint may be suitable for birds up to 1kg in weight. Wings obtained from chickens will be used to compare the properties of our novel splint to those of intact bone and PMMA-splinted bones. Within each pair of wings, 1 humerus will be left intact and the contralateral limb will be osteotomized and randomly assigned to a PLA or PMMA splint. We will test the ability to close the skin over 2mm-thick splints. If the skin cannot be closed without tension, we will produce splints of 1.5 mm thickness. All humeri will be tested in 3-point bending to failure. Stiffness, yield, force and displacement to failure of repaired humeri will be normalized to those of intact contralateral bones for statistical comparisons.

Research Grant: None
Student Support: Western University of Health Sciences
Inhibitory effects of dual antithrombotic therapy by rivaroxaban and clopidogrel on platelet function in cats

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Hypertrophic cardiomyopathy (HCM) is the most common feline cardiac disease. Cats with HCM are at risk of arterial thromboembolism, an often-fatal event with poor prognosis. Despite this outcome, little is known about the synergistic inhibitory effects of rivaroxaban (RVX) and clopidogrel on platelet function. We aim to examine the safety and efficacy of dual antithrombotic treatment using RVX and clopidogrel in comparison to either RVX or clopidogrel treatment. We hypothesize that dual treatment safely reduces platelet-dependent thrombin generation (TG), platelet activation, and platelet aggregation more effectively than single agent treatment of RVX or clopidogrel in cats. Platelet parameters were compared before and after 7 days of RVX (2.5mg PO q24h), clopidogrel (18.75mg PO q24h), or dual treatment. TG on platelet rich plasma was measured by fluorogenic TG assay. Platelet activation, quantified by P-selectin expression, was measured by flow cytometry in the presence or absence of adenosine diphosphate (ADP). Platelet aggregation was measured by ADP-induced whole blood platelet aggregometry. Preliminary data showed no adverse events in cats receiving single agent RVX therapy. RVX treatment lowered P-selectin expression in unstimulated platelets (Mean Fluorescence: 1157 ± 197 vs 821 ± 126, P = 0.004) but augmented platelet responsiveness to ADP (Fold Change: 0.17 ± 0.095 vs. 0.40 ± 0.21, P = 0.002). RVX prolonged (1683 s ± 811 vs 3588 s ± 812, P = 0.01) and diminished (RFU: 5.13x107 ± 4.20 vs 1.64 x107 ± 1.09, P = 0.004) TG. RVX did not alter ADP-induced platelet aggregation. These findings suggest RVX may indirectly inhibit platelet activation by modulating TG. Clopidogrel and dual therapy data generation is ongoing.

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Student Support: National Institutes of Health T35 OD010956

Roles of strain-level differences in Rickettsia rickettsii in vectoring in Arizona and the surrounding region

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The recent unprecedented spread of Rocky Mountain spotted fever (RMSF) in Arizona and surrounding areas has been linked to a novel vector, Rhipicephalus sanguineus (brown dog tick). Since the initial outbreak of RMSF in Arizona in 2003, there have been persistent cases from year-to-year and, in some areas, a fatality rate nearly seven times higher than the national average. Historically, transmission of the causative agent of RMSF, Rickettsia rickettsii, has been associated with two primary tick vectors: Dermacentor variabilis (American dog tick) and Dermacentor andersoni (Rocky Mountain wood tick). While R. sanguineus is the vector in the Arizona region, it has not been identified as a vector of RMSF elsewhere in the US, even though it is present in every state. We have previously identified three genetically and morphologically distinct sub-populations of R. sanguineus across Arizona. Here we examined the prevalence of R. rickettsii in each of those tick sub-populations and looked for variation between ticks collected from locations across Arizona, New Mexico, California, and Mexico. In R. sanguineus tick samples that initially tested positive for R. rickettsii using the highly conserved rOmpA gene, rickettsial intergenic genes were amplified and sequenced to determine strain-level differences across these regions. These sequences were concatenated with rOmpA and rOmpB for phylogenetic analysis. The variation in strains of R. rickettsii may affect the ability R. sanguineus to act as a vector in the Arizona region, but not elsewhere in the US.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program
The effect of TULP4 on behavioural phenotype of Mus musculus

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Tubby-like proteins (TULPs) have been identified as having roles throughout the nervous system, especially during development. *Tubby-like protein 4 (Tulp4)* is the newest member of the vertebrate tubby-like gene family and its function in mammalian species has not yet been fully characterised. While it is a distant member of this family, it is hypothesised that nervous system compromise of an unknown nature may occur as a result of Tulp4 malfunction. To assess the extent to which these animals are able to function neurologically, a series of behavioural and vision tests were performed on a cohort (n = 64) consisting of wild types, heterozygotes and knockouts. Behavioural tests consisted of open field testing; an olfactory assay; novel object; socio-sexual recognition; sociality; familiarity; plus maze; activity wheel; and, distance vision testing. TopView behaviour analysing system was used to assess position of the mice throughout the test and atypical behaviours were coded using the novel software Glitter, developed at Cornell university. Due to the COVID-19 pandemic, the timeline for data collection and processing has been altered, resulting in an incomplete data set and lack of statistical analysis at this time. Current data has revealed a hyper-aggressive and hyperactive phenotype with a consistent predisposition to circling behaviours. Further study will focus on identifying distribution of gene expression in the brain in the view of determining the neurological aetiology of these observations with the potential for functional imaging in future.

Research Grant: Unknown
Student Support: None

T-cell loss and repopulation in the intestinal mucosa following severe ischemic injury

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Intestinal ischemia accounts for over 200,000 annual deaths in humans. Severe disease is due to extensive loss of epithelial cells that disrupts the mucosal barrier and results in bacterial translocation into the systemic circulation. Epithelial regeneration depends on intestinal stem cell (ISC) proliferation. Other mucosal constituents contribute to epithelial regeneration including a subset of T-cells that have been proven to stimulate proliferation of ISCs during homeostasis. However, T-cells’ involvement with ISCs following intestinal ischemia has yet to be studied. Preliminary data showed significant loss of mucosal T-cell numbers following acute ischemic injury and that these cells reappear 48 hours after injury, a time point that we found coincides with peak ISC proliferation. This project aims to create a timeline of T-cell repopulation following a severe ischemic injury to explore the potential role of T-cells during recovery. We hypothesize that ischemic injury induces an acute loss of T-cells that repopulate and home to the ISC niche during early regeneration. To test the hypothesis, 8-10 week-old pigs were subjected to 3 hours of reversible mesenteric vascular occlusion. Animals were euthanized on days 0-3 post ischemia. Intestinal loops were collected at the time of euthanasia from injured and uninjured control jejunum. Immunofluorescence for CD3+ T-cells and Ecadherin+ epithelial cells was used to quantify cell numbers. The lamina propria was divided into three zones: crypt base, crypt neck, and the villus. Ten zones per section were evaluated. Further research will aim to differentiate between subtypes of T-cells (CD8+, CD4+, TCRγδ) and investigate potential signaling between T-cells and ISCs.

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Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070
Analysis of SARS-CoV-2 antibody ELISA test components to assess assay variation: a systematic review

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Antibody tests against SARS-CoV-2 are necessary to 1) confirm infection, 2) stage disease progress, and 3) allow surveillance of population immunity status. An understanding of the components of these tests is essential for reliable test interpretation. The goal of this study was to perform a systematic review to determine the level of standardization between and amongst in-house and commercial COVID-19 ELISAs currently in the published literature. Using commercial software (Covidence, Cochran), 324 studies were assessed by two reviewers, with 31 ultimately retained in the review. Components of test methodology were extracted from included studies when applicable, including format, isotype, test antigen, test sample dilution, patient characteristics, and validation including background antigen controls and test cut-offs. We found few components that were consistent, including sample serum dilution and the use of recombinant SARS-CoV-2 proteins. Most commercial ELISAs used the same serum dilution while for in-house tests there was more variability. Recombinant proteins were used exclusively as test antigens including the S1 domain of the spike protein, the receptor binding domain (RBD) of the spike protein, and the nucleocapsid (NCP) protein. Sensitivity and specificity were variable even when comparing the same test between studies, and a wide variety of cut-offs were used when interpreting results. These discrepancies can contribute to poor test result interpretation, leading to misinformed decisions regarding the spread of SARS-CoV-2.

Research Grant: Fern-Audette Professorship Endowment
Student Support: Florida Veterinary Scholars Program

Economic impact of removing arrival metaphylaxis in the U.S. stocker and feedlot industry

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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.

Research Grant: Foundation for Food and Agriculture Research (FFAR), Association of American Veterinary Colleges (AAVMC), Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction
Student Support: Foundation for Food and Agriculture Research and Association of American Veterinary Colleges
Cardiopulmonary effects of IM alfaxalone-dexmedetomidine-hydromorphone combination in dogs for orchiectomy

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Resources in field and shelter medicine are often limited. Injectable anesthesia provides a short anesthetic period and eliminates the need for expensive machinery. This study evaluated the effect of a singular intramuscular (IM) injection of an alfaxalone-dexmedetomidine-hydromorphone (ADH) combination for anesthesia in dogs undergoing orchiectomy. Eighteen shelter-sourced male intact mixed-breed dogs (6mo-5yrs) were scheduled for elective orchiectomy. Alfaxalone (1.0 mg/kg) was administered IM with dexmedetomidine (15 mcg/kg) and hydromorphone (0.15 mg/kg) to induce anesthesia. Dogs were administered a rescue dose of intravenous propofol mean ± standard deviation (SD) (0.44 ± 0.98 mg/kg) to deepen anesthetic depth during surgery. Atipamezole (75 mcg/kg) was administered IM to reverse dexmedetomidine at 25 minutes post IM administration of anesthesia. Recovery was timed and qualitatively scored. Patients were monitored during surgery for: heart rate (ECG), respiratory rate (capnography), SpO2 (pulseoximetry), pEtCO2, invasive systemic arterial blood pressure, and esophageal temperature every 5 minutes, starting immediately prior to anesthetic induction and ending at time of complete recovery following atipamezole administration. Arterial blood samples were collected for blood gas analysis throughout the surgical procedure. Observed cardiopulmonary effects were minor across individuals and bradycardia was likely due to administration of dexmedetomidine; there was an approximate 40-50% reduction in heart rate throughout the surgical procedure. Hypertension resolved to baseline blood pressure within 10 minutes. ADH combination provided appropriate surgical anesthetic depth for approximately 46.11 ± 17.12 minutes.

Research Grant: Korea Institute of Science and Technology (KIST); BioNet America Inc.; and WesternU CVM Office of Research (Small Grant CVM06)

Student Support: WesternU Scholar

Trans-synaptic tracing reveals functional synaptic competence in maturing hPSC-derived retinal neurons

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Synapse formation in human pluripotent stem cell (hPSC)-derived retinal neurons (RN) has been supported by immunohistochemical and ultrastructural evidence in organoid cultures, and indirectly through electrical recordings and visual function testing following transplantation. However, direct evidence of functional hPSC-RN synaptic connections, which is crucial to understanding the mechanism of action for photoreceptor replacement therapy, has not been definitively established. Here, we describe a high-throughput monosynaptic retrograde viral synaptic tracing workflow to screen for the presence of functional synapses in vitro within hPSC-RN cultures. Glycoprotein-deleted rabies virus (RaV) RaV-mCherry and lentiviral-GFP vectors successfully labeled pre- and post-synaptic retinal neurons, respectively, in dissociated retinal organoid cultures. Functional synapses, as demonstrated by the transmission of RaV-mCherry between neurons, were identified. Synaptic transmission was significantly greater ($P = 0.009$) in experimental cultures than in control cultures, and functional hPSC-PR synapses represented approximately 17% of synapses identified. Although overall synaptic formation rates of hPSC-PRs were expectedly low, this system represents a platform for high-throughput testing of methods to improve hPSC-PR synapse formation in vitro.

Research Grant: Research to Prevent Blindness, Retina Research Foundation, McPherson ERI Trout Chair in Eye Research, Carl and Mildred Reeves Foundation, NIH U54HD090256 to the Waisman IDDRC, and NIH U01 EY027266

Student Support: UW-SVM DVM/PhD Program, NIH U24 EY029890, and NIH/NEI F30 EY31230-01
Examination of paratenic hosts of *Angiostrongylus cantonensis*

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*Angiostrongylus cantonensis*, also known as the rat lungworm, is an emerging pathogen in human medicine in the United States and a parasite with a rapidly increasing geographic distribution. A severe, and sometimes fatal, disease known as eosinophilic meningitis is caused by accidental contamination or ingestion of *A. cantonensis* infective larvae in humans. This can be through the mollusk intermediate host or paratenic hosts. This parasite has become an important public health concern as the cases of human infection expands globally. Literature discussing paratenic host species, location, method of larval identification and potential paratenic hosts were examined. *Angiostrongylus cantonensis* infective larvae were positively confirmed in amphibians, fish, reptiles, flatworms and crustaceans from Southeast Asia, Pacific Islands, the southern continental United States and Hawaii. Larvae detection and identification were completed using morphological traits, tissue analysis, loop-mediated isothermal amplification (LAMP), polymerase chain reaction (PCR) and serological tests. Compiled results document many paratenic hosts for *A. cantonensis* and a growing spread to subtropical regions.

**Research Grant:** None  
**Student Support:** Boehringer Ingelheim

Development of a comprehensive assessment of the risk of *R. rickettsii* exposure to people living in Arizona

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Rocky Mountain spotted fever (RMSF) is a zoonotic, tick-borne disease caused by *Rickettsia rickettsii* and is highly virulent in humans, if left untreated. In 2003, *Rhipicephalus sanguineus* was recognized as the primary RMSF vector in Arizona, in contrast to the common *Dermacentor* spp. vectors. Cases of RMSF in the Southwest United States and Mexico have continually risen over the past few years. Even more concerning, Mexico has reported at least 4000 cases in humans with a mortality rate approaching 40% since 2008. The aim of our research is to develop a comprehensive assessment of the risk of *R. rickettsii* exposure to people and canines living in Arizona, based on a combination of individual dog, population level, and environmental factors. This has been accomplished through multiple techniques, including a focus on the tick vector, the rickettsia, and microclimate modeling, and a focus on the canine itself. Here we report the results of an analysis of canine serum and whole blood taken from dogs across Arizona, California, New Mexico, and parts of Northern Mexico. Serum analysis of 376 samples resulted in 83 positive titers for anti-*R. rickettsii* antibodies. In Arizona, Gila and Hopi counties have historically high human case rates of RMSF and were found to have canine seroprevalences of 62% and 63%, respectively. Our data also reveals that when Arizona canine seropositivity is plotted against corresponding human case rates from each county, there is an exponential correlation ($R^2 = 0.87$). This suggests that the canine host is a major contributing factor, independent of the tick vector itself. This information informs disease modeling and provides new data to help predict and prevent future RMSF outbreaks.

**Research Grant:** REAP Award and Midwestern University Student One Health Award  
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Effect of CpG supplements on teleost resistance to *Yersinia ruckeri* infection

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Aquaculture is now leading the world for seafood biomass produced. The primary species produced in Canada is *Salmo salar*, Atlantic salmon. One common diseases for these fish in freshwater is Enteric redmouth, which is caused by infection via the bacterium *Yersinia ruckeri*. Currently, there are both vaccines and antibiotics available for preventative and post infection treatment, however, their success rates have been varied, costly, and the few antibiotics have generated microbial resistant strains. This study looked to examine the effectiveness of using CpG oligodeoxynucleotides as an immunostimulant to prevent the consequences of infection. Salmon were divided based on feed type and exposure status. CpG ODN was administered with feed orally for a month prior to infection. Post infection mortality was higher in the groups exposed to the CpG ODN feed (*P* = 0.001). Condition scores between the two feed groups before and after infection was not significant (*P* = 0.42). Clinical signs from mortalities included visceral, external, and ocular hemorrhage along with liver congestion and did not differ between groups. CpG ODN are known to elicit an immune response through binding to Toll-like receptor-9, activating and contributing to maturation of dendritic cells. *Y. ruckeri* is known to strongly stimulate inflammatory signals leading to a polarized Th1 lymphocyte response, and it is likely that CpG inclusion further exacerbated this response without a subsequent Th2 homeostatic response as seen in protective responses of vaccinated trout and salmon. Further research should be done to confirm this hypothesis and to determine adjuvants/stimulants which can stimulate the activation of Th2 cells for better protection against ERM.

Research Grant: unknown
Student Support: AVC Veterinary summer research award

The use of computational (*in silico*) tools to predict pathogenicity of missense variants in the horse

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Many equine phenotypes have a hereditary component. A challenge in determining the genetic causes is filtering through variants to distinguish those of clinical significance from benign variants. While null variants result in loss of gene function, missense variants do not always alter gene function. Computational (*in silico*) tools are often used to assess the likelihood that a missense variant is pathogenic (disease causing). However, these *in silico* tools were developed and tested on human genetic variants. Our group previously tested several of these tools on a dog data set, but no data exists for other veterinary species, including the horse. The objective of this study was to determine the performance of seven *in silico* programs (MutPred2, Panther, PhD-SNP, PolyPhen, Provean, SIFT, and SNPs&GO) on the pathogenicity prediction of equine missense mutations. Preliminary results show that the most sensitive programs are Polyphen (93%) and Provean (87%) but this came at a loss of specificity (72 and 78%, respectively) and accuracy (77 and 80%, respectively). The most specific program is SNPs&GO (88%), which was also the most accurate (86%). The poorest performing program is Panther, which had the lowest results for specificity (63%), accuracy (67%), positive predictive value (41%), and Matthews correlation coefficient (0.37). Results for MutPred2 are pending. In conclusion, these *in silico* programs can be used with a varying degree of confidence to predict the pathogenicity of equine missense variants. The best and worst performing programs for equine data were similar to those for canine data.

Research Grant: None
Student Support: University of Minnesota, College of Veterinary Medicine, Office of Graduate Programs
Assessment of the association between NEFA and BHB in dairy cattle during early lactation

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Dairy cows experience a negative energy balance period as part of a normal peripartum period. During this period, cows mobilize body reserves, including fatty acids from the adipose tissue, to support milk production. This adaptation increases the concentration of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) in blood. These metabolites have been associated with a decreased reproductive performance and milk production when in excess in the bloodstream (NEFA, ≥ 0.7mEq/L and BHB ≥ 1.2mmol/L). The objective of this study was to investigate the correlation between NEFA and BHB when measured at the same time or subsequent days, and to determine the association of NEFA and BHB with milk yield and composition at first test. Plasma samples were collected from dairy herds in Minnesota at 3 ± 1 and 7 ± 1 days postpartum. Pearson’s correlations were calculated between both metabolites at the different time points. The overall correlation between NEFA and BHB during the first week postpartum (including 3 and 7 days postpartum) was 0.37 (95%CI: 0.31, 0.43; n = 797). The strongest correlation was observed between NEFA and BHB at 3 days postpartum (r² = 0.48; 95%CI: 0.39, 0.55; n = 378). At day 7 the correlation was 0.41 (95%CI: 0.32, 0.50; n = 420). NEFA on day 3 was associated with increased fat corrected milk (FCM), unlike BHB. FCM increased 0.877 pound for every 0.1mEq/L change in NEFA. Percentage of milk protein decreased 0.045% (95%CI: -0.072, -0.018) for every 0.1 mEq/L increase in d7 NEFA. A decrease in milk fat percentage was observed when both BHB and NEFA (d3 and d7) increased (P < 0.05). These results suggest a weak correlation between NEFA and BHB post-partum and NEFA is associated with differences in milk composition.

Research Grant: Ajinomoto Corporation, Inc
Student Support: Veterinary Summer Scholars Program

Validation of a novel technique to measure rotational instability of the canine stifle

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Cranial cruciate ligament disease (CCLD) is the most common cause of stifle lameness in large breed dogs. Loss of function of the CCL causes translational and internal rotational instability (RIS). While translational motion between the tibia and femur is relatively easy to measure, RIS has proven more difficult and can be confused with tibial torsion (TT). The most common surgical procedure, tibial plateau leveling osteotomy, addresses translational instability, but is not designed to address RIS. Stress compression radiographs do not directly measure rotational instability in CCL injuries. The purpose of this study is to develop and validate a dynamic radiologic technique that quantitates the degree of rotational instability in the stifle joint. We produced a positioning device by importing 3-D CT scans of a Labrador Retriever’s hind limbs into Computer Aided Design (CAD) software and then building the device around those images. This radiolucent device is 3-D printed with Formlabs Tough Resin consisting of adjustable rails and clips, a graduated ring over the stifle and a carbon rod connected to a digital torque meter. It maintains the position of the thigh while applying a standardized torque on the tibia. We will then test our method on 4 hind limbs, obtained from 2 large dog cadavers. We will assess RIS and tibial torsion (TT) in normal limbs and those surgically altered to create RIS, TT or both. All limbs will have standard radiographs and CT scans performed in neutral position and with internal tibial torque applied in a standardized fashion using a novel positioning device.

Research Grant: None
Student Support: Western University of Health Sciences Intramural Fellowship and CVM Summer Research Fellowship
Invasive trophoblast cells of the equine placenta: experts of immune response evasion

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Trophoblast cells make up the outermost layer of the mammalian placenta. The equine placenta has a unique type of trophoblast: the chorionic girdle cells that invade into the endometrium to form the eCG-secreting endometrial cups. Studies of these cells have shed new light on immune recognition and tolerance of the fetal-placental unit in pregnancy. Maternal and paternal major histocompatibility complex class I (MHC-I) antigens are expressed on the surface of the chorionic girdle and the endometrial cup trophoblasts between day 30 and 50 of gestation. The expression of MHC-I antigens stimulates a strong cytotoxic antibody response by day 60 of gestation in almost 100% of mares carrying MHC-incompatible pregnancies. Despite this fierce humoral immune response, the fetus is not rejected, perhaps because of a simultaneous dampening of cellular immune responses in the mare. Trophoblasts of the chorionic girdle can survive, differentiate and maintain their eCG-secreting function after transplantation to ectopic sites in non-pregnant recipient mares, demonstrating that their ability to resist maternal immune attack is inherent in their physiology and not determined by the hormonal state of pregnancy. It is clear that equine invasive trophoblasts are recognized by the immune system of their host; however, they have a way to subsequently dampen the immune response to ensure their survival. If we can understand the exact mechanisms of their immune response evasion, we could possibly find new strategies to limit allograft rejection caused by MHC-incompatibility. The fascinating aspects of equine placentation discovered through these studies highlight the value of fundamental research on domestic animal species.

Research Grant: None
Student Support: None

Evaluation of CXCL1, S100A8, MMP8, and TNF as biomarkers of Mycobacterium tuberculosis infection in humans

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The disease active pulmonary tuberculosis (TB) is caused by the infectious bacterium Mycobacterium tuberculosis. According to the World Health Organization (WHO), M. tuberculosis infects 2-3 billion people across the globe including 13 million in the United States alone. Unfortunately, there are not currently any blood-based, or serum biomarker, diagnostic tests that can differentiate between active pulmonary TB and latent tuberculosis infection (LTBI). Utilizing specific serum biomarkers as diagnostic tools to differentiate LTBI and active pulmonary TB can benefit a large variety of patients infected with Mycobacterium tuberculosis. Diagnostic tests using serum biomarkers can aid in the surveillance and maintenance of LTBI, and help these patients avoid unnecessary antibiotic use. This can help prevent the adverse effects of prolonged antibiotics and limit the development of antibiotic resistance. Patients with active TB will be benefit by receiving a faster diagnosis and treatment. Our pilot studies show that MMP8 has potential as a serum biomarker to differentiate between active tuberculosis infections, latent infections, and healthy patients while CXCL1 does not appear to have significant differences. Further testing will determine if S100A8 and TNF are potential serum biomarkers to differentiate active TB from LTBI.

Research Grant: NIH NHLBI R01 HL145411
Student Support: Boehringer Ingleheim Veterinary Research Scholars Program
Investigating the impact of climate change on leatherback sea turtle hatchling development

Courtney A. Marquette, Debra L. Miller, Jeanette Wyneken

Endangered leatherback sea turtle (Dermochelys coriacea) hatchling development could be negatively affected by climate change. The goal of this study is to examine the effects of temperature and precipitation on hatchling size. Straight carapace length, straight carapace width, and body weight were parameters chosen based on results of past studies and the ability to collect these measures in both live and dead hatchlings. Measurements from live and dead hatchlings from 2019 and 2020 nesting seasons were included. Live hatchlings were recovered from nests protected by predator exclusion cages, and dead hatchlings were recovered during nest excavation after emergence of live hatchlings, or after dying during or shortly after emergence. Full necropsies were performed on the dead hatchlings, but only the external measurements were included for this portion of the study. Data loggers placed inside of nests recorded the corresponding nest temperature every 15 minutes for the duration of incubation time. Precipitation data was collected from local weather stations. We expect that increased incubation temperature correlates with decreased hatchling size. Conversely, we expect that increased precipitation lessens the effects of increased temperature and contributes to more adequate development. Preliminary results of 90 live and 42 dead turtles suggest a correlation with increased temperature and decreased straight carapace length, straight carapace width, and body weight. This study is on-going and is expected to offer insight about another threat leatherback populations face, in addition to accidental catches and plastic pollution.

Research Grant: Royal Caribbean Cruises, Ltd. 4Ocean
Student Support: Boehringer Ingelheim Veterinary Scholars Program (Marquette)

Sexual dimorphism in the forelimb myology of the snow leopard (Panthera uncia)

Shelby Marsh, Samuel Levy, Rebecca Hassur, K.E. Townsend, Heather F. Smith

Snow leopards (Panthera uncia) live in the rugged terrains of Central Asia. With only 5,000 mature individuals left in the wild, snow leopards are notoriously difficult to research and classified as vulnerable by the International Union for Conversation of Nature. Currently, little is known regarding anatomical differences between sexes of P. uncia. In this study, two specimens of P. uncia (one male and female) were dissected. Forelimb myology was documented, muscles mapped, and quantitative data collected. Male snow leopards generally exhibit greater bone mass and more extensive muscle attachments that are useful for male-male competition and situations of increased aggression. Despite the larger body mass of our P. uncia male specimen (39 kg versus 36 kg), most of the forelimb muscle weights of the male were comparable to the female, indicating that the male forelimb muscles were relatively smaller compared to overall body size. We also observed interesting differences in muscle morphology. In the male, m. rhomboideus pars cervicalis was stouter, more robust than m. rhomboideus pars thoracis, and had an expanded origin comparatively, allowing for a greater hold on prey or an adversary. M. triceps brachii caput longum and laterale were almost twice as long as the female’s, resulting in a stronger lever for extension of the elbow. The seven subdivisions of mm. pectorales in the male were organized from superficial to deep, versus eight subdivisions arranged from cranial to caudal in the female. The male’s mm. pectorales did not insert onto the lesser tubercle of the humerus and the muscle bellies were less fused to each other. These differences may be attributed to sexual dimorphism or individuality of the specimen.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Assessing microbiome data integrity from feline fecal samples collected at time points post-defecation

Rachel Mastin, Caity Nelson, Amie Burling, Craig Franklin

Veterinary Research Scholars Program (Mastin, Nelson), Department of Veterinary Medicine and Surgery (Burling), Department of Veterinary Pathobiology (Franklin), College of Veterinary Medicine, University of Missouri, Columbia, MO

The gut microbiome (GM) plays many prominent roles in the health of cats. It aids in the absorption and production of important nutrients, promotes development of the mucosal immune system and prevents intestinal colonization by pathogenic bacteria that may harm the host. Dysbiosis of the GM can be indicative of or result in disease leading to its increased scientific study through the analysis of fecal samples. When analyzing feline fecal samples, common practice is to collect samples at time of defecation; however this may not always be feasible with samples obtained outside of a controlled laboratory environment. As the linkage between the feline GM and disease becomes clearer, the analysis of fecal samples outside of a laboratory setting may become more common when diagnosing disease. This study will use next generation sequencing to compare GM composition at time of defecation and up to 8 hours post-defecation. The goal is to determine the maximum time post-defecation at which reliable GM data can be obtained. Fecal samples will be divided into 7 equal samples at time of defecation and subsequently frozen at 0, 1, 2, 3, 4, 6, and 8 hours post-defecation. DNA of the fecal samples will then be extracted using a DNeasy PowerFecal Kit, and bacterial 16srDNA will be analyzed. The richness, diversity, and composition of the GM of samples taken at various time points post-defecation will be compared to the samples taken at time of defecation. We predict that samples left out for longer than 3 hours will have a GM significantly altered from that of the sample analyzed at time of defecation and will therefore not reliably reflect the feline GM.

Research Grant: Franklin discretionary research funds
Student Support: University of Missouri College of Veterinary Medicine Office of Research

Studying the animal health impacts of climate change: a gap analysis

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The effects of climate change on human health have drawn extensive scholarly inquiry and governmental consideration since the formation of the Intergovernmental Panel on Climate Change in 1990. While many of the emerging climate issues facing humans, such as increased disease ranges, increased rates of extreme weather events, increased thermal stress, and changes in air and water quality, are essentially identical to those of animals, the impacts on animals have historically received little attention. The goals of this literature review are to elucidate how climate change effects are being discussed within animal health sectors and to supply context to determine areas that require action or investigation. Articles gathered from the search will be analyzed based on the animal type, the climate change impact, and the year of publication. As the effects of climate change become more pronounced, the problems facing domestic and wild animals will require more focus, particularly in areas at an increased risk of modification. The northern latitudes are already experiencing vulnerabilities due to climatic shifts and associated ecological impacts on animals. The second part of this project focuses on Alaska, which, according to the Fourth National Climate Assessment, has warmed at twice the rate of the rest of the United States since 1950. A survey was dispersed to Alaskan veterinarians focusing on climate change impacts on animals they serve and where they perceive gaps of knowledge and resources. The analysis and survey provide a baseline understanding of how the scientific community has focused on climate-related challenges to date and where we must direct focus in order to mitigate future instabilities.

Research Grant: One Health Institute
Student Support: CSU Veterinary Summer Scholars Program
Safety and tolerability of African Swine Fever Virus subunit vaccine candidates in commercial pigs

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African Swine Fever Virus (ASFV) is currently the greatest threat to the global swine population. It has decimated the pork industry in much of Asia and developing a vaccine against it is of the upmost importance. While previous studies have shown that protection can be conferred using attenuated ASFV, this method is not safe for the animals. Since attenuated ASFV can induce immunity, this study aims to identify the antigens responsible for conferring protective immunity to swine. Doing so will greatly aid the process of developing a safe and effective vaccine for protection of domestic and feral pigs. To do this, all the ASFV open reading frames have been used to generate multicistronic expression cassettes. This novel approach allows for a more manageable and realistic expression of the whole ASFV proteome in the adenovirus vaccine vector as well as clearer selection of antigens that reactive lymphocytes are binding to in the proteome. The genes encoding the multicistronic expression cassettes have then been cloned into a shuttle vector that allows transfer of the cassettes into a plasmid backbone that is then used to generate a weakened recombinant human adenovirus expressing the ASFV antigens. Once the viruses are assembled, they will be used to formulate an experimental vaccine for immunization of pigs. Following two boosts, three weeks apart, the pigs will be challenged with wildtype ASFV. Antibodies and T cells from survivors will be used to identify the ASFV antigens involved in stimulating protective immunity. Identification of the protective antigens will allow development of a safe and efficacious subunit vaccine for protection of domestic and feral pigs.

**Research Grant:** Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-25041 from the USDA National Institute of Food and Agriculture

**Student Support:** Elanco Veterinary Scholar

Pyometra management practices in the high quality, high volume spay-neuter environment

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Pyometra management in the high quality, high volume spay-neuter (HQHVSN) environment is not well documented. The goal of this study was to identify pyometra management practices and patient outcomes in the HQHVSN environment. An electronic survey was piloted and distributed using Survey Monkey via email lists and social media to HQHVSN clinics. A total of 118 responses were received from June-July 2020. Respondents were distributed across the United States. The majority of clinics were associated with a shelter (75%). Clinics performed a median of 25 spays and neuters per day and 5000 per year. Most clinics (86%) were willing to treat non-incidental pyometras at least sometimes, and nearly all of these clinics (97%) were willing to accept pyometra referrals. Clinics charging a fee (70%) charged a median of $200 for dogs and $125 for cats. Integrating pyometra surgery into the average surgical day was reported as moderately disruptive (12.3%), mildly disruptive (54.4%), or not at all disruptive (33.3%). No respondents indicated pyometra surgery as a major disruption. The majority of patients accepted for treatment were seen the same (50%) or next day (42%). Most clinics (95%) felt they had the resources to appropriately treat most pyometras and that the outcomes were favorable: 98% of the 76% presenting BAR/QAR and treated, 80% of the 20% presenting depressed/non-ambulatory and treated, and 38% of the 4% presenting obtunded/lateral and treated were estimated to survive. The majority of HQHVSN environments seemed willing and able to accept and treat pyometras, and may be a referral resource for private practices where treatment would be disruptive or for clients with financial challenges.

**Research Grant:** None

**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Minimally invasive deep digital flexor tenotomy in the equine limb

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Laminitis is a painful condition involving the delicate laminae of the equine foot that may be treated with medical, surgical, and farriery approaches, or a combination thereof. Tenotomy of the deep digital flexor tendon (DDFT) has been indicated in severe equine laminitic cases to reduce the forces of the DDFT on the coffin bone and decrease the breakdown of the laminae in the hoof. Previously described surgical approaches for DDFT tenotomy involve complete transection via invasive surgical techniques that may result in significant scarring of the surgical site, making subsequent tenotomy of the DDFT difficult or impossible. Other surgical approaches require entry into the flexor tendon sheath which may pose a higher risk for surgical complications. The aim of this study is to investigate whether a minimally invasive technique, modeled after human carpal tunnel and trigger finger tendon transection, may be feasible to perform a tenotomy of the DDFT in horses. Under ultrasonographic guidance, a thread will be percutaneously routed around the DDFT through 2 skin punctures using spinal needles in 20 cadaveric limbs. The tendon will be manually divided by a back-and-forth motion of the thread until the loop emerges from one of the skin puncture sites. Each specimen will be dissected and assessed for completeness of transection and iatrogenic damage via visualization. Descriptive statistics will be reported. We expect that this minimally invasive surgical approach of DDFT tenotomy will provide a feasible alternative with less scarring and shorter surgery time compared to other surgical approaches. Information gathered from this study will allow us to evaluate this technique for future use in clinical equine laminitic cases.

Research Grant: UW-Madison Companion Animal Fund
Student Support: NIH T35 OD011078

Human and animal host phylogenetic evolution of trimeric spike protein gene in SARS-CoV-2 clinical isolates

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SARS-CoV-2 is the virus causing COVID-19, which was first reported in Wuhan, Hubei Province, China in late December 2019. Studies have proposed that the origins of the virus may stem from local bat colonies, where betacoronaviruses possibly serve as a reservoir. Currently, SARS-CoV-2 vaccine efforts target the spike glycoprotein, which are important mediators in host cell receptor binding and cell membrane fusion. However, there is little information available concerning the relationship between the closest SARS-CoV-2 viral relatives spreading through animal populations. The goal of our project is to phylogenetically map the evolution of the SARS-CoV-2 spike protein, of human and animals infected with COVID-19. Understanding the relatedness of COVID-19 circulating animal strains of coronaviruses will assist studies of viral function and thereby contribute to vaccine development. GISAID global database interrogated and full genome sequences of 100 human SARS-CoV2 strains and all available strains isolated from animal species spanning January 2020 to June 2020, were downloaded for this analysis. Sequences were aligned to the S gene and trimmed using MEGA X. A phylogenetic tree will be constructed using clade specific human reference strains from Wuhan, China (original outbreak 11/01/2019-01/31/2020) and cross referenced against all available animal strains. BEAUti & BEAST software will account for time of divergence of animal SARS-CoV-2 and identification of amino acid changes that resulted in human host specificity. We expect that through understanding how zoonotic diseases are related to similar animal strains, we can better assist studies of viral function for vaccine development.

Research Grant: Unknown
Student Support: Unknown
Effect of microalgae, omega-3 and 6 fatty acid supplements on teleost resistance to *Yersinia ruckeri* infection

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*Yersinia ruckeri* is an important pathogen in salmonids responsible for causing enteric redmouth disease (ERM). While vaccines have been developed for ERM, they may not be effective against all biotypes or available in all regions. Additionally, many *Y. ruckeri* isolates are resistant to available antibiotics. Therefore, we are exploring diet manipulation as a mechanism to enhance the immune response to infection with this pathogen. Omega-3 and 6 fatty acids are known to have an impact on a variety of cells of the immune system, but their specific effects on the immune response to *Y. ruckeri* have yet to be defined. The aim of the present study is to determine the effects of omega-3 and 6 fatty acids as well as a microalgae supplement on the ability of salmonids to resist infection with *Y. ruckeri*. To do this, fish were exposed to *Y. ruckeri* and fed either a commercial diet, positive control with microalgae replacement of fish oil, a microalgal oil diet enriched with omega-3, or microalgal oil diet enriched with omega-6 fatty acids. The number of mortalities and occurrence of hemorrhage, external lesions and organ enlargement were recorded. Our results indicate that feeding a diet high in omega-6 fatty acids has a detrimental effect on the ability of fish to resist infection, where this group recorded the highest mortality (90.4% compared to 57.3% in the commercial group) and higher prevalence of many clinical signs of disease. The omega-3 rich diet and positive control performed comparably to each other, however, did not yield significantly different results compared to the commercial diet. The results of this study are important in furthering our understanding of how diet impacts the function of the immune system in fish.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program, Natural Sciences and Engineering Research Council of Canada’s USRA
Student Support: Veterinary Student Research Award Program—Atlantic Veterinary College

Developing parasitology-based teaching tools for use in middle school classrooms: a parasite’s perspective

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K-12 classrooms in many U.S. states are moving toward interactive teaching methods for academic standards in the sciences. This project is targeted toward teaching ecological relationships and types of symbiosis (relationships between species) using web-based tools. Here we present a novel approach for exploring parasite life cycles in middle school classrooms. The strengths of this approach include its interactive capacity and the nature of the parasite life cycle (e.g. as the parasite progresses through its life, it interacts with many species). We hypothesize that students will develop a deep understanding of ecosystems by learning about the relationship between species interactions and parasite life cycles. We aim to 1) evaluate the efficacy of web-based tools in the area of parasitology for teaching middle school science and 2) implement unique curricula wherein students learn about ecological systems from the perspective of the parasite. We use the web-based tools Thinglink and Tikitoki to develop interactive lessons built around parasite life cycles. Teaching plans have been developed into a curriculum that aims to impart a deep comprehension of ecosystems and symbiosis. Classroom implementation will involve student engagement with the lesson and activities to assess the students’ understanding of the material and ability to make relevant connections in the context of new scenarios. We have identified a middle school instructor in Larimer County, Colorado as a collaborative partner. Classroom implementation and testing of the curriculum is ongoing. Future directions include adapting materials to Spanish speaking or bilingual classrooms and developing the curriculum for varying levels of education.

Research Grant: National Center for Veterinary Parasitology (NCVP)
Student Support: CSU CVMBS Dean’s Office, Georgia Norris Scholarship
Characterization of an *Acholeplasma* species isolated from the Florida gopher tortoise (*Gopherus polyphemus*)

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Here we describe a novel *Acholeplasma* species isolated from the nasal passages of the environmentally threatened Florida gopher tortoise (*Gopherus polyphemus*). Clinical isolates (N = 11) isolated from tortoises representing 7 different geographical sites in north central Florida were characterized phenotypically and genetically. The isolates were resistant to penicillin, insensitive to digitonin lysis, produced acid from glucose, filterable through a 0.45 \( \mu \)m filter and did not require serum for growth. All isolates grew well in SP4 medium at a growth range of 30-37°C. On solid agar, all isolates showed a typical fried-egg colonial appearance. Molecular characterization of the 16S rRNA indicated that 10 isolates were grouped together and formed a cluster most closely related to *A. equipharyngis*. However, one isolate was genetically distinct from the other isolates, clustered with *A. hippikon* and likely represents a second species. Based on the isolation source history, phenotypic, and phylogenetic characteristics, we propose the name *Acholeplasma testudinis* sp. nov. for *Acholeplasma* sp. isolated from the nasopharynx of a gopher tortoise. *A. testudinis* is the first described *Acholeplasma* species isolated from a reptilian host.

**Research Grant:** Department of Infectious Diseases and Immunology  
**Student Support:** Florida Veterinary Scholars Program

Whole genome sequencing of animal coronaviruses

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Prior to the COVID-19 pandemic, many coronaviruses of both animals and humans have circulated in the US. Those of human and agricultural importance, particularly ones that shed in less complex sample types such as respiratory fluids, are very well characterized. Other coronaviruses that cause enteric disease are not as well characterized at the genetic level. With the new ability to obtain whole viral genomes from a variety of species using a PCR based enrichment strategy, we can now obtain full length genome sequences from sample types that have abundant host and microbial material such as feces and tissues. This new technique will allow us to better characterize the less well known enteric coronaviruses. In this review, we will outline the application of a sequencing approach based on the tiled amplicon sequencing protocol published by Quick et al. (2017) and Grubaugh et al. (2019) that is ideally suited for the more complex samples. In addition, we will identify gaps in our understanding of veterinary coronavirus diversity as well as establish priorities for further studies. Our review and method will be included in an invited chapter in the second edition of the book Animal Coronaviruses (Springer Protocols Handbooks).

**Research Grant:** Leadership T35 AI007227  
**Student Support:** Leadership T35 AI007227
Trematodes of birds in Canada: a literature review

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The Synopsis of the Parasites of Vertebrates of Canada series has been a widely used and useful information resource for parasitologists, wildlife biologists, and veterinarians since the publication of the first volumes in 1986. An important omission from the series on avian parasites was an issue devoted to the trematodes of birds. Trematodes are parasitic flatworms that have complex lifecycles and infection of wild bird species is relatively common. The primary aim was to summarize and integrate previously reported studies of trematodes present in birds across Canada in order to publish a volume in the monograph series “Parasites of Canada.” The avian trematode volume will list all trematodes (arranged taxonomically) reported in birds in Canada with additional information about the organs parasitized, and the province it can be found. Ninety papers were found using CABDirect, Google Scholar and PubMed with search terms including: trematode(s), fluke(s), helminth(s), platyhelminth(s), avian, bird(s), Canada, and each individual province. Data collected included the trematodes and birds scientific name, province location and organs parasitized. The literature review yielded a total of 338 parasites composed of 87 different genera and 180 different species. Unique trematodes were found in individual provinces, but no provincial niches of trematodes within host bird species were noted. Ongoing surveillance of parasite burdens in wildlife is recommended in order to monitor trends (trematodes in specific birds and provinces, and new trematode species that may arise) as climate change and the introduction of new host and parasitic species through migration, may have an effect on the distribution of trematodes in Canada.

Research Grant: Unknown
Student Support: AVC Veterinary Summer Research Award (VetSRA)

Age estimation in Greyhounds using pulp chamber to tooth width ratio

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In veterinary medicine, there is currently no accurate method for age determination of canine patients after adult tooth eruption. An accurate means to determining age in canines could significantly affect the health care future of many dogs of unknown age who are receiving dentals. The frequency and affordability of dentals are increasing rapidly, thus the number of dogs that could benefit from an equation which could yield age is suspected to be significant. The final adult teeth erupt at about 7 months of age, after which all age estimations are educated guesses at best. Kvaal’s method has been determined a successful technique to estimate age in humans, this method utilizes the pulp chamber to tooth width ratio in dental radiographs. This method was successful as it operated under the anatomical understanding that the pulp chamber width decreases relative to total tooth width as humans age; the same is suspected in canine patients. The greyhound population was selected because it has readily discoverable birthdates due to their racing history. The selection of one breed also allowed for limitation of variables that may be present between breeds. The pulp chamber to tooth width ratio of the mandibular canines and mandibular fourth premolars was then determined using the program J Image. The datum analysis is expected to yield a linear regression equation that will allow birthdate estimation after the eruption of adult teeth. An equation with any level of precision would provide the veterinary community with the only reliable mechanism to age adult canine patients. This study could be expanded to other breeds to determine if accuracy of pulp chamber to tooth width ratio age estimation has species-wide efficacy.

Research Grant: None
Student Support: The Ohio State University College of Veterinary Medicine Summer Research Project
Assessment of language style on autonomy and pet owner decision making

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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.

Research Grant: Boehringer Ingelheim
Student Support: Boehringer Ingelheim

Using survival analysis to examine antimicrobial resistance trends in canine Staphylococcus pseudintermedius

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Increasing antimicrobial resistance (AMR) and possible transmission of resistance genes from animal to human pathogens necessitates the examination of AMR in companion animal pathogens. Changes in the resistance patterns of Staphylococcus pseudintermedius species isolated from canine skin cultures (n = 1,170) from 2007 to 2017 were analyzed using reported minimum inhibitory concentration (MIC) data. MIC distributions were analyzed using survival analysis with growth inhibition as the “event” and antimicrobial concentration as “time.” A Cox proportional hazards regression was used to examine changes in probability of growth inhibition across a range of antimicrobial concentrations to compare MIC trends between years. Antibiograms using CLSI breakpoints show that the prevalence of resistance to antimicrobials is highly variable in S. pseudintermedius. There was a high prevalence of resistance to penicillins with 41 to 89% of isolates exhibiting resistance in a given year while there was a lower prevalence of resistance to cephalosporins (0 to 42%). Overall, resistance to rifampin had the lowest prevalence (0 to 2.5%). Survival analyses provide additional detail of trends of resistance to antimicrobials through time because it can detect changes in the MIC distribution prior to a change in the prevalence of resistance. Differences in MIC distributions across years were observed for amoxicillin/clavulanic acid, ampicillin, cefazolin, cefpodoxime, cefotiofur, chloramphenicol, doxycycline, enrofloxacin, oxacillin, and penicillin. The ability to characterize MIC trends can improve resistance monitoring and antimicrobial stewardship.

Research Grant: None
Student Support: Boehringer-Ingelheim Match
Characterization of spatio-temporal resistance to EGFR-targeted therapy in triple negative breast cancer

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Epidermal Growth Factor Receptor (EGFR) overexpression is common in aggressive cancers, such as triple negative breast. EGFR overexpression and hyperactivation of the downstream MAP Kinase (MAPK) pathway can lead to uncontrolled cell proliferation, making this pathway a promising therapeutic target. Although EGFR-targeted therapies, such as erlotinib, are effective at inhibiting signaling through EGFR and MAPK, resistance inevitably develops in a population of cells. However, it remains unknown 1) whether resistance arises in single cells, groups of cells, or if single resistant cells can influence the response of neighboring cells to EGFR inhibitors, and 2) if growth factors present in the tumor microenvironment, such as IGF, HGF, and FGF, exacerbate resistance. To this end, we used live single cell imaging techniques to monitor activation of signaling through EGFR-MAPK in breast cancer cells using a reporter for MAPK signaling pathway component, ERK. By monitoring ERK activity we were able to track the activity of single cells within a population and perform spatio-temporal analysis of signaling in the presence or absence of EGFR inhibitor. Using this approach, we find that groups of cells exhibit resistance with similar ERK pulse magnitude and duration. These affects result in activation of downstream ERK target genes such as Fra-1 and Egr1 which are associated with proliferation, altered DNA damage response, and metastasis. Cells treated with FGF, HGF, and IGF exhibited minimal difference in pulsatility at their current dose rate compared to the Erlotinib-treated control wells, therefore, the tumor microenvironment may contribute less to resistance of EGFR inhibitors as compared to tumor-derived paracrine signals.

Research Grant: Startup funds provided by Ohio State Comprehensive Cancer Center.
Student Support: NIH T35 Training Grant T335OD012199

Foal outcomes from mares with colic during the gestational period

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Pregnant mares experiencing colic have many factors that could contribute to the survivability of their foal. Previous studies do not agree upon which factors contribute to foal survivability, including factors such as gestational age, presence of systemic inflammatory response syndrome (SIRS), anesthetic duration, and intra-operative hypotension or hypoxemia. The ability for mares to complete their gestation and deliver a healthy foal after colic is an important question asked by many owners and farm managers. The objective of this retrospective study is to determine what factors, if any, contribute to decreased foal survivability in the pregnant mare experiencing colic. We hypothesize that gestational age of 40 days or less will have decreased foal survival and that foal survival will be affected in broodmares experiencing more severe colic, particularly those demonstrating signs of systemic inflammatory response syndrome. Our study reviews cases from the last 9 years of pregnant mares presenting to Louisiana State University with colic. Data such as vital parameters, bloodwork, colic exam findings, operative reports, and development of SIRS was recorded and we are currently awaiting results from our statistical analysis. We hope to find statistically significant factors that contribute to foal survivability in pregnant mares experiencing colic.

Research Grant: None
Student Support: NIH B819
Analysis of AST data to direct antimicrobial stewardship in a veterinary teaching hospital

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Antimicrobial stewardship and appropriate antimicrobial use in veterinary medicine has the potential to directly impact both animal and human health due to the existence of shared bacterial pathogens between veterinary species and humans. Appropriate selection of first line antimicrobials for treatment of common veterinary diseases is critical to decrease development of antimicrobial resistance and relies on knowledge of both existing trends in the isolation of specific bacteria from certain disease processes as well as an understanding of the commonly encountered antimicrobial resistance patterns in those bacterial species. Development of a monitoring system for local antibiogram data has been encouraged as a tool to assist in monitoring these trends, yet minimal data exists in the literature related to development of these systems, particularly in companion animal species. In this study, we analyzed 4 years (2016-2019) of culture and susceptibility data from a single veterinary teaching hospital representing antimicrobial susceptibility testing (AST) of over 3300 bacterial isolates from companion animals. Analysis of this data has identified the most commonly sampled sites (urinary, skin, ear, eye, lower respiratory) and bacterial isolates (Staphylococcus sp., E. coli, Streptococcus sp., Enterococcus sp. and Pseudomonas sp.) resulting in susceptibility testing. Of the Staphylococcus sp. isolated, S. pseudintermedius comprised 70% of the isolates and was the most commonly obtained species overall; 47% of these isolates were considered methicillin-resistant. Antibiogram data compiled on these most common isolates will be utilized in improving the antimicrobial stewardship program for this hospital moving forward.

Research Grant: None
Student Support: NIH T35 Training Grant T335OD012199

Insights into antimicrobial resistance and virulence in Salmonella spp through genomic approach

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Non-typhoidal Salmonella spp. (NTS) are responsible for enteric infections in humans and animals. Considering the fact that reptiles carry and shed NTS through feces, the potential for exotic pets to harbor antimicrobial resistant NTS, a one health concern and needs to be addressed. To fight against the rise in antimicrobial resistance (AMR) and predict outbreaks using virulence matches in serovars from the environment and clinical isolates, we aim to characterize NTS isolates to determine the presence of indicators of virulence and confer AMR using genomic data. We sequenced the whole genomes of 9 NTS isolates from snake fecal samples submitted at Animal Disease Diagnostic Lab Purdue. Comparison of genome data for AMR and virulence gene determinants were performed among the 9 NTS isolates. Antimicrobial susceptibility testing to 17 different antibiotics were evaluated phenotypically according to the Clinical Laboratory Standards (CLSI) procedures. The genetic analysis indicated the presence of several virulence factors such as adherence genes, outer membrane proteins and secretion systems that are of clinical importance. The minimum inhibitory concentration report showed all 9 samples had identical AMR profile with resistance to aminoglycosides and first-generation cephalosporin. Genetic analysis revealed AMR genes conferring resistance to aminoglycoside antibiotics. This data provides insightful public health implications to mitigate outbreaks; gain better understanding of host-pathogen interaction and benefits microbial source tracking and prophylactic planning approaches for clinicians in the future.

Research Grant: Purdue University
Student Support: Purdue University
Spatio-temporal analysis of raccoon rabies animal cases in eastern North America

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In the U.S., raccoon rabies virus (RABV), a zoonotic pathogen, is endemic along the Atlantic coast from Maine to Florida. Limited studies have suggested that rabies disease dynamics could vary along a North to South gradient, as well as along an urban to rural gradient; raccoon ecology can vary by latitude or human population density. Therefore, we examined whether rabies case numbers vary according to latitude, anthropogenic development, and season. We predict that latitude will have an effect on the seasonality of rabies cases with northern ecoregions having greater seasonal clusters than in southern ecoregions. Additionally, we predict the number of cases will be higher in urban settings and occur more throughout the year than in more rural and suburban areas. Using data from 2006 to 2017 from selected US states within the defined enzootic area, we compiled monthly cases of raccoon RABV at the county level and then classified each county as either urban, suburban, or rural based on the US census definition. Using generalized linear mixed-effect models, data were analyzed to test for differences in case numbers between urban, suburban, and rural counties and whether these differences change latitudinally and seasonally. In future work, we will use a hurdle model to understand the role of covariates on two important processes that describe spatial disease dynamics: occurrence and prevalence given occurrence. Ultimately, by contributing to our understanding of rabies disease ecology, we hope to assist in the optimization of disease control techniques in the future.

Research Grant: University of Minnesota, College of Veterinary Medicine, Office of Graduate Studies
Student Support: None

Evaluation of differential gene expression in the canine FGF19 signaling pathway

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Fibroblast Growth Factor 19 (FGF19) is a hormone produced in the intestines of both humans and dogs in response to the presence of bile acids. FGF19 signaling in the liver inhibits bile acid production and promotes glycogenesis and protein synthesis. In humans, bolus feeding of a high fat diet changes gene expression patterns in the FGF19 metabolic signaling cascades. To investigate whether this is true in dogs with long-term high fat/high protein diet feeding, we will evaluate the gene expression profile dataset GSE22945 from 12 healthy beagle dogs fed a high fat/high protein, animal derived diet or a low fat, moderate protein, plant derived diet. The GEO2R platform will be used to compare the FGF19 associated gene expression pathways between the 2 groups.

Research Grant: None
Student Support: NIH T35 Training Grant
Challenges to IUCN guideline implementation in the rehabilitation and release of trafficked primates in Peru

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The rehabilitation and release of nonhuman primates after confiscation from illegal wildlife trafficking has implications for conservation, animal welfare, and public health. Risks associated with primate release include ecosystem disruption, inability of released primates to engage in normal foraging and social behaviors, and pathogen spill over. The International Union for the Conservation of Nature (IUCN) has several guidelines for the rehabilitation and release of confiscated primates to minimize such risks. Little is known about the use of these guidelines during primate confiscation, rehabilitation, and release or about the challenges faced by those who attempt to implement such guidelines in specific contexts. As one of the leading sources of Neotropical primate trade in the world, Peru has a primate population particularly vulnerable to the negative consequences of confiscated primate release. This study used semi-structured interviews and structured questionnaires of 19 people involved in primate confiscation, rehabilitation, and release in Peru and found that awareness and implementation of the IUCN guidelines are minimal. Opportunities to increase guideline implementation in Peru include expanding government involvement and support, adapting guidelines to specific contexts and locations, and establishing a platform for increased communication, cooperation, and research amongst those performing this work.

Research Grant: Westmoreland Primate Conservation Fund
Student Support: Westmoreland Primate Conservation Fund and SAVMA Travel Grant

Videofluoroscopic and manometric assessment of lower esophageal function in dogs with idiopathic megaesophagus

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Idiopathic megaesophagus (ME) is a poorly understood disorder characterized by abnormal dilation of the esophagus and carries a poor prognosis due to the high risk of aspiration pneumonia. A recent study of dogs with idiopathic ME identified incomplete relaxation of the lower esophageal sphincter (LES) via video fluoroscopic swallow studies (VFSS) in 70% of dogs, mimicking the syndrome of esophageal achalasia in people. Confirmation of esophageal achalasia can only be determined via high-resolution manometry (HRM) quantitative pressure analysis of the LES and esophageal body by passing a pressure-sensitive catheter transnasally into the esophageal lumen. This study aims to critically evaluate LES function in a cohort of dogs with idiopathic ME utilizing VFSS and HRM. Five dogs with idiopathic ME have undergone VFSS at UC Davis VMTH using liquid barium boluses and barium admixed with food of varying consistencies. Evidence of achalasia-like syndrome was characterized by a bird beak effect in 4/5 dogs, complete (3/5) or delayed (2/5) emptying of liquid boluses into the stomach, a mean height of the esophageal barium column of 4.2cm (range 2.4-6.4cm), and esophageal barium retention > 60 min post-procedure in 5/5 dogs. Primary esophageal peristaltic contractions were absent in all dogs with liquid boluses. These preliminary results are consistent with an achalasia-like syndrome and HRM is pending to confirm the syndrome. Classification of idiopathic ME via HRM will help facilitate surgical or medical interventions to improve LES function, decrease esophageal dilation, and reduce aspiration pneumonia.

Research Grant: Center for Companion Animal Health (CCAH)
Student Support: NIH T35 Training Grant T35OD010956
Do early life seizures chronically alter socially relevant rat ultrasonic vocalizations?

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Early-life seizures (ELS) are associated with Autism Spectrum Disorder (ASD); however, due to the lack of effective treatments for ELS, the role of ELS in ASD remains unclear. A core feature of ASD is deficit in social behavior. To isolate the impact of ELS on social communication, we probed the behavioral consequences of a single episode of kainic acid-induced early-life seizures (KA-ELS) in male and female Sprague Dawley (SD) rats. The rat is a social animal with a complex behavioral repertoire, including ultrasonic vocalizations (USV), which are a crucial component of rodent communication. Previous work has demonstrated that rats subjected to ELS display deficits in behaviour in response to socially relevant stimuli including USV’s and scent. As a means of further exploring this deficit, emission of USV’s during a social interaction task were recorded. The social interaction task permits social communication between animals in the presence of a protective barrier. Spectrograms, which provide a visual representation of animal USV’s, were utilized as a means of validating and deciphering complex USV characteristics, a novel method of assessing communicative content. Following confirmation of inter-rater reliability on a 14-category call schematic, USV recordings of ELS rats in a social interaction task were analyzed to determine potential deficits in expressive auditory communication. The results of these findings will not only be critical in the progression of the study of communication deficits associated with ELS but will also provide the groundwork for future studies which intend to measure complex USV characteristics.

Research Grant: Unknown
Student Support: Veterinary Summer Research Award

Hematologic reference intervals in cockatiels (Nymphicus hollandicus)

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Cockatiels are the most common avian companion in the USA with more than 20 million birds recorded in 2017. The purpose of the study was to establish hematologic reference intervals (RI) for cockatiels, explore age and sex differences, and assess parameters not previously reported in this species (plasma protein, fibrinogen and thrombocyte concentrations). Healthy cockatiels (60 males and 60 females, 2-11 years old) from the captive breeding colony at the University of California, Davis were included in the study. Blood samples were collected in K2EDTA tubes and erythrocyte, leukocyte and thrombocyte concentrations were determined via automated analyzer (ADVIA 120), hemocytometer and stained blood smear evaluation, respectively. Data was analyzed for outliers, distribution, descriptive statistics, and RI via Reference Value Advisor, a set of macro-instructions for Microsoft Excel. Females had significantly higher concentrations of total leukocytes, heterophils, basophils, lymphocytes, monocytes and plasma protein compared to males. Therefore, sex-specific RI were generated. A significant inverse linear correlation was noted between total leukocyte concentration and age ($P = < 0.0001$, $r^2 = 0.13$) and lymphocyte concentration and age ($P = < 0.0001$, $r^2 = 0.26$). Finally, lymphocytes were found to be the predominant leukocyte type. The reported RI are uniquely robust given the large number of animals included, the balanced sex and age distribution, and the stringent control over individual health status. The results of this study will strengthen the diagnostic power of the complete blood count in cockatiels.

Research Grant: The Richard M. Schubot Parrot Wellness & Welfare Program
Student Support: Walker/Musser Endowment Fund via Students Training in Advanced Research (STAR) at UC Davis
**Does weight loss affect tissue fibrosis in the obese murine mammary gland?**

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Obesity is a metabolic condition characterized by chronic adipose tissue inflammation which increases the risk of developing mammary cancer in women. Additionally, the obese mammary gland has increased adipose tissue fibrosis and immune cell recruitment and is associated with breast tumor progression and metastasis. Although it is recommended that obese women undergo a weight loss regimen to reduce their risk of breast cancer, the impacts of weight loss on the mammary gland are not well understood. We predict that weight loss will reduce mammary gland fibrosis and recruitment of two immune cell populations, macrophages and fibrocytes. Briefly, we will use a mouse model to examine the effects of weight loss on the mammary gland. Picrosirius red staining, to label collagen, will be coupled with immunohistochemistry to monitor changes in tissue fibrosis and immune cell recruitment. We generated an obese mouse model by feeding a high fat diet for 16 weeks, which resulted in significant weight gain in mice; we then induced weight loss by feeding a low-fat diet for 5 weeks. Collagen deposition was increased in mammary glands of obese mice relative to lean mice, suggesting that obesity contributes to increased mammary gland fibrosis, but the impact of weight loss on fibrosis remains unclear. Elucidating the relationship between weight loss and tissue fibrosis in the obese mammary gland will lead to a better understanding of obesity’s contributions to breast cancer progression and may guide future treatment recommendations.

**Research Grant:** NIH/NCI R01 CA227542  
**Student Support:** UW-Madison Food Research Institute

**BREATHE: better racing and exercise in air that horses enjoy**

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The link between air pollution and human health is well established; public advisories are issued when pollutant concentrations exceed acceptable levels set by the United States Environmental Protection Agency (EPA). Air pollution is an unquestionable threat to public health. However, the veterinary community has only recently investigated its influence on animal health. Equine athletes have exceptional performance abilities and respiratory requirements. They consume roughly ninety-two liters of air per minute at peak performance, thus making them uniquely exposed to hazardous air pollutants. In this study, we aim to identify the magnitude of effect of inhaled hazardous air pollutants - fine particulate matter (PM$_{2.5}$), ozone (O$_3$), and nitrogen dioxide (NO$_2$) - on equine race speed at three California racetracks. We selected 109 staked races throughout the race seasons at Santa Anita, Del Mar and Golden Gate Fields tracks from 2015-2019. We collected performance, meteorological and air pollution data from Equibase, National Oceanic and Atmospheric Administration (NOAA), and the EPA, respectively. Multiple regression analysis will be used to explore the relationship of air pollutants, weather, and performance parameters with equine speed. We hypothesize this study will demonstrate the utility in monitoring performance and environmental data, such that informed strategies can be implemented to mitigate occupational and welfare hazards to equine athletes.

**Research Grant:** None.  
**Student Support:** Colorado State University, College of Veterinary Medicine and Biomedical Sciences
Prevalence of within-group statistical comparisons in randomized controlled trials of dogs and cats

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Randomized controlled trials can test the effects of treatments on the outcomes by either between-group or within-group comparisons. In human clinical research, between-group comparisons are considered more appropriate than within-group comparisons due to regression to the mean, confounding of the outcome due to natural improvement over time and inflation of type I statistical errors. The purpose of the present study was to evaluate the prevalence of within group comparisons in veterinary literature and how often conclusions were drawn from these comparisons. All randomized trials published in 15 journals on dogs and cats in the years 2017 and 2018 were eligible for inclusion in the study. The RCTs were evaluated individually by two separate investigators for multiple factors pertaining to the outcome classification and statistical comparison used. After completion of the data extraction by each investigator, the results were then compared to reach a consensus. There were 3640 outcomes extracted from the 307 articles that were evaluated. Of the outcomes that had a statistical analysis reported, two third of them were between group comparisons (2083/3004; 69.3%, 95% CI: 67.7 to 71.0%), while one third of them were within-group comparisons (921/3004; 30.7%, 95% CI: 29.0 to 32.3%). There were 636 outcomes that were not statistically compared by either method. We found that within-group comparisons are frequently used in RCTs, though less common than between-group comparisons. We advise readers to be aware on how the authors reach their conclusions to make informed clinical decisions and recommend future research limit within-group comparisons.

Research Grant: None
Student Support: Oklahoma State University College of Veterinary Medicine

M. kansasii: evolution, diversity, and diagnostics

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Mycobacterium kansasii is a pulmonary mycobacterial infection that mimics M. tuberculosis, M. bovis and the M. avium complex pathologically and histologically. Distinguishing these infections is extremely difficult with current diagnostic techniques in cattle. Our lab has whole genome sequenced and assembled a M. kansasii isolate from a bovine granuloma. I aim to annotate the genome, and once complete, place it on a phylogenetic tree relative to other M. kansasii lineages, M. bovis and members of the M. avium Complex. With this information, I will search for regions in the genome that are unique for M. kansasii and could be targeted for more specific diagnostics. Furthermore, I will investigate virulence-associated regions and compare them to the MTB complex. In order to accomplish these goals, I will use the RAST server to annotate our genome. Selected genes and differentiating sequences will be pulled from our M. kansasii genome as well as from reference isolates, concatenated, and aligned with MEGA X. The alignment will be used for phylogenetic analysis as well as for highlighting regions of difference between species. Selected regions of difference will be examined and confirmed unique by BLAST, and potential diagnostic targets proposed from this pool.

Research Grant: Boehringer Ingelheim and the Graduate School at Michigan State University
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University
Prevalence of bacteremia in clinically healthy dairy calves

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Diarrhea is a leading cause of death in pre-weaned dairy calves, which is often a result of intermittent bouts of septicemia. In preliminary research focusing on heifer calves with diarrhea, 9.3% (10/108) of diarrheic calves and 14.8% (4/27) of clinically healthy calves were bacteremic. This finding may suggest that clinically healthy calves experience intermittent bouts of bacteremia due to high intestinal permeability early in life. Currently, there is no available research on the prevalence of bacteremia in healthy calves. The objective of this study is to determine if clinically healthy neonatal calves experience bouts of bacteremia. We hypothesized that younger calves would be more likely to experience bouts of bacteremia due to higher intestinal permeability when compared to older calves. Healthy calves were enrolled based on health scoring criteria including temperature, dehydration, navel score, fecal score, and depression. Aseptic jugular and saphenous venous blood samples were collected from the same calves at 2-7 days of age and again at 21-27 days of age. Isolates from positive blood culture bottles were evaluated with mass spectrometry to determine bacterial species. Our preliminary results show that the prevalence of bacteremia in healthy calves at 2-7 days of age is 17.4% (4/23). We expect that there will be a higher prevalence of bacteremia when calves are at 2-7 days of age when compared to 21-27 days of age, due to a higher likelihood of intestinal permeability. The significance of this potential outcome can lead to novel approaches for treatment and prevention of unrecognized bouts of bacteremia in calves.

Research Grant: None
Student Support: COBA/ Select Sires Summer Research Fellow

Anesthetic management and physiologic effects in pangolins: a systematic literature review

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Pangolins, the most heavily trafficked mammals on Earth, are underrepresented in the scientific veterinary literature. Anesthetic procedures have been performed successfully on pangolins; however, little information on their physiology while under anesthesia and comparative efficacy and safety studies of different anesthetic protocols have been published and remain anecdotal in nature. Pangolins’ unique, specialized morphology and physiology necessitate a much more species-specific approach to anesthetic procedures and monitoring. Their unique anatomy impede tracheal intubation, which in turn precludes securing a patent airway on a routine basis. Pangolins have lower metabolic rates and are subject to greater fluctuations in body temperature compared to most other mammals. A variety of injectable anesthetic protocols have been used in pangolins; however, the use of a gas anesthesia using chamber induction and maintenance using a fitted face mask is the preferred method of immobilization for minimally invasive procedures. Anesthetic monitoring can be especially challenging for pangolins, necessitating systematic evaluation of their physiological response while under anesthesia in order to empower clinicians to provide best-practices, evidence-based care for these animals during veterinary procedures. Given their conservation status and possible significance in the transmission and evolution of SARS-CoV-2, maintaining pangolin health while under human care is critically important. This review provides a foundation for wildlife and zoological centers to develop informed anesthetic protocols and highlights gaps in existing published reports where there is a need for future research.

Research Grant: None
Student Support: Mack Fund
A retrospective study of meropenem administered via regional limb perfusion for septic synovitis in horses

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Septic synovitis is life threatening in horses. Early therapy is key, with antibiotic therapy administered prior to sensitivity reports. Due to risk of bacterial resistance, meropenem may be used as initial therapy. Meropenem is a critically important antibiotic in human medicine, making veterinary use divisive. Therefore, verifying meropenem’s efficacy over other antibiotics used in intravenous regional limb perfusion (IVRLP) treatment is essential for human and equine medicine. Equine patient medical records from the last four years at a single veterinary teaching hospital were examined. Cases treated with meropenem or gentamicin via IVRLP for septic synovitis were retrospectively analyzed for demographics, diagnostics, treatments, and outcomes. Twenty-three meropenem (MER) and thirty-seven gentamicin (GEN) treated horses were analyzed; demographic information was similar between groups. In the MER group, nine horses received meropenem only; the remainder received another antibiotic initially then changed to meropenem. Structures included joints (MER = 13/GEN = 17), tendon sheaths (MER = 5/GEN = 8) and navicular bursae (MER = 2/GEN = 6). Overall survival to discharge was 86% (52/60), with MER 91% (21/23) and GEN 84% (31/37). Twenty-four of twenty-six bacterial isolates obtained from culture were reported as sensitive to imipenem. However, laboratory-reported susceptibility to other antibiotics such as ceftiofur (n = 22/26), ampicillin (n = 18/26), amikacin (n = 15/26), or gentamicin (n = 12/26) was also frequently present. Clinicians should be aware that while meropenem appears to be an effective treatment for septic synovial structures, less critical antimicrobials may also be a viable treatment option.

Research Grant: None
Student Support: Boehringer Ingelheim

A review on herpesviruses in Neotropical primates

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There exist 204 distinct species and subspecies of Neotropical primates, 21 of which are listed as critically endangered, 28 as endangered, 30 as vulnerable and 9 as near threatened. As these species continue to decline, exacerbation of infectious diseases, such as herpesviruses, have become an increasing threat. Much is known about the clinical manifestations, zoonotic potential and overall characterization of herpesviruses in Old World monkeys. However, there is insufficient data describing the effects and potential consequences of herpesvirus infections in New World monkeys, specifically Neotropical primates. This study aims to review and summarize the current literature on herpesviruses in Neotropical primates and identify areas of research that require further exploration to refine pre-existing guidelines for rehabilitation, conservation and herpesviruses management in these species. Preliminary findings show that herpesvirus infections are a common source of morbidity and mortality in these species and that they are unique due to their ability to transition between phases of latency and reactivation in affected hosts, integrate into host chromosomes and for their oncogenic potential. Furthermore, considering that herpesviruses usually manifest as mild or subclinical disease in their natural hosts, in contrast to fatal manifestations in non-natural hosts, the literature is lacking sufficient data to describe the potential consequences of inter-species viral transmission among Neotropical primates. This review will focus on the transmission, zoonotic potential, detection methods, pathogenicity, virulence, phylogeny, evolution and case reports of herpesviruses in Neotropical primates.

Research Grant: None
Student Support: NIH T35 Training Grant by Cummings School of Veterinary Medicine at Tufts University
Investigation of genetic variations associated with hereditary diseases in three wild felid species

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Multiple ocular coloboma in the Snow leopard, transitional cell carcinoma in the Fishing cat, and polycystic kidney disease in Pallas’s cat are all suspected to have a major genetic component. The wild populations of these felid species are either vulnerable or nearly threatened and the breeding populations maintained in captivity are critical to their survival. The exact genetic variants causing these disease processes are yet to be discovered. The aim of this study is to identify candidate genetic variants causing the health concerns in these three felids to help guide the captive management of each species, i.e. their species survival plan. The hypothesis is that each disease has a major genetic component and are likely to have autosomal recessive modes of inheritance. DNA from the wild felids has been submitted for whole genome sequencing, including a normal and affected fishing cat, an affected Pallas cat, and several normal and affected snow leopards. Sequence data analyses are underway to produce a variant call file to identify potentially causal variants. GoldenHelix Varseq software will assist the identification of likely variants. Causal variants are expected to be homozygous in affected cats and either heterozygous or absent in normal cats. These variants should also be absent in the 99 Lives dataset, which includes ~300 domestic cats and ~30 felids of various species. PCR will be used to amplify the candidate variants in the cat used for whole genome sequencing to validate their presence and other affected felids. The genetic variants can be tested in all the members of each felids species survival plan to help eradicate the health problems and to potentially monitor felids in the wild as well.

Research Grant: University of Missouri, College of Veterinary Medicine, Gilbreath McLorn Endowment
Student Support: Endowment by IDEXX-BioAnalytics

Imidacloprid interferes with ovarian antral follicle growth, gene expression, and steroidogenesis

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Following removal of organophosphorus pesticides from the agricultural market, neonicotinoid pesticides (NPs) became widely used worldwide. NPs are systemic neurotoxicants used to exterminate sucking insects, termites, and fleas. Their ubiquitous and rapidly increasing use results in large-scale environmental accumulation and chronic exposure of non-target species. It is unknown whether imidacloprid (IMI), the most popular NP, is an ovarian toxicant. This study tested the hypothesis that environmentally relevant levels of IMI interfere with antral follicle growth, steroidogenesis, and receptor expression. Antral follicles were dissected from ovaries from CD-1 mice (31-41 days old). Follicles were cultured in media treated with dimethyl sulfoxide (DMSO; vehicle control) or IMI (0.2 µg/ml, 2 µg/ml, 20 µg/ml, and 200 µg/ml). Antral follicle growth was measured after 48 h in culture; follicles and media were collected separately. Antral follicle RNA was used in qPCR reactions to quantify gene expression of steroidogenic genes. IMI (0.2 µg/ml) inhibited follicle growth, whereas IMI (200 µg/ml) increased follicle growth compared to control. IMI at various concentrations altered expression of the aryl hydrocarbon receptor (Ahr), both estrogen receptors (Esr1 and Esr2), pro-apoptotic and anti-apoptotic factors, cell cycle regulators and steroidogenic enzymes. These data suggest that IMI might follow a nonmonotonic dose response curve, as do many endocrine disruptors. IMI also interferes with cell proliferation, apoptosis, and steroidogenesis.

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Student Support: NIH T32 Training Grant T32ES007326
Role of macrophages in tissue repair in Acomys

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While blastema-mediated morphogenic regeneration is common in amphibians; mammalian wound repair is usually accomplished through repair characterized by scarring. One of several exceptions to this is the African spiny mouse, *Acomys cahirinus*, which regenerates completely after ear, skin and muscle injury. We performed a literature review to describe the interaction between macrophages and the tissue extracellular matrix in *Acomys* following injury. This review summarizes differences in extracellular matrix (ECM) protein composition, including collagen and fibrin, and protein expression compared with control species. Differences in regulation of cell cycle proliferation, ECM remodeling, inflammation and scar formation were also summarized. We also collate data on macrophage distribution and phenotype in *Acomys* and control species following injury. These studies provide further evidence that macrophage phenotype is intimately associated with regenerative capacity in various tissues, and positions *Acomys* as an ideal model to investigate the role of macrophage phenotypes in tissue regeneration in order to inform strategies to prevent scarring in other mammals and humans.

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Student Support: Leadership T35 AI007227 NIH

Genetic mechanisms of resistance to BTK inhibition in canine diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is a common, aggressive cancer affecting both humans and dogs, and effective therapies without toxicities are lacking. In a previous study, our laboratory performed a clinical trial to investigate the efficacy of BTK inhibition in canine DLBCL using the BTK inhibitor acalabrutinib. The objective response rate was 25%; thus, most patients demonstrated primary resistance. In the current study, we performed whole exome sequencing of canine lymphoma cells before and after acalabrutinib therapy to discover mutations in major drivers of B-cell cancers that could propagate cell survival and proliferation despite proximal BCR inhibition with acalabrutinib. We identified 242 unique variants affecting 202 different genes, of which 39 were high impact mutations and 203 were moderate impact mutations. On average, there were 21.7 variants (median = 21.5 variants). We found that four genes, *CSMD1*, *DDX3X*, *MYC*, and *SETD2*, were more frequently mutated in patients that achieved stable disease, while two genes, *FRAS1* and *ENSCAFG00000030258*, tended to be mutated in patients with a partial response to therapy. Six genes, *MAP3K14*, *CSMD1*, *DDX3X*, *POT1*, *SETD2* and *ENSCAFG00000030258* tended to be mutated in samples of patients that had received chemotherapy prior to initiating acalabrutinib therapy, compared with chemotherapy-naive dogs. However, due to the small sample size, these findings were not statistically significant, and further investigations must be performed to verify trends. These data will guide future investigations to determine how specific mutations and genes alter molecular pathways driving lymphoma cell survival and drug resistance.

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Student Support: American Veterinary Medical Association
SIRT5 inhibition causes increased oxidative stress and impairs tumor progression and metastasis

Irma Fernandez, Yashira Negron Abril, Jun Young Hong, Min Yang, Sushabhan Sadhukhan, Viviana Maymi, James Mullmann, Richard Cerione, Hening Lin, Robert Weiss

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The metabolic reprogramming that occurs in cancer cells creates dependencies that can be exploited for treatment. Our work establishes a new strategy to treat cancer by targeting a critical metabolic regulator, mitochondrial sirtuin 5 (SIRT5). SIRT5 is over-expressed in breast cancers and other malignancies, leading us to hypothesize that SIRT5 promotes cancer progression. We found that SIRT5 loss inhibits anchorage independent growth of human cancer cells. To examine how SIRT5 loss impacts tumorigenesis in vivo, we utilized MMTV-PyMT transgenic mice, which develop mammary adenocarcinomas and lung metastases. Sirt5 knockout (KO) MMTV-PyMT mice had increased survival, decreased tumor size, and delayed lung metastasis, as compared to Sirt5 wild-type (WT) MMTV-PyMT controls. Current studies are focused on the molecular mechanism by which SIRT5 promotes tumorigenesis and metastasis. SIRT5 KO cancer cells have reduced levels of important antioxidants such as NADPH and GSH, and higher levels of reactive oxygen species (ROS). These results indicate that SIRT5 KO cells experience greater levels of oxidative stress and suggest that SIRT5 could be promoting breast cancer by mitigating ROS. Importantly, pharmacological SIRT5 inhibition also impaired mammary tumor growth in both transgenic and human breast cancer xenograft mouse models. Considering that SIRT5 KO mice are generally normal, these data establish SIRT5 as a promising target for treating breast cancer.

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Student Support: NIH F30 CA250451

A retrospective medical record review of benign prostatic hyperplasia in a population of client-owned dogs

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Benign prostatic hyperplasia (BPH) is a disease of intact male dogs and men resulting in prostatic enlargement and leading to sexual and urinary dysfunction. Screening and diagnosis involve rectal palpation and diagnostic imaging. However unlike in men, screening, diagnosis, and treatment of BPH in dogs is inconsistent and understudied. Strong correlations between inflammation and symptom severity have recently replaced the historical dogma that prostate size is the main contributor to symptom severity in men. Correlation of prostate volume, bacterial infection, and symptom severity in dogs has not been addressed in current literature. We performed a retrospective medical review in a canine population that received gold standard screening and diagnostics, to better characterize BPH. Cases were assessed for prostate size, presence of bacterial infection, severity of symptoms, and response to treatment. We calculated expected prostate dimensions with the given weight and age to determine if there was enlargement. We also used a BPH severity score (Zambelli et al.) to quantitatively measure and compare severity of symptoms. We also describe a case series for those cases with follow up, to examine the effects of treatment on prostate size and BPH severity score. We expect symptom scores to be higher in aged dogs and those with bacterial infection. Preliminary data supports significantly increased symptom scores in presence of bacterial infection. Further characterization of BPH in dogs will improve examination of prostatic disease in this species and also the use of dogs as an animal model for human BPH.

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Student Support: NIH T35 OD011078
Barriers to veterinary care access and perceptions of video-chat appointments during COVID-19

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Although one can assume that the COVID-19 pandemic, which has forced veterinary clinics across the country only to provide essential services, has impacted access to veterinary care, there is currently no existing data to analyze this presumptive phenomenon. The present cross-sectional study uses a survey-based method to assess the veterinary needs of clients with low socioeconomic status during the COVID-19 pandemic. The survey gathers client perceptions regarding the potential barriers and incentives of synchronous telemedicine appointments at Tufts at Tech Community Veterinary Clinic as a method to address these disparities. The target sample size for the survey is 200 active clients. Participants were randomly sampled from a spreadsheet of active clients. Participants were either emailed or contacted via phone until the target number of 200 clients was achieved. The survey was made available in both English and Spanish. Clients who completed the questionnaire received a free examination credit on their Tuft at Tech account as a thank you for participation. Descriptive statistics will be analyzed and reported based on the findings from the survey. Findings will help to inform development of telemedicine services at Tufts at Tech in the near future.

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Student Support: PetSmart Charities, Tisch Student COVID Response (TSCR) Summer Program

Determining what narrative composition features foster antimicrobial stewardship

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Encouraging the responsible use of antibiotics is beneficial for the long-term healthcare of animals and people. Communicating about antibiotics and important specific issues like antimicrobial resistance (AMR) and antimicrobial stewardship (AMS) offers science communicators a variety of challenges, one of which is how exactly to design messages that might promote behavioral change and maintenance of actions relevant to AMS. In the present study, we examine one particular tool that has been identified as useful in science communication about complicated topics: narratives. Narratives refer, generally, to stories that people tell that exemplify personal experiences. Strategic use of narratives has been employed by science and health communicators in past work to promote behavior change. In the current study, we use an experimental approach to determine what narrative features (e.g., character details, story trajectory characteristics, narrator attributes) influenced people’s willingness to adopt or maintain AMS behaviors (e.g., responsible antibiotic use and disposal). The study combines human coding of narrative features with audience evaluations of individual narratives to determine what objective, modifiable narrative features contribute to differences in people’s willingness to engage in AMS. Additionally, the data will offer a test if narratives (overall) influence people’s AMS behavioral expectations more than non-narrative messages. The end result of the study will contribute to a growing evidence regarding how narrative communication can be used to reach various audiences about complicated scientific topics.

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Student Support: Iowa Livestock Health Advisory Council (ILHAC)
Examining differences in the microbiomes of shelter cats, foster cats, and cats in a stable home environment

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Veterinary Research Scholars Program (Nelson, Mastin), Department of Veterinary Pathobiology (Franklin), and Department of Veterinary Medicine and Surgery (Burling), College of Veterinary Medicine, University of Missouri, Columbia, MO

The gut microbiome (GM) plays a huge role in the health of animals and people, with differences in the GM being linked to susceptibility to gastrointestinal diseases, cancer, and even neurological disorders. The GM is susceptible to environmental influences with marked changes resulting in dysbiosis which can be associated with clinical disease. Cats are susceptible to multiple intestinal diseases which may occur due to a change in the environment and it is conceivable that changes in the GM may play a role in these clinical conditions. The objective of this study is to examine the GM of cats in multiple environments and identify the key changes in both the species and overall composition. To accomplish this, we are collecting feces at multiple time points from cats when in shelters, when entering foster homes, and when in a stable home environment. DNA is isolated and subjected to next generation sequencing to determine the richness, diversity, and composition of the GM. We expect to see key GM changes that will serve as a foundation for future studies on GM-based treatments of disease.

Research Grant: Franklin discretionary research funds.
Student Support: University of Missouri College of Veterinary Medicine Office of Research.

Hoof kinetic changes at the impact phase of the walk before and after routine trimming in horses

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Lameness is a painful and prevalent ailment in horses, impeding their athletic ability and quality of life. Hoof imbalance is a proposed component of lameness development in horses. Hoof pathologies affect the horse’s distal limb as well as proximal structures of the horse’s body, further contributing to lameness. Farriery and trimming of the hoof can help correct hoof unevenness and prevent injury, making farriery critical to equine practice. The act of hoof trimming is significant as it alters hoof confirmation, which can change hoof biomechanics. However, due to limited research on hooves, farriery has been primarily based on opinion and experience. Understanding hoof biomechanics is crucial to improving farriery and treatment of hoof pathologies. To broaden the knowledge of horse hoof biomechanics, it is important to understand the force and pressure exhibited by horses’ hooves. Pressure plates (PPs) are an accurate way to gather data on hoof kinetics. Studies have examined hoof kinetics at the midstance phase of the walk using a PP, but fewer studies have used PPs to examine hoof kinetics at the impact phase of the walk. Comparing the PP data at the impact phase before and after trimming provides information on how farriery can affect forehoof biomechanics. The objective for the study was to investigate the effect of routine hoof trimming on the forehooves at the impact phase of the walk. The data may increase current knowledge on impact phase kinetics in horses, which could help prevent impact phase related hoof pathologies and lameness. Preliminary results will be presented at the NVSS poster session.

Research Grant: Arabian Horse Foundation and Western University of Health Sciences
Student Support: Boehringer Ingelheim
Pilot study evaluating oral transmission of *Trypanosoma cruzi* in domestic cats

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Natural *Trypanosoma cruzi* transmission to domestic cats is suspected to primarily occur via ingestion of kissing bugs containing infective metacyclic trypomastigotes (MCTs); however, this route has not been empirically investigated. Here, we conducted two separate experiments assessing oral transmission of *T. cruzi* in laboratory-reared domestic cats. In the first experiment, we examined transmission by ingestion of kissing bugs fed previously on an experimentally infected cat (*T. cruzi* culture strain SYLVIO X-10). In the second experiment, we examined transmission by oral administration of *T. cruzi* in axenic culture. In the first experiment, two cats were inoculated via the intraperitoneal route with culture-derived ~20,000 MCTs; three weeks post-inoculation, 12 kissing bugs (*Rhodnius prolixus* nymphs) were fed on one parasitemic cat (as detected by PCR). Fed bugs were held for 69 days to allow development of MCTs in the digestive tract; six bugs were tested by PCR and three bugs each were fed to two cats. After ingestion of bugs, cats were monitored weekly for clinical signs and for parasitemia by cytology, hemoculture, and PCR. The bugs tested by PCR were negative for *T. cruzi* DNA. Cats that ingested bugs remained clinically normal, and parasite was not detected in circulation by any method for five weeks. These results suggest that, although susceptible to infection, cats may not be suitable reservoir hosts to feeding kissing bugs with the *T. cruzi* strain used. In the second experiment, the same cats were orally challenged with 2.5 X 10^7 MCTs in culture. The study is ongoing and results are pending. To the authors’ knowledge, these are the first experiments assessing oral *T. cruzi* transmission routes in domestic cats.

**Research Grant:** Oklahoma State University College of Veterinary Medicine  
**Student Support:** Oklahoma State University College of Veterinary Medicine

*OPA1* mutation and gender differences in retinal nerve fiber layer in rhesus macaques (*Macaca mulatta*)

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Dominant optic atrophy (DOA) is frequently the product of a genetic mutation in the *OPA1* gene that results in vision impairment in people worldwide. *OPA1* mutations affect mitochondrial networks, which disrupts protein functions in retinal ganglion cells. Currently, there are no large animal models of DOA that exist to our knowledge. Gene therapy and novel pharmaceutical interventions are promising strategies to treat DOA, and pre-clinical large animal disease models would accelerate these studies. Recently, we discovered non-human primates (NHPs) at the California National Primate Research Center (CNPRC) with *OPA1* mutations predictive of disease. Based on observations of retinal nerve fiber layer thicknesses (RNFLT) from human patients, including individuals with the *OPA1* gene mutation, it was hypothesized that circumpapillary RNFLT in Rhesus macaques (*Macaca mulatta*) will be reduced in *OPA1* mutants versus wildtype animals and wildtype males would have a lower RNFLT versus wildtype females. To test these hypotheses, we utilized 6 NHPs with *OPA1* mutations and 28 wildtype NHPs. These NHPs underwent ophthalmic exams that include a Heidelberg Engineering OCT-ONH Scan to measure RNFLT at 768 points equidistant from optic nerve head (ONH). Results of the ONH scans were analyzed using unpaired Welch’s t-tests. There were significant differences between the *OPA1* and wildtype groups in RNFLT (*P* < 0.05), where *OPA1* mutants had thinner RNFLT. Additionally, when compared against gender, significant differences in thickness between males and females (*P* < 0.05) were observed, where males had thinner RNFLT. Thus, NHP models of DOA will be useful to expand our understanding of this disease and test novel therapeutic interventions.

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**Student Support:** Student Training in Advanced Research (STAR) Fellowship
Survey development to address risk factors for feline oral squamous cell carcinoma

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Oral squamous cell carcinoma is the most common oral cancer in cats. Despite the lack of effective treatment options and poor prognosis, only one study has evaluated risk factors. Limitations of the study and lack of follow up since its publication 17 years ago suggest that the risk factors identified, secondhand smoke, canned feline diets, canned tuna, and flea collar use, as well as the potential for other risk factors, should be further investigated. Clay clumping cat litters pose a potential risk factor for feline oral squamous cell carcinoma (FOSCC) as they may contain crystalline silica, a type 1 carcinogen that has been associated with an increased risk of developing lung cancer in humans. In this study, an online survey will be sent to owners of cats with FOSCC or chronic renal disease diagnosed within the past 10 years. Environmental risk factors that will be assessed in the survey include exposures to different types of litter, food, flea/tick control products and secondhand smoke. The identification of risk factors will offer insight into preventative measures for cat owners. Multivariate analysis and relative risk ratios will be conducted to determine associations between examined factors and FOSCC. We expect to validate the findings of the previous study. In addition, due to the role that crystalline silica plays in lung tumor development, we hypothesize that cats exposed to clumping cat litter will also be at an increased risk for FOSCC.

Research Grant: None
Student Support: Boehringer Ingelheim

Development of an interactive equine neurology simulator to enhance veterinary student problem solving skills

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Perhaps the most important milestone for North American veterinary students is successful completion of the North American Veterinary Licensing Examination (NA VLE). This online, rigorous clinical decision-making test is required for students to obtain licensure to practice. Preparation and successful completion of the NAVLE is a major source of stress to veterinary students and continued development of educational tools to enhance student confidence and problem-solving skills are indicated. While fourth year veterinary students are required to complete clinical rotations that allow them to practice clinical decision making, the volume and type of cases seen on rotations are unpredictable. This is especially true in the large animal hospital at the Atlantic Veterinary College. Therefore, we propose development of an online interactive equine simulator for enhancing veterinary student confidence and clinical decision-making skills. Our pilot study will focus on skills related to equine neurology including anatomy, lesion localization, diagnosis, and treatment. This project will be completed in three steps: development of the online decision tree matrices, veterinary student feedback on simulator functionality, and a controlled study evaluating student performance before and after using the case simulators. We hypothesize that students would feel more competent and confident in clinical decision making after some practice using the online case simulator in which they can “choose their own adventure”. Additionally, we hypothesize that students who have used our simulator will have a deeper understanding and better recall of equine neurology compared to students who have not.

Research Grant: None
Student Support: AVC Veterinary Summer Research Award (VetSRA)
Non-canonical NF-κB signaling in lymphatic vessels regulates immune homeostasis in the lung

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Lymphatic vessels play a central role in immune homeostasis as they transport immune cells from tissues to peripheral lymph nodes (LNs) where immune responses occur. Emerging evidence indicates that non-canonical NF-κB signaling in the lymphatic endothelial cells (LECs) that line lymphatic vessels plays key roles in the development and immunological function of LNs. To study non-canonical NF-κB signaling in LECs in vivo, we generated mice conditionally lacking the critical non-canonical NF-κB kinase IKKα by crossing Ikkα<sup>F/F</sup> and Lyve-1-cre mice. Remarkably, the resulting Ikkα<sup>LYVE-1</sup> mice lack all peripheral LNs; however, we found peri-bronchiolar and perivascular accumulations of lymphocytes in the lungs of these mice. Analysis of these structures revealed compartmentalized zones of B cells, T cells and DCs together with stromal cell, lymphatic, and vascular remodeling. Altogether, these novel spontaneous bronchus-associated lymphoid tissues (sBALTs) strongly resemble inducible (i)BALT that occurs in response to infection (e.g. influenza) or inflammation and allergy (e.g. COPD, asthma). Notably, areas of the lungs free of sBALT appeared normal by pathological examination, had normal lymphatic vessels, and no cellular infiltration or edema. Moreover, mean linear intercept (MLI) analysis revealed that the overall integrity of the airways and alveoli was intact and undamaged. Our findings therefore establish that LEC-intrinsic IKKα regulates the normal immune homeostasis of the lung and that its loss leads to formation of highly organized BALT structures. Moreover, unlike the previously described pathology associated with iBALT in inflammatory diseases, sBALT in Ikkα<sup>LYVE-1</sup> mice forms in otherwise healthy and undamaged lungs.

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**Student Support:** NIH/BI Summer Veterinary Scholars Program

Myostatin deletion improves muscle function in a female obese mouse model

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Obesity is well characterized as being accompanied by cardiometabolic disease (vascular dysfunction, hypertension, diabetes, etc.). Exercise is the front-line defense against obesity, but many obese patients are unable to exercise at a level that conveys cardiovascular benefit. As such, it would be therapeutically valuable to find treatments that can mimic exercise and its accompanying cardiovascular benefits. Myostatin is a negative regulator of muscle mass that is upregulated in many muscle wasting diseases, including obesity. This study sought to determine the effect of myostatin deletion on obesity-derived cardiometabolic/muscle dysfunction in a mouse model of obesity (the db/db mouse). In adult female mice, myostatin deletion did not alter weight intragroup, but did improve glucose homeostasis in obese mice, as measured by HbA1c, fasting plasma glucose, and a glucose tolerance test (IGTT). Myostatin deletion also improved muscle function in obesity, with twitch force, tetanic max force, and force frequency all increasing compared to lean control. Interestingly, while myostatin deletion did improve overall force production in obesity, it did not improve fatigability. Taken together, myostatin inhibition in females may prove an effect mechanism for improving muscle function and cardiometabolic health in obesity.

**Research Grant:** None
**Student Support:** Boehringer Ingelheim
Systematic review of vaccination approaches to combat SARS-CoV-2 (COVID-19)

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The current pandemic of COVID-19 has stimulated massive efforts to develop vaccines against SARS-CoV-2. There are no approved antiviral therapeutic strategies or vaccinations to combat the virus, but several investigational vaccines have entered clinical trials, while other candidates are in preclinical stages. This systematic review aims to investigate how the vaccine candidates in development are expected to provide immunity to the host in different ways. The objectives of this review are to both analyze the vaccines in development to combat COVID-19, and to assess the different ways they aim to provide immunity against the virus. The search strategy for this systematic review contained three concepts to capture relevant references. The search strings used in PubMed and Web of Science databases encompassed these three concepts, which were COVID-19, vaccine, and immunity. The primary search, preformed on May 26th, yielded 2073 articles for primary title and abstract screening. After removal of duplicates and irrelevant studies 17 articles remained for review. Of the 16 pre-clinical trial studies included, seven used in-silico based vaccine technology as the study design. Four pre-clinical trials that were retained used animals to test potential vaccine candidates. This review indicates that there is limited peer reviewed information regarding the development of COVID-19 vaccines and their immune responses, indicating the possibility of various vaccines coming to market without peer review.

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Student Support: University of Florida College of Veterinary Medicine (FVSP)

To kill O. ophiodiicola: efficacy of terbinafine nebulization in infected snakes and in vitro antifungal drugs

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Ophidiomyces ophidiicola (Oo) is an emerging fungal disease of snakes that threatens wild populations in both Europe and eastern United States (US). The disease was first reported in 2006 in timber rattlesnakes in the Northeastern US and has since caused high morbidity and mortality in wild snakes, with diagnoses of Oo occurring in more than 30 species of snakes from a large geographic area. In May 2018, an Oo epizootic occurred in an Arizona reptile sanctuary several months after incorporating a large collection of snakes from North Carolina. Despite many reports of Oo infection and the growing list of susceptible species, treatment options are scarce and unverified. Our study aimed to evaluate the efficacy of terbinafine treatment in captive snakes naturally infected with Oo during the Arizona epizootic and to test the growth inhibition of select antifungal agents against Oo in vitro. We followed 13 snakes through diagnosis, treatment, and post-treatment sampling. At the onset of the epizootic, 23 snakes had skin lesions and were PCR positive for Oo or were highly suspected to have Oo infection based on the presence of dermatitis and Oo-positive cage mates. Of these, 13 snakes (56.5%) received treatment that included at least one round of 2-4 weeks of terbinafine nebulization. Seven snakes (53.8%) died during or after treatment and five remained PCR positive for Oo on postmortem skin. Currently, six snakes (46.2%) are alive, four of which are asymptomatic. Further research will evaluate the efficacy of terbinafine treatment for Oo infection in snakes and determine, through in vitro growth inhibition studies, if other antifungal agents may be better treatment options.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program
Identifying spatial drivers of avian influenza virus using true prevalence estimations

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Spatial and temporal patterns of wildlife diseases can be used to investigate underlying drivers of disease spread and to inform management decisions and disease surveillance plans to prevent infection of domestic animals. However, uncertainty in diagnostic tests used on wildlife and clustering of sampling in space and time can create a biased view if only apparent prevalence, calculated strictly from observed values, is considered. Bayesian statistical frameworks serve as a solution to biased apparent prevalence calculations, by allowing uncertainty in diagnostics and sampling to be incorporated into estimates of true prevalence. Here, wild migratory waterfowl were sampled for the presence of avian influenza virus (AIV) and multiple Bayesian statistical models were used to make true prevalence estimates for all times and locations. The model with the best fit to the data is selected using deviance information criterion (DIC) procedure, with the inference that the selected model best captures the underlying drivers creating the observed pattern of AIV. Out-of-sample model validation is used to validate how well the selected model reproduces observed patterns.

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Student Support: USDA Animal Health and Disease Scholar Program

Development of animal disease surveillance utilizing free-text electronic veterinary medical records

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Early detection through surveillance is critical for control and prevention of outbreaks caused by disease or foodborne illness. Veterinary electronic medical records (EMR), if strategically analyzed, could aid in animal disease surveillance. However, EMR data are often in an unstructured free-text format. The project objective was to develop a signal-detection algorithm that integrated medical knowledge for disease surveillance using EMR. We used 2017 to 2019 EMR of Purdue University Small Animal Hospital for this research. We searched for historical pet food recalls and disease outbreaks in companion animals during study period to compile a list of pathogens, chemicals and corresponding clinical signs. We investigated clinical signs and used an established vocabulary library to define search terms. We then used NVivo to convert the chief complaint data to quantitative measures using the search terms. The results were analyzed using the investigated algorithm and rules for signal detection. A total of 60,226 non-duplicate records were analyzed. Among the 11 defined search terms, search term pertaining to vomiting resulted in most consistent signals. Among 31 detected time periods, 55% were classified as plausible aberrations and 45% as plausible false negatives. Review of medical records found 11% errors from NVivo query with most due to irrelevance to the project objective. Development of a disease surveillance application involving free-text EMR is still a work in progress, however the potential it presents as an effective and robust tool should be recognized, and thus explored further.

Research Grant: None
Student Support: Purdue University College of Veterinary Medicine
Tumour grading and histopathology image analysis using artificial intelligence in veterinary pathology

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Grading schemes are objective tools used by pathologists to evaluate tumours and help predict their biological behaviour. They evaluate histologic features such as mitotic figure counts, degree of cellular or nuclear pleomorphism, and necrosis in order to predict the future behaviour of the tumour. For some tumours, grading systems are imperfect or are lacking in some form. Some suffer from intra- and interobserver variability, others lack consistent correlations between histologic features and outcome. Validation studies are limited in veterinary medicine due to small case numbers, limited or absence of follow-up data, and lack of standardized treatment regimes. Having validated and robust grading schemes for tumours enables better outcome prediction which ultimately allows for better individual treatment. In the past ten years, artificial intelligence and trained computer algorithms have gone from handwriting recognition to complex image analysis tasks. Computer algorithms can detect metastases, count mitotic figures, and detect areas of interest. This study is a literature review collecting available information to equip us to begin programming algorithms to help grading tumours which lack good grading schemes. Convolutional neural networks (CNNs), one of the most powerful image analysis tools currently available, are trainable algorithms with multiple layered functions. Pre-designed, ready to train CNNs are available both as open-source and as paid software, making them accessible for a grading scheme study. In subsequent stages of this project, the ability of a CNN will be explored to grade tumours of veterinary importance to improve prognostication and thus, patient care.

Research Grant: None
Student Support: Veterinary Student Research Awards (VetSRA)

Determining the cost-effectiveness of VacSIM through a swine flu market analysis

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Influenza A virus of swine, known as swine flu, is a significant respiratory pathogen due to being globally ubiquitous, having the potential for transmission from pigs to humans, and causing substantial economic losses to the swine industry. Current swine flu vaccines elicit low levels of efficacy and require multiple doses to induce high antibody titers. Vaccines on the market for this disease include FluSure XP by Zoetis, Sequivity by Merck, and Ingelvac Provenza by Boehringer Ingelheim. VacSIM is a slow immunization delivery method that enhances vaccine efficacy and host protection due to the gradual release of vaccine antigens. The goal of this study is to determine if the addition of VacSIM delivery to swine flu vaccines can improve their immunogenicity, providing a cost effective approach to mitigating industry losses. Data for the market analysis was collected from vaccine manufacturers and professionals in the swine industry. This information is currently being evaluated to determine how the costs of VacSIM, about $0.50/dose, will affect vaccine manufacturing revenue and the swine production industry. Experimentally, over 6000 doses of VacSIM have been administered to water buffalo and cattle in vaccine trials with no adverse events, showing evidence that VacSIM is safe for use in livestock. The VacSIM delivered Schistosoma japonicum vaccine elicits 56% protection against challenge in water buffalo and reduces fecal parasite egg output by 57%, significantly reducing disease transmission. These findings suggest that VacSIM has the potential to improve both the humoral and cell-mediated immunity of vaccinated swine while having the potential to reduce commercial losses to livestock producers.

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Bald eagle morbidity and mortality in the southeastern United States: a five-year retrospective

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Bald eagles (*Haliaeetus leucocephalus*) have historically been at risk of anthropogenic causes of mortality, most commonly through sources of trauma (e.g., collisions, electrocution, and gunshot). As both apex predators and facultative scavengers, bald eagles also frequently suffer from toxicoses, notably lead, anticoagulant rodenticides, and insecticides. However, published reports on causes of mortality in bald eagles in the southeastern U.S. are scarce. We retrospectively evaluated diagnostic findings of bald eagle cases submitted from 21 states (primarily southeastern) from January 2015 to June 2020 and categorized primary and contributing causes of mortality. Diagnostic findings included gross and histopathology and case-specific ancillary test results when performed. Among 269 bald eagles examined, non-infectious causes of mortality (213; 79.2%) were more common than infectious (20; 7.4%), with 36 (13.4%) that died of unknown causes. The majority (127; 47.2%) of noninfectious causes were attributed to trauma, most commonly blunt force (e.g., vehicular collision; 28). Seventy-five (27.9%) eagles died from toxicoses, including lead (44), anticoagulant rodenticides (18), and avian vacuolar myelinopathy (i.e., cyanobacterium toxin; 5). In addition, numerous toxicants were detected at low-levels in 110 birds but the significance remains unclear. Infectious causes were most often viral (10; most commonly, West Nile virus). This study demonstrates challenges faced by bald eagles in the southeastern U.S. and emphasizes potential impacts of human-associated activities on eagle populations. Further, the findings highlight the need better understand the significance of low-level toxicant exposure in bald eagle populations.

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Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

The interaction between *Cryptosporidium parvum* and mucosal immunity in calves

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Cryptosporidiosis is a common protozoal infection affecting neonatal calves in the cattle production industry. It results in watery diarrhea and has a high morbidity and mortality. However, healthy adult cows are asymptomatic and seemingly unaffected by the parasite. The mechanism behind the contrasting disease severity in neonates and adults is poorly understood. Consequently, there is no effective treatment or preventative for infection. A probiotic or vaccine would be of significant value to producers, as it would increase quality of life for calves and reduce costs associated with calf illness and loss. This research strives to further characterize the relationship between the host and *Cryptosporidium parvum*, in order to contribute to the long-term goal of a vaccine. The specific aim of this study was to determine the effect of *C. parvum* infection on intestinal epithelial cells. To accomplish this, we used bovine intestinal tissue from healthy adult dairy cattle slaughtered for human consumption at a local slaughter house. We then cultured these bovine enteroid cells and infected them with *C. parvum*. The last step is to measure and describe cytokine production using MILLIPLEX MAP for IFN-γ and IL-2 to assess CD4+ activity and Fas ligand expression using qPCR as a function of CD8+ activity. The results of this study will help provide insight into the adult intestinal immune response to *C. parvum* and guide development of a cryptosporidiosis vaccine.

Research Grant: None  
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Identification of parasites with metagenomic barcoding confirms microscopy and detects additional organisms

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Although microbiome research has led to an explosion of knowledge about prokaryotic communities and their influence on host health, a parallel revolution for eukaryotic communities has yet to be realized. The field of parasitology in particular would benefit from a metagenomic method for characterizing eukaryotes. We used a metagenomic barcoding approach to detect eukaryotic parasites in fecal samples using published pan-eukaryotic primers. After creating a validation set of parasites, we extracted genomic DNA, amplified a common barcode region (18S ribosomal RNA gene, hypervariable region V9), and deep sequenced the amplicon. This approach identified 100% (n = 6 helminths, n = 6 protozoans) of organisms in the validation sample, 75% (9/12) to the species level and 25% (3/12) to the genus level. We then repeated the process with samples from wild nonhuman primates where matched fecal samples had been previously characterized by microscopy in published studies, adding steps to enrich for parasite DNA. Results were analyzed with a combination of mothur, qiime, and custom python scripts using the SILVA132 database. The percentage of host reads ranged from 2.77% to 5.1% with mean = 3.79% and median = 3.45%. In one representative sample, 5.24% of filtered reads mapped to a commensal ciliate species found via microscopy and 4.42% mapped to a gut protozoan not identified by microscopy but expected to be present based on PCR-based diagnostics. This work demonstrates the potential of a metagenomic barcoding approach for identifying eukaryotic gastrointestinal parasites.

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Effects of intermittent hypoxia and obesity on breathing and central nervous system inflammation in mice

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Sleep apnea and obesity are on the rise in populations throughout the world. Extensive investigations show that both diet and the hypoxia caused by apneic events play a role in propagation of metabolic disease and sleep apnea, suggesting a complex reciprocal relationship between sleep apnea and obesity. Diets high in saturated fat (HFD) have been demonstrated to lead to a clinical condition called metabolic syndrome, which has been associated with the diagnosis of sleep apnea. Sleep apnea leads to intermittent reductions in breathing throughout sleep, which causes intermittent reductions in systemic oxygen tension (chronic intermittent hypoxia; CIH). Sources are conflicted on correlations between these two states, with some stating CIH worsens metabolic state and others demonstrating worsened apneic phenotype with HFD. Here we tested the hypothesis that high fat diet (HFD) leads to an increase in apneas during presumptive sleep, which is exacerbated by chronic intermittent hypoxia (CIH). Further, we examined the consequences of HFD and CIH on the development of central nervous system inflammation. Following six weeks of continuous high fat diet or normal chow, mice were exposed to either normoxia or CIH (90 sec 10.5% O2, 90 sec 21% O2, 12 hours per day) for 14 days. Breathing during the sleep cycle was measured using whole body plethysmography immediately prior to and following CIH, and analyzed for apnea occurrence. At the completion of the study, brainstem and spinal cord were collected for assessment for inflammatory gene expression. If our hypotheses are supported, these findings may have implications for the management of individuals with obesity or sleep apnea syndrome.

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Lessons learned from the Pamir Mountains: what conservancies can do to save endangered species

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The Pamir Mountains of Tajikistan are a harsh landscape home to livestock herders and several endangered species. Around the world, limited resources and grazing availability can lead to conflicts between marginalized communities and the surrounding wildlife, so viable solutions that balance the economics of herding and conservation interests must be found. In order to find such solutions in Tajikistan, we worked with three Pamiri villages to conduct herd health assessments and household livelihood questionnaires. Of the 155 questionnaires completed, respondents perceived the most important herd health issues to be parasitism (45%), specified etiologies such as foot and mouth disease (FMD) and brucellosis (34%), respiratory illness (21%), and neurological/ocular disorders (12%, n = 155 households). That being said, serological analyses found that only 0.9% of small ruminants had been exposed to FMD, and 0.9% to Brucella (n = 1077 samples), which differed vastly from herder’s perceptions. Perceived livestock rearing challenges include predation, as well as limited access to veterinary assistance, medicine, and livestock markets. Rearing livestock was the main income source for the villages, but many animal products (e.g. wool/hide) are underutilized. For these communities, the primary interventions we suggest are: improving access to veterinary care and medicine, implementation of an anti-parasitic regimen, and exploring income avenues for underutilized animal products. We believe that these strategies have the potential to maximize production and improve community livelihoods, without risking the survival of surrounding endangered wildlife.

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Student Support: Veterinary Investigator Program—T35 ODO010941 (NIH)

Investigation of canine cancer immunotherapies utilizing NK cells and tumor-targeting monoclonal antibodies

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Canine immunotherapies are not widely available and research to show their efficacy is in its early stages. Human immunotherapy studies are utilizing an innate immune cell population known as natural killer (NK) cells. These cytotoxic lymphocytes can target cancer cells by recognition of antibodies specific to tumor antigens. This process is referred to as antibody-dependent cell-mediated cytotoxicity (ADCC), and it is a key mechanism of action for many tumor-targeting therapeutic monoclonal antibodies (mAbs) in humans. ADCC occurs when the Fc portion of an antibody attached to a tumor cell is engaged by an Fc receptor on NK cells, leading to their release of cytolytic factors and anti-tumor cytokines. Our focus is on enhancing ADCC by canine NK cells to increase the efficacy of anti-tumor therapeutic mAbs. 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Analysis of infectious bronchitis virus survey throughout the southeastern United States

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Infectious bronchitis virus (IBV) is a gamma coronavirus that causes an economically significant upper respiratory tract disease in commercial poultry. There are many serotypes of IBV, and it mutates often leading to the emergence of more serotypes, making it difficult to control the disease via vaccination. For this reason, even though nearly every chicken is vaccinated against IBV, disease is prevalent in commercial poultry operations. In an effort to understand how prevalent variant IBV is in vaccinated flocks, a surveillance program was established in the Jordan lab. Southeastern poultry companies were asked to submit samples for PCR analysis. Samples were tested for all known IBV serotypes, and with a “generic” positive/negative test that could detect potentially new serotypes. The results were analyzed by what serotypes were found in each state, region, company, complex, and vaccine program used by the companies. Through the surveillance program, we found that nearly all samples were positive for virus by PCR. This was primarily vaccine origin, indicating that vaccines linger in flocks for much longer than previously expected. Non-vaccine origin viruses were also detected. These potential “challenge” viruses were of multiple serotypes including a group of unknowns. These unknown samples may be novel variants or mutations of vaccines that aren’t detected by the PCR assay, but these findings support the ideology that IBV mutates rapidly. Multiple vaccine programs with many different serotypes were used by companies submitting samples in this survey, yet non-vaccine origin viruses were still detected proving that IBV vaccines are very specific and do not offer full protection against heterologous viruses.

Research Grant: NIH T35 Research Grant
Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD01043314

Intranasal midazolam effective dose 50 and its effect on circulating corticosterone levels in zebra finches

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Sedation in birds is important for safe handling and minimizing stress. Intranasal (IN) midazolam is commonly used in birds for this purpose, but the minimum effective dose in zebra finches has not been determined. A previous unpublished Oklahoma State University study investigated the effects of 13 mg/kg midazolam on circulating corticosterone levels (cCORT), a marker of physiological stress. Unexpectedly, instead of decreasing cCORT, midazolam caused a relative increase in comparison to the placebo. The causes remain unclear, but we hypothesize that high dose of midazolam may induce the release of cCORT. The current study has two phases. The 1st phase’s objective was to determine the effective dose for 50% of the population (ED50) of IN midazolam using the Dixon Up-and-Down method. Each animal received a variable dose selected based on the response (sedation vs. no sedation) of the previous animal. A total of 14 birds were used and the ED50 was calculated as 1.67 mg/kg. The 2nd phase’s objective was to determine the effects of 13 mg/kg of IN midazolam (n = 10 finches), the ED50 (1.7 mg/kg, n = 10), and a saline placebo (n = 10) on cCORT. This was a masked, randomized trial. Blood samples were collected at T0 min, treatments were administered and animals were placed in a cloth bag for 20 min, and blood samples were again collected at T20 min. The samples will later be used to measure cCORT via ELISA. We hypothesize that cCORT will rise with 13 mg/kg of IN midazolam and with the placebo but decrease with the ED50. This study will help clinicians give a more appropriate dose of midazolam to zebra finches and will determine if midazolam decreases physiological stress.

Research Grant: Oklahoma State University (OSU) Veterinary Clinical Sciences departmental funds and the OSU Dr. Kristie Plunkett Exotic Animal Fund
Student Support: OSU Summer Research Training Program sponsored by Boehringer Ingelheim
Retrospective analysis of laboratory data as prognostic factors for survival in canine splenic hemangiosarcoma

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Hemangiosarcoma (HS) makes up 5-7% of malignant tumors in dogs and has a poor prognosis due to metastatic disease. HS originates from pluripotent endothelial cells and relies on angiogenesis for growth. Initial slow growth allows time for invasion of surrounding tissues and hematogenous dissemination. Visceral HS of the spleen or right auricle is more common than non-visceral HS, which develops in the skin or muscle and has a slightly better prognosis. Treatment of visceral HS with surgery results in an average survival time of 1-3 months, and with chemotherapy, survival time can be up to 6 months. However, reliable factors to help predict survival have been difficult to elicit. In this study, electronic medical records from cases of splenic HS in dogs presenting to a veterinary teaching hospital from 2010-2020 were analyzed to determine if signalment data, CBC, and serum chemistry values had a significant relationship to overall survival time and therefore could be utilized as potential prognostic markers. Twenty-three cases of splenic HS met inclusion criteria and were divided into three groups: G1, < 90 days survival; G2, 90-180 days survival, and G3, > 180 days survival. Kruskal-Wallis was used for group comparisons. As expected, presence of the tumor in multiple organs (multicentric) and gross metastatic disease were both significantly different between groups ($P < 0.5$). In addition, serum phosphorus levels were decreased in G2 compared to G1 and G3. This retrospective analysis supports the presence of metastasis as a useful prognostic indicator in canine HS. Other potential prognostic markers of HS in dogs are being analyzed, including IHC and microRNA-based markers using the archived biopsy samples from these cases.

Research Grant: None
Student Support: Boehringer Ingelheim, Purdue College of Veterinary Medicine

Proxy measurements of insulin sensitivity, glucose, and insulin concentrations in healthy neonatal foals

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While reference intervals for adult horses are vital in a clinical setting, they are often inappropriately extrapolated to neonates. The objectives of this investigation were to establish normal ranges for proxy measurements of insulin sensitivity, blood glucose (BG), and insulin concentrations in healthy foals, determine correlation with minimal model analysis, and differences between horses and neonatal foals. Blood samples were collected from healthy neonatal foals less than 72 hours in age and healthy adult horses. Reference intervals were calculated from BG and insulin concentrations while nursing and after a 1-hour fast. Proxies were calculated after a 1-hour fast in neonates and 12-hour fast in adults. Proxies were compared between foals and horses, and neonatal proxies were correlated with minimal model analysis in foals. BG concentrations in nursing foals and after a 1-hour fast were 209-236.3 mg/dL and 153.8-169.5 mg/dL, respectively. Insulin concentrations before and after fasting were 0 to 102.91 μIU/ml and 0 to 33.60 μIU/ml, respectively. Insulin to BG ratio (IG) and Modified Insulin-to-Glucose Ratio (MIRG) were significantly lower ($P < 0.0001$), and BG to Insulin Ratio (FGIR) was significantly higher ($P = 0.0007$) in foals compared to adults. Proxy measurements did not correlate to calculated indices of minimal model analysis (insulin sensitivity and acute insulin response to glucose) in neonatal foals, in contrast to studies in humans and horses. Results of this study show dramatic differences in energy regulation in neonatal foals, which indicate that further investigation of dynamic responses to exogenous insulin and glucose in foals is needed.

Research Grant: Ohio State Racing Commission OSU CVM Equine Intramural Funds
Student Support: OSU Veterinary Student Summer Research Program NIH T35
Survey of amyloidosis cases among different wildlife and zoo species

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Amyloidosis regroups a range of diseases (i.e. protein-folding disorders) characterized by a buildup of amyloid fibril deposits in one or multiple organs. The pathogenesis and pathologic findings of amyloidosis can vary widely due to the nature of the precursor protein that is highly prone to misfold and aggregate. In veterinary medicine, there are ten proteins known to form amyloid deposits in various organs. Amyloid deposits, when large enough, cause loss of structure and function of organs. There are no treatments currently available. This review aims to compare amyloidosis cases amongst different wildlife and zoo animals to determine which species are particularly susceptible to amyloid formation and which protein is commonly involved. Systemic amyloidosis, specifically AA amyloidosis, is most often reported in wildlife and zoo species. It is caused by serum amyloid A (SAA) protein, an acute phase protein, and may be triggered by chronic inflammation, endotoxin, or stress. AA amyloidosis may be transmitted between animals and between species according to studies involving birds, cheetahs, and Japanese quails. Transmission of AA amyloid occurs in a similar fashion than prions, through a seeding-nucleation process via feces or vectors that contain misfolded AA. It is relevant in institutions such as zoos, where multiple species are housed in close proximity, potentially increasing chances of transmission of AA amyloidosis. Additionally, this review includes guidelines for reporting single or multiple cases of amyloidosis. Learning more about the diversity, transmission, and pathogenesis of known amyloidogenic proteins and species prevalently affected may provide more insight into prevention and treatment of amyloidosis.

Research Grant: Unknown
Student Support: Funding for HP provided by NIH T35 OD016477 award to Michigan State University

Refinement of rat ultrasonic vocalization call characteristics and its application in assessing welfare

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Rats remain one of the most commonly used animals in research to date, however, optimal housing conditions for these animals are still debated. Recently, the Canadian Council on Animal Care recommended that all rats be housed in cages with sufficient space for vertical stretching, of which double level cages are the most common. Previous work has indicated that analysis of ultrasonic vocalizations (USVs) emitted by rats can be used as a non-invasive method to assess welfare of rats housed in different cage types, however, this was restricted to comparing call frequency (22 vs 50 kHz) distributions. Beyond these simple/broad categorizations, complex rat USV call patterns are beginning to be characterized and identified, which may provide further insight into the affective state of the rat. In this study, call classifications were refined to improve inter-rater reliability and correlation to calls occurring in juvenile Sprague Dawley rats. The refined call classification scheme was used to score recordings obtained over a period of 18 days from rats housed in “enriched” double-decker (DD) cages and rats housed in “standard”, single-level cages (SC). Call rates and frequencies were also assessed. Preliminary results indicate that more complex calls are associated with single-level cages than double-decker cages, and the frequency of calls varied over time and cage type.

Research Grant: None
Student Support: Sir James Dunn Animal Welfare Summer Student Research Award
**Phylogenetic analysis of poultry-associated isolates of type A *Clostridium perfringens***

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**BACKGROUND:** Whole-genome sequencing (WGS) of poultry-associated type A *Clostridium perfringens* isolates was utilized to identify pathogenicity loci, core and variable genes. The isolates were obtained from fecal, eggshell and fluff, and carcass rinse samples taken during a national poultry epidemiological survey. The WGS data were used for various comparative genomic analyses to identify similarities and differences among these strains according to ecological niche.

**RESULTS:** Evolutionary relatedness of strains was determined using genome hierarchical clustering based on functional assignments from pfam, COG, and KEGG databases. Clustering of isolates based on their ecological niche was robust and repeatable using the UniFrac approach in mothur. To subsequently identify differentially abundant genomic features according to ecological niche, several approaches were taken. First, abundance tables of pfams, COGs, and KEGG pathways for each genome were used to identify significant differences by niche with parametric and non-parametric statistical tests in R. Several genes were found that were differentially abundant in isolates from the three different ecological niches. Of particular interest were those generally over-represented in the fecal isolates associated with different glycosyl hydrolases, a recombinase, and a sortase. Next, UniFrac analyses of specific gene phylogenies confirmed significant phylogenetic clustering by ecological niche. In conclusion, WGS was able to identify significant differences in genomic features even among closely related *Clostridium perfringens* strains. Most of the genomic differences were related to carbohydrate binding and metabolism, and may influence virulence.

**Research Grant:** USDA NIFA grant, research pool  
**Student Support:** USDA ADH Research Scholarship

**Discovery of the male American alligator cloacal anatomy through 3D imaging reconstruction***

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Across the world, there are 23 species of crocodilians, including two species native to the United States. These two species are the American alligator (*Alligator mississippiensis*) and the American crocodile (*Crocodylus acutus*). In 1967, the American alligator was listed as an endangered species due to overhunting and habitat destruction, but through extensive conservation programs and controlled harvest throughout its habitat range, the species made a remarkable comeback and was taken off the endangered species act in 1987. Unfortunately, there is still habitat destruction of wetlands through drainage and development that threatens the American alligator to this day, which is problematic as these animals are a keystone species in the ecosystem of the southeastern states. To continue increasing the population of American alligators both in the wild and on captive farming operations, it is imperative that the reproductive anatomy, as well as, the physiology behind copulation is studied. Currently, there is limited knowledge on these subjects because the American alligator copulates underwater, making it difficult to study the physiology behind copulation and insemination. Similar to previous studies of other crocodilian species, the purpose of this study was to delineate the specific anatomy of the male American alligator cloaca and phallus. Determining the tissue make up of these reproductive structures helps shed light on the physiology behind copulation and may lead to improved reproductive management methods. Through magnetic resonance imaging, histological sectioning, and gross dissection, we reconstructed the male cloaca. Each tissue type was 3D reconstructed from the MRI using Seg3D imaging software.

**Research Grant:** Crocodile Specialist Group FHVS-SRAS Grant  
**Student Support:** Veterinary Research Scholars Program
Spontaneous background arrhythmia assessment in canine, swine, and non-human primates

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New compounds used to pursue advancements in drug therapy can be accompanied by life threatening side effects. Drug-induced arrhythmia, in particular, is a serious concern. The use of ECG telemetry analysis in preclinical studies to evaluate drug safety is an important process in the development of new medications. However, some arrhythmias occur naturally and can be influenced by diurnal effects, interindividual differences, and species-specific differences which confound these studies. To address this critical issue, we studied the incidence of spontaneous background arrhythmias in healthy canine, swine, and cynomolgus monkeys. Our goal is to improve the accuracy of pharmacologic and toxicologic drug studies by expanding our ability to discern background arrhythmias from drug induced arrhythmias. To accomplish this, retrospective analysis of continuous 24 h telemetry data from male canine (n = 12), swine (n = 12) and cynomolgus monkeys (n = 12) were analyzed for arrhythmias using Rhythm Express software (VivaQuant, LLC). Arrhythmia type, incidence, and time of day were identified and interindividual comparisons were made within and between species. We determined variability of ventricular arrhythmias, atrial arrhythmias, and premature junctional complexes for all three species and found indications of diurnal effects on arrhythmia incidence distinct to each species. Differences between individual of the same species regarding arrhythmia incidence were also found. Time of day and interindividual differences may play a key role in the future design of predictive preclinical drug studies. By understanding diurnal effects and normalizing arrhythmia incidence in pre-treatment individuals we can better evaluate risk.

Research Grant: NIH T35 OD016477 award to Michigan State University
Student Support: None

A review of African black-footed penguin (Sphenicus demerus) chick mortality at the Maryland Zoo

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The Maryland Zoo currently houses the largest colony of endangered African black-footed penguins (Sphenicus demerus) in North America and is actively involved in efforts to understand the factors that affect chick mortality in zoo-housed breeding colonies. The purpose of this study was to further classify and understand the pathologic processes contributing to African penguin chick mortality in a captive-bred colony. To conduct this retrospective analysis, pathology reports for African black-footed penguins under 3 years of age from 2006 to 2020 (N = 27) were obtained from the Johns Hopkins comparative pathology database. Chicks were sorted into age groups and general causes of death were assigned based on the major gross and histopathologic findings. Of the 27 chicks, 59% died before reaching fledging age (3 months) and 26% died within seven days of hatching. Causes of death included failure to thrive (26%), trauma (15%), respiratory disease (15%), gastrointestinal disease (11%), congenital defects (11%), avian malaria (7%), hemorrhage/blood loss (7%), myocarditis (4%), and CNS disease (4%). The chicks whose cause of death was failure to thrive (N = 7) underwent additional histologic examination to further investigate lesions that contributed to mortality. The findings of this study will provide a more thorough assessment of chick mortality at the Maryland Zoo and inform future efforts aimed at enhancing chick survival in zoo-housed breeding colonies.

Research Grant: None
Student Support: Boehringer Ingelheim
Expression profiling of proteins in the retinal pigment epithelium

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Age-related macular degeneration (AMD) is the leading cause of blindness in older adults in the developed world. While the pathological mechanism(s) has not been elucidated, converging evidence from multiple studies implicate mitochondrial dysfunction in the retinal pigment epithelium (RPE). The purpose of this project is to use a proteomic approach to identify protein changes associated with donors at progressively more severe stages of AMD. These results will provide novel insights into pathways and processes that are disrupted with AMD and offer clues to the disease mechanism. I initially optimized sample preparation of RPE cells from human donors for mass spectrometry (MS) analysis of RPE proteins. The initial experiments included biochemical assays to quantify protein yield after testing variables such as buffers and wash conditions. After optimizing conditions, we identified > 2400 proteins from the RPE using the established optimal conditions. To ensure reproducibility of our results, we performed seven technical replicates and found that the coefficient of variance between samples was 4.7% ± 5%. To provide an estimate of the biological variability, we performed MS analysis on seven samples from individual donors who had the same stage of AMD. When comparing the content of specific proteins between donors, results showed biological variability of 36% ± 27% between quantification of proteins. Having optimized the conditions, I processed 75 samples of donors with varying stages of AMD. Unfortunately, MS results were not usable due to an unknown polymer contamination that was observed in the spectra. Future experiments will entail identifying the source of polymer to avoid contamination in sample processing.

Research Grant: NIH-NEI RO1 EY026012, Anonymous Donor for Macular Degeneration Research, Helen Lindsay Family Foundation
Student Support: Boehringer Ingelheim Veterinary Scholars program, NIH-NEI RO1 EY026012-S (Diversity Supplement)

CWD outreach in Minnesota tribal communities using a community-based participatory research approach

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) that infects cervid species including deer, elk, caribou, and moose. CWD is similar to other TSE diseases including scrapie in sheep, bovine spongiform encephalopathy in cows and Creutzfeldt-Jakob disease in humans. CWD was discovered in Colorado in the 1960s and has spread to 26 US states, Canada, Norway, Finland, Sweden and South Korea. CWD was identified in Minnesota in farmed elk in 2002 and wild white-tailed deer in 2010. CWD continues to be identified in farmed and wild cervids across Minnesota, becoming endemic in wild white-tailed deer in southeastern counties. As CWD spreads, more communities are at risk of being affected by CWD, including seven Anishinaabe (Chippewa, Ojibwe) and four Dakota (Sioux) tribes. To keep human and cervid populations healthy, while maintaining traditional hunting practices, culturally appropriate wildlife disease outreach is critical. It is important to develop a relationship and engage each tribe to develop the most culturally appropriate CWD outreach. A community-based participatory research approach ensures synergistic partnership with community participation and researcher accountability. To lay the foundation for this multi-tribal CWD surveillance, management, and outreach project, we began by interviewing tribal biologists. Through these interviews we can assess the needs for CWD surveillance and management on tribal lands, as well as what CWD-associated outreach the tribes have had and the best practices for disseminating future outreach to coincide with surveillance. Results are forthcoming and will provide necessary background for future CWD outreach, surveillance and management within each tribe.

Research Grant: 2020 LCCMR Environment and Natural Resources Trust Fund Emerging Issues
Student Support: University of Minnesota, College of Veterinary Medicine Summer Scholars
Assessment of warming effects and dynamics of dairy methane emissions using a novel greenhouse gas metric

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Carbon dioxide (CO$_2$) and methane (CH$_4$) are two of the most important greenhouse gases (GHG) responsible for global warming. CO$_2$ accumulates in and continues to warm the atmosphere even if rates of CO$_2$ emission decline. In contrast, CH$_4$ is removed from the atmosphere after 10 years and does not accumulate. Under decreasing rates of CH$_4$ emission, more CH$_4$ is removed than is replaced, leading to a cooling effect. The climate impacts of cumulative pollutants such as CO$_2$ and short-lived climate pollutants (SLCP) such as CH$_4$ are often compared using Global Warming Potential (GWP), a metric that converts other GHG into CO$_2$-equivalent emissions. However, GWP has been criticized for its misrepresentation of SLCP dynamics, including its inability to capture the cooling effect of declining SLCP emissions. Because livestock GHG emissions are predominated by CH$_4$, accurate understanding of warming effects of animal agriculture currently, as well as under reduced methane emissions, is essential. A new usage of GWP, known as GWP*, has been developed to more accurately quantify CO$_2$-equivalent SLCP emissions. Using GWP* and GWP, we characterized historic CH$_4$ emission dynamics from dairy cattle in California and the USA to compare estimates by the two metrics, predicted future CH$_4$ emissions under Business As Usual and reduction scenarios, and modeled warming effects of these emissions scenarios. We found that GWP*-derived CO$_2$-equivalent CH$_4$ emissions reached zero under BAU scenarios and were negative under reduced CH$_4$ emissions scenarios. Cumulative GWP*-derived estimates also followed similar dynamics to warming effects profiles of CH$_4$ emissions, suggesting that GWP* is a more appropriate metric for quantifying SLCP in GHG inventories.

Research Grant: None.
Student Support: University of California, Davis School of Veterinary Medicine—Ritchie Hill endowment funds

Reliability and observer bias of qualitative behavioral assessment in dogs from commercial breeding kennels

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Subjective scoring methods such as Qualitative Behavioral Assessment (QBA) may inform welfare assessments, but observer bias may influence ratings. This study investigated: 1) whether QBA could be reliably used to score the emotional states of dogs from commercial breeding kennels (CBKs) and 2) the effects of knowledge about the dogs’ sourcing and empathy for animals on veterinary students’ interpretations of dogs’ emotions. Five observers experienced in dog behavior analysis and trained to use QBA terms scored 25 videos of dogs to assess inter- and intra-rater reliability. There was moderate to good agreement (0.40-0.80) across all terms and raters. Using an online survey, students at two Midwestern Colleges of Veterinary Medicine (n = 71) used the same 20 terms to score dogs from 8 videos and completed an Animal Empathy Scale (AES). Students were randomly assigned to informed/uninformed groups relative to being told that the dogs were from CBKs. The level of agreement on each QBA term was lower for students than for experienced observers (< 0.40 for 3/20 terms). Principal component analysis (PCA), used to reduce the QBA variables, extracted 4 components (PCs) explaining 69.2% of the variance. Regression models were used to investigate the effects of treatment group and AES on the 4 PC scores. Being informed about the source of dogs did not impact QBA scoring, but higher AES was associated with scoring dogs as being more sociable/explorative (PC1). Overall, QBA training and experience in dog behavior analysis improved scoring reliability. As in previous studies, empathy towards animals impacted scoring of animal emotions. The findings suggest that QBA scoring should be used cautiously to avoid bias in assessing welfare.

Research Grant: The Stanton Foundation
Student Support: Boehringer Ingelheim, Purdue University College of Veterinary Medicine
Disease prevalence among beef cows submitted for necropsy to a veterinary diagnostics laboratory

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There is currently a lack of data available that summarizes the prevalence of pathologic findings that may contribute to mortality of adult beef cows. The purpose of this study was to evaluate the prevalence of pathologic findings among beef cows submitted to the University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL) for necropsy. This retrospective clinical case study was conducted by reviewing necropsy data from reports submitted between 2017-2019. Cases were selected based on predetermined inclusion criteria, including female bovine, 24 months or older, and a nondairy breed. Both gross pathology and laboratory findings were recorded. All diagnoses were categorized based on system and pathologic agent type. All data were descriptively analyzed. To date, 300 reports have been reviewed and 57 cases have met the criteria for inclusion. Of the included cases, a total of 105 diagnoses were made with a median of 2 diagnoses per cow (range: 1-5). The median age of cows included was 5 years (range: 2-15 years). The most commonly diagnosed disorders were categorized as gastrointestinal (22/105, 21%), followed by respiratory (21/105, 20%). Overall, bacteria (35/105, 33%) were the most common agents associated with the diagnoses. These data may prove valuable for veterinarians treating beef cows in the state of Missouri and may assist them in diagnostics, treatment, and prevention recommendations.

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A service dog dyad approach to cultivating positive affect and reducing negative affect associated with PTSD

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Use of service dogs for veterans with posttraumatic stress disorder (PTSD) has gained popularity over the past few years despite little empirical evidence evaluating its efficacy. Traditional PTSD interventions, while effective for many, often have large drop-out rates in the veteran population. Previous literature has indicated that the service dog/veteran dyad can be used in conjunction with traditional interventions to assist veterans in improving their well-being and increase their likelihood to remain committed to their PTSD treatment. This study is intended to provide empirical data to evaluate a service dog’s potential effectiveness in improving overall well-being for veterans with PTSD. In order to understand well-being in the veteran population, emotion and affect were measured via ecological momentary assessment. We hypothesize that veterans paired with service dogs have higher positive affect scores and lower negative affect scores when compared to the waitlist group. T-tests were performed in SPSS to compare the affect and emotion scoring within each group and between groups. Future studies will use linear mixed effects models in SAS to analyze data. PTSD in the veteran population remains difficult to treat and the use of service dogs as a complement to traditional interventions may provide an effective tool to address affect and emotion of veterans with PTSD.

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Student Support: Boehringer Ingelheim, Purdue College of Veterinary Medicine
Evaluation of potential biomarkers in canine atopic dermatitis after treatment of canine mesenchymal stem cell

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Canine atopic dermatitis (cAD) is a chronic, recurrent inflammatory, and pruritic skin disease affecting 15-20% dogs globally. This skin disease is most prevalent and diverse in clinical presentation in dogs, with characteristics most-commonly associated with IgE-mediated hypersensitivity to environmental allergens. Its pathogenesis is associated with a complex of interactions and multifactorial disease involving immune dysregulation, skin barrier defects, environmental factors, genetic predisposition, and allergic sensation. Currently, all fast-acting symptomatic therapy, elimination of allergen, and allergen-specific immunotherapy is often associated with serious adverse effects. Recently, a human clinical phase I/II study demonstrated that subcutaneous delivery of allogeneic MSCs improved human AD without serious adverse effects. In this study, we examined subcutaneous delivery of allogeneic adipose-derived canine MSCs to treat cAD. 15-patients were enrolled and randomly grouped into three groups: a placebo group, a low-dose MSC treatment group, and a high-dose MSC treatment group. The three groups were injected subcutaneously, than followed with a comprehensive evaluation (a cytokine panel, mRNA, RNA, and IgE) at different time points (baseline-pro-canine adipose MSCs injection; 3 months, 6 months, and 12 months post-canine adipose MSCs injection). Some of these parameters may serve prognostic markers of cAD.

Research Grant: VetCell Therapeutics, College of Veterinary Medicine Advance Scholar Research Program, Western University of Health Science
Student Support: 2020 Summer Fellowship Grant, Western University of Health Science

Canine central nervous system metastatic melanoma: a retrospective analysis and comparative review

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Over 100,000 patients are diagnosed with malignant melanoma (MM) every year, and 10-40% of those cases will develop cerebral metastases. MM usually metastasizes to the frontal lobe and causes seizures in 33% of patients - with an average time to onset of neurologic signs of 3.5 years. In dogs, MM makes up 7% of malignant cancers, but neither the incidence of CNS metastasis nor features associated with these cases, are known. This study aims to determine the clinicopathologic features associated with canine CNS metastatic MM. A retrospective review of cases seen at the UC Davis Veterinary Medical Teaching Hospital from 1985-2019 was performed using the inclusion criteria of a diagnosis of MM and histopathological examination of the CNS. Of the 67 cases identified, 30% (n = 20) had CNS metastases, mostly in the brain (n = 18) and less commonly in the spinal column (n = 2). Within the brain, the temporal lobe was the most common site of metastasis (25%, n = 5). However, most dogs had multiple metastatic lesions (n = 13), averaging at least three across the CNS. Neurological signs were observed in 70% (n = 14) of dogs with CNS metastasis, with most dogs (n = 11) developing signs following a primary diagnosis (median of 187 days) and a minority of dogs (n = 3) developing neurological signs as the first indication of MM. Seizures were the most common sign, seen in 64% (n = 9) of clinically presenting dogs. These findings suggest that canine and human MM patients share similar CNS metastasis incidence rates and clinical presentation but differ in site predilection and time to onset of neurologic signs. Furthermore, this study reveals key features of canine CNS metastatic MM, which will guide clinical management of these patients.

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Student Support: Wittenburg/Toedebusch Labs
Interactions of CD4 T cells and CD8 T cells during female reproductive tract chlamydial infection

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*Chlamydia trachomatis* is the most commonly reported sexually transmitted disease in the United States, causing upper reproductive tract pathology in a subset of untreated women. We have recently found that protective CD4 T cells induced by intranasal (IN) chlamydial infection may reduce pathology by inhibiting the pathogenic CD8 T cells following intravaginal (i.vag) chlamydial challenge. The long-term goal of this study is to characterize mechanisms underpinning such inhibitory effects. In this project, we will use flow cytometry and determine the frequencies of naive (CD44lo, CD62Lhi), central memory (CM; CD44hi, CD62Lhi), or effector memory (EM; CD44hi, CD62Llo) CD4 T or CD8 T cells in spleens at various time periods following intranasal or intravaginal chlamydial infection. Mice infected i.vag will be analyzed on days 9, 16 and 20 after infection, whereas IN infected mice on days 14 and 30 after infection. On day 14 after IN infection, spleens from infected mice displayed a significantly increased frequency of CM CD4 and CD8 T cells and EM CD4 T cells when compared to the mock-infected wild type animals. No significant differences were found in the other analyzed populations between infected and mock-infected animals. Analysis of the other time periods is ongoing. Results from this study will serve as a foundation to obtain cells at appropriate time-periods for future studies on CD4 T cell-mediated inhibition of CD8 T cells during chlamydial infection.

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**Student Support:** Boehringer Ingelheim Veterinary Scholars Program

Evaluation of immune markers of leptospirosis in Townsend’s chipmunk populations on a land gradient in Oregon

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Leptospirosis is a complex bacterial zoonosis prevalent in rural and urban environments worldwide. Wild rodents are important hosts for *Leptospira*, which they shed into the environment infecting humans and other animals. Leptospiral infections in rodents are generally subclinical and difficult to identify. Our objectives were 1) to evaluate whether circulating leukocytes can serve as immune markers for *Leptospira* infection in wild rodents, and 2) to examine the effect of land use on the relationship between leukocyte profiles and *Leptospira* infection. Antibody titers to *Leptospira interrogans* were measured via microagglutination test in plasma collected from Townsend’s chipmunks (*Tamias townsendii*) captured along a gradient of forest management in Oregon between 2011 and 2013. Chipmunks were classified as presumptive positive or negative for *L. interrogans* infection based on combined antibody titer levels and serial titer changes for serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona. Within each of the three forest management levels, we compared total circulating leukocyte counts, as well as absolute neutrophil, lymphocyte, and monocyte counts in *Leptospira* positive (n = 63) and negative (n = 95) chipmunks using t-tests. For chipmunks captured more than once, we visually examined changes in leukocyte profiles over time and related cell counts to antibody titers. We did not detect any relationships between leukocyte profiles and *Leptospira* infections at any of the forest management levels. Additional work is needed to characterize the immune response of wild rodents to infection with *L. interrogans* and understand the impact of land use on the host-pathogen dynamics.

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**Student Support:** Boehringer Ingelheim
Epidemiology of staphylococcal mastitis in primiparous heifers on a pasture-based dairy

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This study evaluated the epidemiology of staphylococcal mastitis in primigravid heifers on a pasture-based dairy. Approximately 14 d prior to expected calving date, 294 cross-bred heifers were systematically assigned to 3 groups (n = 98 each) based on pre-partum mammary secretion sampling: 1) all 4 quarters, 2) 2 diagonal quarters, or 3) none collected. Skin swabs of all teats of each animal were also collected. After calving, foremilk samples were aseptically collected from all quarters of all cattle once weekly for 3 weeks. All samples were cultured. Staphylococci were genotypically identified by rpoB sequence and pulsed-field gel electrophoresis. Relative risk (RR) and attributable fraction of population (AFP) were used to associate pre and post-partum data. Among all samples, Staphylococcus chromogenes and Staphylococcus aureus prevailed. Staphylococci were isolated from 19% (232/1176) of teat skin samples. Only 57 of 232 quarters had data to compare teat skin to pre-partum IMI; 3 quarters had the same staphylococcal strain in both samples. Similarly, only 41 of 232 quarters had data to correlate teat skin to post-partum IMI; 3 quarters had the same strain. The RR of post-partum S. aureus IMI or non-aureus staphylococcal (NAS) IMI when the teat skin had S. chromogenes was 0.5 and 1.0, respectively. The RR and AFP of post-partum IMI relative to pre-partum IMI with the same staphylococcal strain were 11 and 77%, 43 and 86%, and 12 and 68% for all staphylococci, S. aureus, and NAS, respectively. Pre-partum staphylococcal IMI can persist into lactation, and diagnosing pre-partum IMI may be a useful tool for predicting IMI at calving. Isolation of staphylococci from teat skin was not predictive of post-partum IMI.

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Student Support: Student support: USDA NIFA, Animal Health project 1017880

Agent-based modeling of antimicrobial resistance dissemination in beef cattle

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Feedlots commonly use metaphylactic treatment on cattle upon arrival to decrease the prevalence of Bovine Respiratory Disease (BRD). However, this mass treatment of cattle may be contributing to the dissemination of antimicrobial resistance (AMR) throughout feedlots. This study evaluates the effect that metaphylactic administration of antibiotics may have on the dissemination of AMR within enteric bacteria and the pathogens that cause BRD. Transmission experiments were conducted on a 70-head feedlot by inoculating 5 steers with a commensal Escherichia coli strain. We quantified the interactions of these steers by using real-time location data collected during the transmission experiments. This empirical data was then used to develop and calibrate an agent-based model for E. coli transmission in Netlogo 6.1.1, a specialized software for creating agent-based models. Transmission of Pasteurella multocida, a causative agent of BRD, was also implemented into the model. We further added antibiotic administration and antibiotic resistance dynamics to simulate the effects of metaphylactic antibiotic use on a feedlot. This model specifically evaluates the use of Tulathromycin, a macrolide antibiotic that E. coli and P. multocida can confer resistance to via horizontal transfer. Within our model, we will evaluate the effects of individual treatment vs. metaphylaxis on disease and antibiotic resistance outcomes while considering differing Minimum Inhibitory Concentrations (MICs) for P. multocida and E. coli. We will also evaluate if there are consequences to not using metaphylactic treatment within the herd.

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Student Support: NIH T35 Training Grant 2T35OD011070-11
Temporomandibular joint pathology of wild carnivores in the western USA

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4,663 skull specimens from: southern sea otter (*Enhydra lutris nereis*), harbor seal (*Phoca vitulina*), California sea lion (*Zalophus californianus*), northern fur seal (*Callorhinus ursinus*), walrus (*Odobenus rosmarus*), polar bear (*Ursus maritimus*), North American brown bear (*Ursus arctos*), California mountain lion (*Puma concolor couguar*), California bobcat (*Lynx rufus californicus*), grey fox (*Urocyon cinereoargenteus*), kit fox (*Vulpes macrotis*), and grey wolf (*Canis lupus*) were macroscopically examined for dental and temporomandibular joint (TMJ) pathology. The presence of TMJ osteoarthritis (TMJ-OA) varied across species: 4.1% of southern sea otter, 34.5% of harbor seal, 63.5% of California sea lion, 20% of northern fur seal, 60.5% of walrus, 9.2% of polar bear, 13.2% of North American brown bear, 19.8% of California mountain lion, 0% of California bobcat and grey fox, 5.9% of kit fox, and 11.6% of grey wolf specimens had lesions consistent with TMJ-OA. TMJ-OA was significantly more prevalent in males over females in walrus, North American brown bear, polar bear, and California mountain lion (*P* < 0.001, *P* = 0.005, *P* = 0.005, *P* = 0.004). No other species showed a sex predilection. Adult specimens were significantly more affected than young adults in the harbor seal, fur seal, walrus (all *P* = < 0.001) kit fox (*P* = 0.001). Grey wolf young adults were significantly more affected by TMJ-OA (*P* = 0.047) more affected by TMJ-OA. Of the twelve species analyzed, three species, the harbor seal, northern fur seal and polar bear had a significant increase in the prevalence of TMJ-OA if their teeth had attrition and abrasion (*P* < 0.001, *P* < 0.001, and *P* = 0.033, respectively). TMJ-OA can lead to morbidity and mortality in wild animals but its etiology is not yet fully understood.

Research Grant: School of Veterinary Medicine—Lider endowment funds
Student Support: University of California Davis, Students Training in Advanced Research

A comparison of targeted-selective and rotational treatment strategies for controlling parasites of horses

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A common strategy for managing parasites in horses is rotational treatment (RT), where anthelmintics of different classes are administered to all horses in an alternating manner at frequent intervals. Another approach advocated by most equine parasitologists is targeted selective treatment (TST), where horses are dewormed based on the needs of the individual and time of year. This study sought to compare these two methods in a herd of horses. Two groups of adult horses pastured separately were assigned to either RT (n = 23) or TST (n = 14). Fecal egg counts (FEC) were examined for all horses monthly for 3.5 years. Horses in the RT group were dewormed every 2 months regardless of FEC results, alternating between pyrantel (PYR) and oxibendazole (OBZ). Horses in the TST group were treated individually with a combination of PYR + OBZ if FEC was ≥ 200 eggs per gram. Additionally, all horses were treated once annually with moxidectin (MOX) + praziquantel. The effect of these dissimilar treatment frequencies on drug efficacy, egg shedding trends, and cost were evaluated. Excluding the annual MOX treatment, horses in the TST and RT groups received an average 1.3 and 5.1 treatments per year, respectively. Despite the greater treatment frequency, RT horses did not have lower FEC. Additionally, more than 85% of FECs from low egg shedding TST horses remained low even without treatment, and no horses showed signs of parasitic disease anytime during the study. Interestingly, the efficacy of PYR and OBZ decreased in both groups similarly, despite the large difference in treatments. These results suggest that TST was just as effective in controlling parasites and maintaining horse health as RT, with much lower costs and labor required.

Research Grant: None
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine
Effects of vitamin E status on ROR\(\alpha\) and LXR expression in dorsal root ganglion neurons of young mice

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Approximately ninety percent of Americans do not meet the recommended intake requirements for vitamin E (vitE). Severe deficiency associated with mutations in tocopherol transfer protein alpha (\textit{TTPA}), affecting vitE transport, leads to ataxia with vitE deficiency (AVED). AVED localizes to the dorsal root ganglia (DRG), with symptoms developing during childhood. ROR\(\alpha\) and LXR are important nuclear receptors linked to vitE. Nuclear receptors are ligand-regulated transcription factors that are activated by lipid-soluble signals, regulating lipid metabolism. ROR\(\alpha\) and LXR were hypothesized to be alternatively expressed in the DRG depending on vitE status. We explored the effects of vitE status on the age-dependent expression of ROR\(\alpha\) and LXR in DRG neurons using the \textit{Ttpa}-null mouse model. Six experimental groups were evaluated via qRT-PCR: \textit{Ttpa}\(^{+/+}\) and \textit{Ttpa}\(^{-/-}\) at 1 mo. of age on a basal diet and 6 mo. \textit{Ttpa}\(^{+/+}\) on a basal diet, 6 mo. \textit{Ttpa}\(^{-/-}\) on a vitE deficient diet and both 6 mo. \textit{Ttpa}\(^{+/+}\) and \textit{Ttpa}\(^{-/-}\) mice on a highly supplemented vitE diet. There were no differences in expression levels of \textit{Rora} (encodes ROR\(\alpha\)) or \textit{Nr1h3} (LXR\(\alpha\)) across experimental groups. There was no effect of age on \textit{Nr1h2} (LXR\(\beta\)) within genotypes. However, \textit{Ttpa}\(^{+/+}\) mice maintained on a highly supplemented vitE diet had significantly higher DRG expression of \textit{Nr1h2} than \textit{Ttpa}\(^{-/-}\) on a vitE deficient diet (\(P = 0.03\)). Therefore, vitE status affects LXR\(\beta\) expression in DRG neurons and may be a therapeutic target for AVED.

Research Grant: National Institutes of Health
Student Support: “Students Training in Advanced Research” (STAR) Fellowship

Effects of nutrient restriction and day of gestation on beef heifer hematology during late gestation

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Hematological changes during pregnancy are likely an adaptation to sustain fetal development. Undernourishment is often a challenge for late gestation beef heifers and cows due to poor forage availability, but the impact of nutrient restriction on maternal hematology during advancing pregnancy is unknown. To investigate this, crossbred beef heifers were individually fed a nutrient restricted diet (NR: \(n = 9\)) which provided 70% metabolizable energy and crude protein requirements or a control diet (CON: \(n = 9\)) which provided 100% from day 160 of gestation until calving. Heifers were fed a sorghum Sudan hay-based diet supplemented to meet treatments using a Calan Broadbent feeding system. Jugular blood samples were collected every 6 weeks, beginning before treatment initiation (day 158). Whole blood samples were analyzed using a Sysmex XT- 2000i-V CBC + fibrinogen analyzer at the UM Veterinary Medicine Diagnostic Laboratory. A mixed model in SAS 9.4 was used to determine the effects of nutritional plane, day of gestation, and their interaction. There was a decrease (\(P \leq 0.02\)) in WBC, RBC, HGB, HCT, RDW-CV and reticulocytes, but an increase in MCV and MCH (\(P \leq 0.04\)) from day 158 to 201 of gestation. Heifers fed the NR treatment had less (\(P \leq 0.003\)) MCV and MCH, but greater (\(P = 0.003\)) MCHC. Of all samples, 16.7% of WBC, 11.1% of RBC and 2.8% of RDW-CV were above reference intervals. However, 11.1% of MCV and 19.4% of MCH were below reference intervals. Platelet count decreased (\(P = 0.003\)) in NR heifers from 158 to 201 days, but remained similar (\(P = 0.42\)) for CON. These preliminary results suggest that beef heifers undergo hematological changes as late gestation progresses, which may be affected by nutrient restriction.

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Preclinical assessment and analysis of anti-sema4D treatment for osteosarcoma

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Osteosarcoma (OS) is the most common primary malignancy of bone and is most commonly diagnosed in adolescents. Despite advances in treatment, survival rates for OS patients have not improved in more than forty years. While immuno-therapy has shown promise in other cancer types, this therapeutic approach remains relatively unexplored for OS. Interestingly, semaphorin type IV D (SEMA4D) and its cognate PLXNB family receptors are endogenously expressed at high levels in many human solid tumor types including breast, colon, and ovarian cancer, and are commonly associated with poor patient prognoses. Inhibiting SEMA4D-PLXNB receptor interactions represents a novel immunotherapeutic strategy to promote immune cell infiltration, reduce tumor progression, and decrease instances of tumor metastasis in SEMA4D+ OS. We treated immunocompromised and immunocompetent mouse models of OS with either anti-SEMA4D or an isotype control. Beginning prior to cell engraftment, tumors were measured twice weekly until sacrifice. Tumors from both treatment groups were analyzed via flow cytometry and immunohistochemistry to evaluate for immune cell changes. Tumor measurements of mice suggest that anti-SEMA4D treatment induces a substantial anti-tumor response with a dramatic decrease in tumor volume in treated animals. Preliminary results indicate that there is increased immune cell infiltration of anti-SEMA4D treated tumors. Reduced tumor progression is mediated through increased immune cell infiltration, enhanced T cell killing, and recruitment of pro-inflammatory immune cells. Additionally, reduced tumor progression is impacted by blocking SEMA4D-PLXNB receptor interactions, which inhibit oncogenic signals within the tumor microenvironment.

Research Grant: Team Nat—Children’s Cancer Research Fund
Student Support: U54

Endogenous lactate clearance as a potential marker to predict anaerobic athletic capacity in polo horses

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Polo is a game in which the player depends mostly in the ability and stamina of her/his horse to be able to compete at a high level. However, there is no accurate method of assessing the athletic capacity of these horses and in many cases, it is subjective to the opinion of the riders or trainers. Therefore, in this study, we explore the possibility of utilizing lactate clearance along with an equine fitness monitoring system (E-trakka) to predict the athletic capacity of horses. Serial blood L-lactate measurements were taken from horses during and after completing a standardized exercise test (SET) utilizing a point of care lactate meter. The SET consisted of two sessions; first, a three-minute warm-up that included trots, gallops, stops, and turns, followed by another three-minute workout that included gallops, stops, turns, and sprints. Lactate was measured immediately after each session, followed by a series of fifteen-minute lactate measurements to calculate lactate clearance percentages. In order to compare horses’ L-lactate clearance percentages to their anaerobic capacity, we used the E-trakka to calculate the performance capacity of each horse during the SET. An initial pilot study suggests that anaerobic metabolism can be reached utilizing the established SET and performance capacity each horse is exerting during the workout can be calculated based on the data provided by the equine fitness monitoring system E-trakka.

Research Grant: None
Student Support: None
Morphology of the giraffe (*Giraffa camelopardalis*) external ear

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The giraffe (*Giraffa camelopardalis*) is the largest living ruminant. Giraffes are members of the order Artiodactyla, the even-toed ungulates, which includes cattle, goat, sheep, antelope, deer, and pronghorn. The current study examines how the structure of the external ear of the giraffe compares to other mammals of similar morphology, namely equids and bovids. Our findings reveal that the giraffe’s auricle is proportionally larger and wider than other artiodactyls, and is composed of an auricular and annular cartilage. In comparison to other ungulates, the specific arrangement of the muscle relationships is more similar to equid anatomy rather than the closely related bovid. All muscles associated with the auricular, annular, and scutiform cartilages are identical to those of other mammals in their origin, insertion, and function; however, there are notable structural changes, as well as additional muscle bellies or attachment points that are not described in other mammals. Some of the muscles and associated auricular structures are displaced laterally and ventrally due to the ossicone protuberances on the dorsal cranium. There is a prominent scutiform cartilage with muscle attachments on the dorsal and ventral surface lying superficial to the temporalis muscle. Caudal and rostral auricular arteries are present without an extensive capillary network that would be required for auricular thermoregulation. We aim to provide a guide for those working with giraffes in medical or research settings to assist in the health and survival of endangered and threatened giraffe subspecies.

**Research Grant:** Midwestern University College of Veterinary Medicine  
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program

Polyomavirus-associates spindle cell sarcomas in mallard duck (*Anas platyrhynchos*)

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An adult female mallard duck (*Anas platyrhynchos*) was brought to the Wildlife Rehabilitation Center of Minnesota; upon physical examination two masses were noted, one on the duck’s thorax under its left wing the other on the distal phalanx of the second digit of the right foot. The duck was humanely euthanized and the masses were submitted formalin-fixed for histologic examination. Both tumors had a lobular organization with newly formed neoplastic cartilage separated by densely cellular interlacing bundles of neoplastic spindle cells. Numerous neoplastic cells, particularly the chondroblasts, had intranuclear inclusion bodies in both tumors. Ultrastructural examination confirmed that the inclusion bodies were of viral origin. The viral particles were most consistent with polyomavirus based on the median size of approximately 44nm and presence of paracrystalline viral arrays. PCR for confirmation of the identification of polyomavirus and further classification is pending. This case report provides evidence that avian polyomaviruses are associated with tumor formation under natural conditions.

**Research Grant:** None  
**Student Support:** Unknown
Comparing the spread of H3N8 canine and equine influenza viruses, and control of the viruses in horses

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Equine influenza virus (EIV) H3N8 strain emerged in 1963 and is still circulating among horses and other equids in many parts of the world today. Canine influenza virus (CIV) emerged as a variant of EIV in 1999 and was only prevalent amongst dogs in the USA until it died out in 2016. It is not understood why these viruses show different patterns of distribution. This raises questions about influenza viruses and their ability to adapt and spread in different hosts in their populations. This study uses viral sequencing to understand H3N8 distribution, which was classified by location based on continents and pre-divergence. Phylogenetic analysis showed reassortment of H3N8 EIV with pre-circulation EIV since its emergence with clear splitting into Eurasian and American-like clades, but with relatively frequent transfer of viruses between different geographic regions. CIV and EIV genes co-evolved independently, and H3N8 CIV showed no reassortment with other viruses. Overall, this study uses evolutionary variation to track the spread of the viruses, which may allow new approaches of surveillance and control of the EIV H3N8 viruses. In particular, the idea that there is repeated transfer of viruses between continents, and into the USA, may be key to the continuing circulation of the viruses, and better quarantine and testing strategies may allow the virus to die out naturally in the USA (or other regions).

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Student Support: NIH grant

Evaluation of antimicrobial activities of phytophenols against pathogens that cause liver abscesses in cattle

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Liver abscesses occur in finishing cattle fed high-grain, low-roughage diets. Cattle with abscessed livers do not show any clinical signs and are detected only at slaughter. Liver abscesses, which account for 67% of all liver abnormalities in cattle slaughtered, in the US, are of major economic concern to the beef industry. Fusobacterium necrophorum, Trueperella pyogenes, and Salmonella enterica are the main etiologic agents. Currently, the control of liver abscesses is based on in-feed use of antibiotics. The emergence and dissemination of antimicrobial resistance to antibiotics use in animals is a public health concern. Plant-based phenolic compounds, called phytophenols, are known to have antimicrobial properties. Our objectives were to evaluate antimicrobial activities of phytophenols on the liver abscess pathogens. Phytophenols extracted from black sorghum bran, sumac sorghum bran, rosemary, green tea, grapeseed, green coffee, organic goji berry, matcha tea, and yerba mate were tested. The phytophenols were extracted using 75% aqueous acetone and total phenolic content was determined in a spectrophotometer. Bacteria were cultured in Mueller-Hinton broth (Salmonella and Trueperella) or anaerobic brain-heart infusion broth (Fusobacterium) with and without phytophenols, at 6, 12, 24, and 48 hours and bacterial concentrations were determined. Also micro-broth dilution method was used to quantify the inhibition. Phytophenols from green tea, grape seed, rosemary, sumac sorghum and black sorghum inhibited T. pyogenes. Further studies are ongoing to investigate different concentrations of phenolic compounds on the pathogens. Phytophenols that inhibit the pathogens may have the potential to control liver abscesses.

Research Grant: Collaborative Sorghum Investment Program and Global Food Systems, Kansas State University
Student Support: Kansas State Veterinary Research Scholars Program
Osteomyelitis: animal models review and fosfomycin-loaded chitosan hydrogel therapeutic

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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 BHI plates contaminated with S. aureus using: PBS, chitosan hydrogel, PBS with high dose (0.05mg) fosfomycin, chitosan hydrogel with high dose fosfomycin, 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.

Research Grant: Unknown
Student Support: NIH T35 Grant 5T35OD010432

Prostate cell type mapping in intact male dogs leads to finding that aging alters cell type producing COL1A1

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The identity and spatial distribution of prostatic cell types has been determined in humans but not in dogs, even though aging- and prostate-related voiding disorders are common in both species and mechanistic factors, such as prostatic collagen accumulation, appear to be shared. Sequential immunohistochemical stains were used to identify specific prostatic cell types and determine their distribution in the capsule, peripheral, periurethral and urethral regions of the young intact canine prostate. Prostatic cells identified using this technique include perivascular smooth muscle cells, pericytes, endothelial cells, luminal, intermediate, and basal epithelial cells, neuroendocrine cells, myofibroblasts, fibroblasts, fibrocytes, and other hematolymphoid cells. To enhance rigor and transparency, all images are available through the GUDMAP database at https://doi.org/10.25548/16-WMM4. The prostatic peripheral region harbors the largest proportion of epithelial cells. Aging does not change the density of hematolymphoid cells, fibroblasts, and myofibroblasts in the peripheral region or in the fibromuscular capsule. Aging changes the procollagen IA1 positive prostatic cell identity from a myofibroblast to a fibroblast. Hematolymphoid cells and myofibroblasts are often identified as sources of collagen in tissues prone to aging-related fibrosis. We show that these are not the likely sources of pathological collagen synthesis in older intact male dogs. Instead, we identify an aging-related shift in the prostatic cell type producing COL1A1 that will help direct development of cell type and prostate appropriate therapeutics for collagen accumulation.

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Student Support: TL1TR002375 and F30DK122686
Breed impact on hematological and biochemical values for Thoroughbred, Quarter Horse, and Andalusian foals

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Hematological studies for the reference intervals for foals have been conducted, but the impact of other parameters, such as age and breed are not yet understood. Previous studies have shown that normal blood values differ among breeds in adult horses, but available data on such differences in foals are limited and often outdated. Understanding differences in normal values between breeds, and age-based reference intervals, is critical for accurate diagnosis of diseases and treatment of neonates and foals. Data from 81 foals (26 Thoroughbreds, 32 Quarter Horses, and 23 Andalusians) was collected via jugular vein draw on day different days (days 2, 7, 30,90, 180, and 365) within a 1-year period. This project aims to provide more insight of breed differences under normal living conditions in the general population as compared with data provided from university teaching hospitals using teaching herds. Our preliminary results showed significant differences in the following values among breeds: White Blood Cells, Red Blood Cells, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Total Proteins, Albumin, Globulin, Aspartate Aminotransferase, Alkaline Phosphatase, Gamma-Glutamyl Transferase, Blood Urea Nitrogen, Creatinine, Phosphorus, Calcium, Glucose, Potassium, Cholesterol, Creatinine Phosphokinase, Lactate Dehydrogenase, Fibrinogen, Immunoglobulin G, Magnesium, Alanine transaminase, Sorbitol Dehydrogenase, Total bilirubin, Direct Bilirubin, and Indirect Bilirubin. These findings may assist clinicians to better interpret laboratory data and evaluate/treat neonates and foals.

Research Grant: Third-party funding by Antech Diagnostics
Student Support: Boehringer Ingelheim Scholars Program

Survey of perspectives on veterinary diagnosis and management of laminitis in the field

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Laminitis is a painful condition of the equine foot that may occur with endocrine disease, inflammation, or increased weight bearing on one limb. In practice, most cases are associated with endocrinopathy. Diagnosis and management of endocrinopathic laminitis differs from management of other types of laminitis. Practitioners were surveyed using an 18 item web-based questionnaire to determine how they incorporate diagnostic testing into identification of the underlying cause of endocrinopathic laminitis and how treatment for the endocrinopathy influences time to improvement of laminitis. 174 practitioners started the survey, although response rate varied, with only 135 practitioners completing the survey. Most (74%) were equine only, with 26% mixed practitioners. Practitioners performed the following diagnostics in some or all cases at first examination: clinical examination (99%), radiographs (93%), basal ACTH (69%) complete blood count and biochemistry (68%), insulin (unfasted 57% or fasted 28%), dynamic ACTH testing (27%), or dynamic insulin testing (9%). 66% of practitioners indicated that their diagnostic approach has changed since graduation. 60% of practitioners reported that treatment for PPID improved time to resolution of laminitis, and 70% reported that treatment for EMS improved resolution. Practitioners reported that treatment for PPID (99%) or treatment for EMS (100%) prevented further laminitis episodes. These findings suggest practitioners are aware of an association between endocrinopathy and laminitis, but may not be comprehensive in endocrine assessment upon initial visit. Treatment of EMS and PPID were considered effective in improving resolution of disease and preventing further laminitic episodes.

Research Grant: None
Student Support: NIH T35 Training Grant 5T35OD011151-17
Expression of MHC-II on equine peripheral blood eosinophils

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Eosinophils are leukocytes with various functions, but most notably play a role in parasitic infections and allergic inflammation. However, research has shown that eosinophils can express major histocompatibility complex class II (MHC-II), indicating that eosinophils can function as antigen-presenting cells (APCs). As APCs, eosinophils can activate and modulate an immune response to airway antigens, such as with asthma, but this has not been investigated in the horse. Equine asthma can be characterized by a spectrum ranging from mild inflammatory airway disease (IAD) to severe recurrent airway obstruction (RAO). Due to the horse’s large blood volume, isolation of these rare cells is possible and may serve as a model for comparative eosinophil research. Our lab has successfully isolated equine peripheral blood eosinophils with 90% purity and 78% viability using discontinuous density centrifugation. The next steps will be to use antibody labeling and cell staining with equine-specific monoclonal antibodies, and flow cytometry to determine the presence of MHC-II expression by eosinophils. Equine eosinophil research will help further our understanding of the roles that eosinophils play in disease and may serve as a model for humans with common diseases like asthma.

Research Grant: Midwestern University College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Analysis of the 2017-2019 registry summary for the ACVECC VetCOT trauma initiative annual report

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The ACVECC-Veterinary Committee on Trauma (VetCOT) Registry is a multi-institutional data collection effort, geared towards standardizing and collating clinically useful data describing small animal trauma. Few multi-center data registries exist in veterinary medicine due to the challenge of data consolidation among hospitals, yet their importance is monumental. Trauma is a leading cause of death in dogs across all age groups.1 Novel, epidemiological information may be clinically useful to improving mortality rates, as access to collated data on veterinary patient trauma provides useful information for a multitude of prospective epidemiological studies. Utilizing the web-based data capture system, RedCAP, data were recorded by 30 veterinary trauma centers across the world, capturing over 40,000 cases since it’s establishment in 2013. The registry has since been revised and was summarized in 2017. This report summarizes cases presenting to their trauma centers between April 2017 and December 2019.


Research Grant: Unknown
Student Support: Unknown
Infection number and duration shape how pathogens affect the gut microbiota

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Changes in the gut microbiota during pathogen infection are often predicted to influence disease outcomes. However, studies exploring whether pathogens induce microbiota shifts have yielded inconsistent results. This suggests that variation in infection, rather than the presence of infection alone, might shape pathogen-microbiota relationships. For example, most hosts are coinfected with multiple pathogens simultaneously, and hosts vary in how long they are infected, which may amplify or diminish microbial shifts expected in response to a focal pathogen. We used a longitudinal study of free-ranging African buffalo (*Syncerus caffer*) to examine whether (i) coinfection with bovine tuberculosis (*Mycobacterium bovis*, TB) and gastrointestinal nematodes, and (ii) the duration of TB infection, modified effects of single pathogens on the gut microbiota. By accounting for the interaction between TB and nematodes, we found that coinfection altered single pathogen-associated changes in microbial abundance. Furthermore, the duration of TB infection predicted more variation in microbial diversity than the presence of TB. These findings demonstrate that acknowledging infection heterogeneities may be crucial to understanding the relationships between the gut microbiota and infectious diseases.

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**Student Support:** NSF DGE-1545433

Risk of infectious disease in university day care centers

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Almost 25% of American children under the age of five attend organized daycare arrangements. These children have a greater likelihood of encountering infective pathogens in comparison to those who do not attend due to their close contact with other children, toy sharing, and toilet training. The role of disease in infant microbial maturation and succession remains unexplored. The aim of this study was to better understand the relationship between daycare environments and disease exposure. An extensive literature review was performed, and retrospective analysis of exposure reports and disease events were collected and analyzed from three daycare rooms at The Ohio State University. A total of 33 exposure notices and 190 total fever or diarrheal disease events were obtained. Results demonstrated a consistent increase in disease reporting in January, with lower numbers being reported in the summer months. The most common diseases reported from exposure notices were conjunctivitis (6) followed closely by hand-foot-and-mouth disease, influenza, and roseola (5 each). In the future, these findings will be supplemented with longitudinal stool collection and microbiome analysis, guardian surveys, and environmental sampling analysis from multiple daycare locations at The Ohio State University.

**Research Grant:** Ohio State University Infectious Disease Institute  
**Student Support:** OSU CVM Summer Research Program
Assessing potential for antiviral resistance in feline herpesvirus

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The most common cause of infectious ocular disease in cats is Feline Herpesvirus (FHV-1). FHV-1 is often treated with antiviral medications at a range of doses and frequencies, but it is unknown if the use of these medications in cats with FHV-1 can lead to mutation of the virus and development of antiviral resistance. The aim of this study is to assess FHV-1 isolates from shelter cats for evidence of development of antiviral resistance after treatment using Next-Generation Sequencing, with subsequent analysis of viral genomic variation. The hypothesis of this study is that although point mutations in the isolates may develop following antiviral therapy, it is unlikely that any of these mutations will lead to the development of clinically important antiviral resistance. Forty shelter-housed cats with signs of FHV-1 ocular disease will be screened with conjunctival swab rt-PCR to confirm the presence of the virus. Virus isolation will then be performed using swabs taken before and after 7 day treatment with antivirals (twice daily topical ganciclovir or topical cidofovir or oral famciclovir), as well as placebo medications. In order to screen for sequence variants which could cause resistance, full genome sequencing of FHV-1 isolates focusing on UL23/30/42 genes will be performed. Preliminary results will be presented. Following analysis, we expect to find that the virus has an overall low genomic diversity with minimal evidence of mutation following the use of antivirals. Overall, we expect these findings will further support the use of antiviral medications in cats with FHV-1 and alleviate concerns regarding potential development of resistance when these medications are used twice daily in shelter-housed cats.

Research Grant: Morris Animal Foundation and Stokes Healthcare
Student Support: Morris Animal Foundation and LSU School of Veterinary Medicine Summer Scholars Program

Understanding the role of specific methanogens in methanogenesis and their bacterial associations in the rumen

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Enteric methane emissions from ruminants account for a substantial portion of anthropogenic greenhouse gas emissions and are considered a net energy loss to the ruminant host. This methane is solely formed by archaea (methanogens) taking up hydrogen released by other microbes during microbial fermentation in the rumen. Research efforts to mitigate methane emissions are ongoing, but a thorough understanding of methanogenesis and associations between specific methanogens and bacteria is needed in order to advance mitigation efforts. The goal of this study is to understand and quantify individual methanogens and associated bacteria in the rumen and determine to what extent mitigation affects these microbes. First, specific primers were acquired or developed to target methanogens such as Methanobrevibacter species, Methanosphaera, and Methanomassillicoccales representatives. Primers were also acquired or developed to target bacterial populations such as Succinivibrionaceae, Clostridium, Bacteroidales, Bulleidia, Ruminococcus, and Prevotella. Second, the identified primer pairs will be validated in the laboratory and the individual microbes will be quantified in rumen samples. Third, these microbes will be quantified and compared between archived rumen samples from dairy cows that were supplemented with a potent methane inhibitor, 3-nitroxypropanol, and cows that were not supplemented. Finally, the qPCR results will be compared to previously collected 16S, metagenomic, and metatranscriptomic data to gather a comprehensive understanding of these microbes and their functional contribution towards methanogenesis. Conclusions will be drawn about methanogen-bacterial associations and their impact on methane emissions.

Research Grant: USDA-NIFA grant and Pennsylvania Department of Agriculture pilot grants
Student Support: NIH-BI Veterinary Summer Scholar Program
Equine colic can be caused by many different types of lesions, each drastically impacting the course of treatment and prognosis. Identification of clinical markers associated with colic lesion, duration, management, progression, and treatment outcome could aid clinicians in more effectively diagnosing and treating colic patients. Previous studies have evaluated peripheral and abdominal lactate, heart rate, and peripheral white blood cell count as predictors of colic lesion, treatment, and outcome. However, there is little literature specifically analyzing peritoneal fluid neutrophil counts and their correlation to colic disease factors. As neutrophils are an established sign of acute inflammation, and can exert damaging effects within tissues, analyzing their quantity in peritoneal fluid could strengthen colic diagnosis and better guide treatment progression. In this retrospective study, the medical records of 446 colic horses from the past 10 years were analyzed. Peritoneal fluid white blood cell and neutrophil counts will be compared to type of lesion, duration of colic signs, development of complications such as ileus or SIRS following treatment, and treatment outcome. We hypothesize that peritoneal fluid neutrophil counts and percentages will be higher in horses with strangulating and inflammatory lesions, lesions with longer durations (> 24 hours), and in cases that develop SIRS or ileus, necessitate surgical management, or require euthanasia due to disease severity or poor prognosis. Identification of such associations in colic patients may allow for earlier or better interventions, provide more information on what to expect during the course of treatment, and help render a more accurate prognosis.

Research Grant: Research Funding: UGA College of Veterinary Medicine
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Variability found in NSAID prescription patterns at Thoroughbred and Standardbred racetracks in Pennsylvania

Phenylbutazone, a non-steroidal anti-inflammatory drug (NSAID), was recently found to be associated with an increased risk for catastrophic injury in racehorses. The frequency of NSAID usage in racehorses has not been previously described. In this study, NSAID prescriptions reported for racehorses at four racetracks in PA were analyzed to investigate the hypothesis that prescription patterns are affected by multiple covariates, including racetrack, breed (Thoroughbred vs Standardbred), time of year, indication for treatment, and veterinarian. Data were collected from treatment logs from four racetracks in Pennsylvania (two Standardbred and two Thoroughbred) from June 2018-August 2018. The frequency of administration and the NSAIDs prescribed were characterized for all racetracks and for each subgroup of interest. Phenylbutazone was prescribed the most frequently (65.0%), followed by flunixin (26.1%) and ketoprofen (8.8%) at all four racetracks; however Standardbreds received a higher percentage of other types of NSAIDs (33.4%) than Thoroughbreds (22.9%; p < 0.0001). NSAIDs were prescribed more frequently in July than in June and August (p < 0.001). As expected, the most common indication was musculoskeletal soreness, which was treated primarily with phenylbutazone (63%) and flunixin (30%). It was the only indication that was also treated by multiple other NSAIDs such as ketoprofen, firocoxib, and diclofenac. The percentage of NSAIDs prescribed varied greatly by veterinarian. Ongoing studies are expanding the number of racetracks and years available for analysis and will investigate if specific NSAID regimens are associated with an increased risk of catastrophic injuries.

Research Grant: Pennsylvania State Horse Racing Commission
Student Support: NIH/Boehringer Ingelheim Summer Research Program
Trichloroethylene modulates hepatic and blood gene expression in experimental primary biliary cholangitis

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Human exposure to Trichloroethylene (TCE) is linked to the development of hepatic autoimmune disease. Surprisingly, in studies seeking to define the TCE mode of action, TCE displayed unanticipated therapeutic action. Specifically, TCE treatment reduced hepatic injury and inflammation in dnTGFBRII mice, which develop hepatic autoimmunity resembling Primary Biliary Cholangitis (PBC). We sought to identify transcriptomic signatures (Illumina RNA sequencing) driven by development of PBC-like autoimmunity in dnTGFBRII mice, in both blood and liver, and tested the hypothesis that TCE treatment altered the expression of active genes. Female 8-week old Wild type and dnTGFBRII mice were treated with TCE (0.5mg/ml) or vehicle (1% ethoxylated castor oil) for 12 or 22 weeks. Active genes were identified statistically and as those genes with $\geq 1.5$ fold increase or $\leq 0.6$ fold reduction for specific comparisons. In dnTGFBRII mice treated with vehicle, 1,572 genes were differentially expressed in the blood and 1,167 in the liver. Among these genes, 113 genes in blood were affected by concurrent TCE exposure, and 55 were affected in the liver. Although a surprisingly small fraction of genes were altered by TCE, induction of genes encoding select chemokine-chemokine receptor pairs were inhibited by TCE exposure. The results indicate that TCE inhibits the induction of specific genes associated with development of experimental PBC. The results suggest potential mechanisms whereby TCE may inhibit development of liver pathology in experimental PBC.

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**Student Support:** NIH grant R5R25HL103156-10

A systematic review of environmental sustainability in veterinary practice

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Upon acceptance to the veterinary profession, one recites an oath swearing to protect animal health and welfare, conserve animal resources, and promote public health. Consequently, veterinary professionals must responsibly implement sustainable changes in their daily practice to mitigate climate change, an indisputable source of traceable, negative impacts on both animal and human health. Since the medical field has proactively taken the first steps in conducting a literature review regarding environmental sustainability in the context of human hospitals, the objective of this paper is to replicate the methods of this review through the lens of veterinary medicine. The goal of a review of this magnitude is to outline concise and achievable eco-friendly changes to the way veterinary medicine is practiced. However, results of this search illustrate the drastic void in experimentally derived, evidence-based clinical guidelines pertaining to environmental sustainability in all forms of veterinary practice. Given this absence, recommendations were extrapolated from empirical research in engineering, human hospitals, and behavior change. Critical next steps to fill this literature gap include integrating research on climate change impacts on animal health in the veterinary education curriculum, establishing an evidence-based sustainability certification as the gold standard for clinics, and expanding the scope of research on the veterinarian’s role in mitigating climate change. As a profession, veterinarians declare to serve animals, humans, and their environment; this crucial pillar of one health professionals cannot fall behind the curve driven by the force of climate change.

**Research Grant:** CSU One Health Institute

**Student Support:** CSU Veterinary Summer Scholars Program
Canine transmissible venereal tumor in Grenada, West Indies: a retrospective study

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Canine transmissible venereal tumor (CTVT) is a naturally occurring neoplasia affecting female and male dogs worldwide. Previous CTVT studies in Grenada were limited to dogs with neoplastic conditions or dogs presented to a veterinary diagnostic laboratory. This study aimed to determine the occurrence and risk factors of CTVT in a wider population of owned dogs presented to the university’s Small Animal Clinic between 2008 and 2018. Data on the age, breed, gender, and gonadectomy status were retrieved from an electronic database and analyzed using logistic regression. Of the 7180 dogs presented during the period, 102 dogs (1.4%) were diagnosed with CTVT. Affected dogs varied in age between 2 and 18 years (median 7 years, interquartile range 5-9 years) and included 7 purebreds, 54 Grenadian pothounds, 37 mixed-breed dogs, and 4 dogs with missing breed information. The number of female, male, intact, and neutered dogs diagnosed with CTVT was 49, 53, 52, and 50, respectively. A higher predisposition was observed in Grenadian pothounds (OR = 22.9, 95% CI 10.4-50.8) and mixed-breed dogs (OR = 9.2, 95% CI 4.1-20.8) in comparison to the purebreds. Neutered dogs (OR = 2.2, 95% CI 1.5-3.4) were at an increased risk of CTVT than intact dogs. Age (OR = 1.0, 95% CI 0.9-1.0) and gender (OR = 0.8, 95% CI 0.5-1.2) were not identified as significant risk factors. The percentage of dogs with CTVT in this study represents a crude estimate of the CTVT prevalence in Grenada. Further studies including both owned and free-roaming dogs are required to get true estimates of prevalence and incidence of this neoplasia in the region. Our results indicate that breed and gonadectomy status are significant risk factors for the occurrence of CTVT in Grenada.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Trap-neuter-return and return-to-field programs for managing community cats in Florida shelters

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Florida’s 154 shelters collectively take in more than 200,000 cats each year, most of which are unowned community cats admitted as strays. In 2019, only 72% of cats had live outcomes, compared to 88% of dogs. Trap-Neuter-Return and Return-to-Field programs sterilize, vaccinate, ear-tip and return healthy un-owned shelter cats to the location of origin as an alternative to euthanasia or shelter crowding. The purpose of this study was to characterize use of TNR/RTF programs in Florida shelters for managing community cats. Shelters were surveyed to correlate previous year cat intake and outcome data with use of TNR/RTF programs. Interim analysis included surveys received from 92 of the 154 animal shelters known to be operating in Florida. A total of 51 shelters (55%) performed some form of community cat management via sterilization and returning to the field. TNR/RTF was more performed by both municipal (23 shelters; 25%) and private shelters (28 shelters; 30%). Other medical procedures included in TNR/RTF were rabies vaccination, (51 shelters, 100%), FVRCP vaccination (42/82%), FeLV and FIV testing (32/63%), internal parasite control (30/58%), external parasite control (42/82%), treatment of illnesses/injuries (46/90%), and humane euthanasia for debilitating conditions (51/100%). The most common minimum age/weight for cats in the TNR/RTF programs was 8 weeks old/2lbs (39%). Identification of sterilization status included ear-tipping in 51 shelters (100%) and tattooing in 43 shelters (84%). Shelters with TNR/RTF programs had higher live outcome rates for cats than shelters without them. In conclusion, TNR/RTF was a commonly utilized tool in Florida shelters and was associated with increased feline lifesaving.

Research Grant: Maddie’s Fund
Student Support: Maddie’s Fund, Department of Small Animal Clinical Sciences
Multi-regional phylogenetic characterization of skunk amdoparvovirus (SKAV)

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Amdoparvoviruses (APVs, family Parvoviridae) cause persistent infections in many small carnivores, with outcomes varying from subclinical to fatal disease. Skunk amdoparvovirus (SKAV) was first reported in 2017, and has been detected at high prevalence (65-87%) in striped skunks (Mephitis mephitis) across North America. Most infections are subclinical but fatal disease has been observed, and host spillover into mink has been reported. The APVs exhibit remarkable genetic diversity, but genetic factors mediating disease manifestations and host species jumps are unknown. The objective of this study was to clarify the genetic characteristics and range of diversity within SKAV by analyzing viral genomes from multiple geographic regions (California, New England, and British Columbia). We hypothesized that this would reveal geographic clustering of sequences, with regions of relative conservation and hypervariability across the ~4500nt SKAV genome. Cases were detected by conventional PCR, and full SKAV coding sequences were obtained by overlapping PCR amplifications and Sanger sequencing. Phylogenetic analysis revealed broad genetic diversity with clear geographic clustering, and demonstrated that diversity in SKAV is driven by relative variability in the non-structural (NS) gene, with relative conservation in the capsid-coding (VP) gene. Conservation of the capsid sequence is enigmatic, but has also been observed in related viruses and proposed as a mechanism to promote antibody-dependent enhancement (ADE) of infection and establishment of persistent infection. Structural gene conservation in SKAV could indicate that ADE is a potentially conserved mechanism of infection across the APVs.

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Student Support: AVMA/ AVMF Second Opportunity Research Fellowship, UC Davis STAR Fellowship

Prevalence and diversity of Cryptosporidium spp. in veterinary students completing their ambulatory rotation

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Cryptosporidium is a protozoal parasite that is spread through oocysts in infected feces. Outbreaks are caused by contaminated food, water, or contact with infected animals or people. Infections are usually self-limiting but can be serious in the immunocompromised or in people with underlying health conditions. Cryptosporidium spp. outbreaks have been reported in veterinary students at several different universities. These outbreaks typically occur after contact with infected calves during labs or other clinical experiences. The purpose of this pilot study is to investigate the genetic diversity and prevalence of Cryptosporidium in veterinary students completing their ambulatory rotation at a large veterinary teaching institution and to determine risk factors for exposure. This study will identify potential cases using a questionnaire about gastrointestinal symptoms. Stool samples will be collected from potential cases and analyzed using PCR to detect Cryptosporidium spp. The genetic diversity present between cases will also be examined to determine if there is a farm of origin for the cases. Overall, this study will investigate anecdotal evidence of cryptosporidiosis in fourth year students and will inform decision making on future precautions for ambulatory students.

Research Grant: None
Student Support: NIH T35 Training Grant through Cummings School of Veterinary Medicine at Tufts University
Antibody levels against CDV and CPV-2 in dogs vaccinated via nomograph vs traditional vaccination protocols

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Canine Distemper Virus (CDV) and Canine Parvovirus-2 (CPV-2) are deadly viruses that affect canine populations worldwide. Vaccination schedules have been developed to protect dogs by effectively inducing antibody production once maternal antibodies have diminished. Traditional vaccine protocols recommend vaccinating a puppy every 3-4 weeks until 16 weeks of age, with the expectation that blocking maternal antibodies will no longer negatively affect immunization by that age. However, the range of titers seen in breeding bitches varies widely, and thus maternal antibody neutralization of modified live viral vaccines remains a significant cause of immunization failure. A nomograph allows a tailored vaccination schedule and earlier follow-up testing to ensure immunity for the puppy. Dam titers measured at the beginning of colostrum production and a conservative estimate of antibody transfer are used to calculate expected antibody degradation in the pup, as based on known half-life. Using nomograph information, vaccinations are timed for the first possible successful immunization and again when maternal antibody is highly likely to be completely degraded. Protective levels for actively produced antibody have been established via experimental challenge of immunity. The aim of this study is to compare humoral protection rates in puppies one year of age or less to determine differences between traditional and tailored vaccine schedules. Results of this study show that puppies under one year of age are significantly more likely to be unprotected against CPV-2 and CDV than are adult dogs. This difference is not seen in puppies vaccinated which have had a nomograph completed for their dam.

Research Grant: None
Student Support: University of Wisconsin Companion Animal Vaccine and Immune Diagnostic Service Laboratory

Correlating Salmonella serogroup with clinical findings in horses at the NC State veterinary hospital

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Salmonella is an enteric pathogen of horses, and clinical presentation can range from subclinical carriers to severe diarrhea and death. Diagnosis is confirmed with microbiological methods including PCR and culture; clinical signs and neutropenia also highly suggest the pathogen. There is little research investigating the role of serogroup as an indicator of clinical outcome. This retrospective study investigates associations between Salmonella serogroups in positive fecal samples and CBC and chemistry panel outcomes, performed within 2 days of the first positive fecal sample, as well as the presenting clinical complaint at admission. Sixty-four Salmonella-positive cases from 2018 to 2020 were considered; serogroups were available for 42 of these isolates. Predictably, the majority (81%) of cases presented for colic, and 34% of cases tested positive for Salmonella more than once during their stay. Fifty percent of horses presented with hyperglycemia. When comparing bloodwork results to expected ranges, Serogroup B was associated with high band neutrophils (1037 ± 243 cells/μL), low lymphocytes (744 ± 173 cells/μL) and were the only serogroup with normal monocytes (250 ± 183 cells/μL), neutrophils (3479 ± 2243 cells/μL) and fibrinogen (233 ± 115 mg/dL). Serogroup C was associated with high glucose (124 ± 6 mg/dL), BUN (18.7 ± 1.6 mg/dL) and creatinine (1.57 ± 0.63 mg/dL). High BUN and creatinine values were associated with euthanasia ($P < 0.004$) and trended with diarrhea ($P < 0.1$). High monocytes and band neutrophils trended with higher euthanasia rates ($P < 0.08$). This information can contribute additional considerations to make the best possible treatment plan for horses with salmonellosis at the NC State Large Animal Hospital.

Research Grant: None
Student Support: NC State University Veterinary Scholars Program
Abundance and diversity of ticks in different habitat types within parks in Athens-Clarke County, Georgia

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Tick abundance and diversity data provide important information about tick-borne disease risks for people and pets. In Georgia, several tick species are vectors of pathogens of medical and veterinary concern. However, there have been few studies on ticks in Athens-Clarke County, GA, particularly within parks and natural areas where humans and pets may have contact with ticks. The objectives of this study were to 1) determine the number and diversity of ticks in parks/natural areas in Athens-Clarke County, 2) determine their phenology and habitat preferences, and 3) determine if species abundance or diversity are related to any spatial associations or habitat connectivity. From May-July 2020, we conducted over 1,000 tick drags in eight parks/natural areas to collect host-seeking ticks from the environment. Each tick drag was 100 meters long and 6 replicates of three different habitat types (field, edge, and forest) were sampled. We found two tick species: *Amblyomma americanum* (lone star tick) and *A. maculatum* (Gulf coast tick). Of note, we did not find the invasive tick *Haemaphysalis longicornis* (Asian longhorned tick) which was recently found in border counties of TN and NC. Nearly all ticks (97.5%, n = 435) were *A. americanum* and remainder (2.5%, n = 11) were *A. maculatum*. Although *A. americanum* were found in all sampled habitat types, *A. maculatum* were primarily found in grass/field habitats. These data are an important first step in educating the public on ticks in local community parks and where tick prevention strategies should be implemented to decrease the risk of tick-borne diseases.

Research Grant: None

Student Support: Foundation for Food and Agriculture Research Vet Fellowship, UGA College of Veterinary Medicine

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Dual software analysis of 24-48-hour holter monitoring of eight arrhythmic dogs

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Michigan State University

OBJECTIVE—Holter monitoring or ambulatory electrocardiogram (AECG) is a common method of ventricular arrhythmia detection and monitoring. Software analysis is used to evaluate these events. Accuracy is imperative in this software since a small difference can affect treatment and screening. Will two different software analyses of ventricular arrhythmias be similar and/or effect clinical course.

ANIMALS—Eight dogs with ventricular arrhythmias of various breed and age.

METHODS—Dual 24-48-hour AECGs were performed on all eight dogs. The Pathfinder SL 3-lead system and the Forest Medical Trillium 5-lead system. A dual attachment was done to the same electrode on all dogs. Recovered data was evaluated by boarded veterinary cardiologists. Ventricular arrhythmias were reviewed by a 3rd year Michigan State University veterinary student. Descriptive statistics will be used to review and show results.

PRELIMINARY RESULTS—Preliminary results show minimal difference, but analysis is still ongoing.

CONCLUSIONS—Based on preliminary results, further investigation with a larger sample size and a more specific demographic may be necessary.

Research Grant: NIH Grant 5T35OD016477-19

Student Support: Student Funding provided by NIH Grant 5T35OD016477-19 to Michigan State University
Multi-tissue transcriptomic analysis of BRSV-infected cattle using machine learning and statistical approaches

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Bovine respiratory syncytial virus (BRSV) is a viral pathogen that replicates in upper and lower airways, contributing to bovine respiratory disease (BRD). Transcriptomic evaluation of the host response to BRSV across multiple tissues may elucidate underlying mechanisms involved in BRD. Our objective was to identify genes and enriched pathways associated with BRSV infection through machine learning (ML) and statistical applications. Raw sequenced reads from 65 bovine samples across six tissue sites (n = 36 BRSV, n = 29 control) were assembled with the ARS-UCD1.2 genome assembly in a HISAT2/Stringtie2 pipeline. Gene counts for ML analysis were transformed and analyzed with MLSeq, utilizing six ML algorithms. Cross-validation parameters were applied in a 70:30 training/testing ratio. Statistical analysis of raw counts was performed with edgeR likelihood-ratio testing with a q-value cutoff of 0.05. WebGestalt, Reactome, and String were utilized for downstream analysis. ML analysis revealed 150 genes that classified individuals with 80.0-100.0% accuracy. edgeR analysis identified 188 differentially expressed genes; 53 genes were shared across ML and edgeR analyses. Pathways involving type I interferon signaling, IL-10 signaling, DDX58/IFIH1 regulation, and MHC-I antigen presentation were increased in BRSV-infected cattle. ATP synthesis via the respiratory electron transport and temperature regulation pathways were decreased in BRSV-infected cattle. Using machine learning and statistical approaches, we described distinct genes and pathways important for understanding clinical BRD associated with BRSV infection. This approach provides new information regarding genomic mechanisms relevant to BRD pathogenesis and immunity.

Research Grant: None
Student Support: Mississippi State University College of Veterinary Medicine (DPPM)

Reduced organo-somatic indices in fish exposed to lead-contaminated sediment

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Lead (Pb) is a pervasive heavy metal implicated in a wide spectrum of toxic effects including metabolic disturbances, impaired hemapoiesis and anemia in fish. We hypothesized that organisms exposed to high doses of Pb would exhibit signs of organ cytotoxicity such as cellular necrosis and an elevated inflammatory response. To test this hypothesis, we examined the organo-somatic indices (OSI) of fish from three rivers in southeast Missouri (Big River, Flat River, Meramec River) that have been impacted by historic mining and contain a gradient of elevated in-stream sediment concentrations of Pb (50-1200 mg/kg dry weight). OSI from fish collected at 3 sites with sediment Pb < 20 mg/kg dry weight were used as a reference. Spleen and hepatopancreas OSI were calculated as (organ mass/total body mass)*100 for three species of fish (Lepomis megalotis, Moxostoma erythrurum, and Ambloplites rupestris). OSI for spleen (mean ± SD; 0.097 ± 0.059%) and hepatopancreas (0.652 ± 0.155%) of Lepomis megalotis from contaminated sites were lower than the Castor reference site (0.134 ± 0.046% and 1.016 ± 0.243%, respectively) and may indicate necrosis. OSI for other species were limited to fewer sites and the relationship to sediment Pb concentration was unclear. Variation due to site-specific Pb concentrations and Pb bioavailability at each site should be considered. Tissue damage suspected by differences in OSI among sites will be confirmed with histopathological assessment. The relationship between OSI and Pb concentration in fish fillets and blood are currently under investigation. Decreasing OSI in response to Pb exposure could serve as an early indicator of mortality, poor body condition, and impaired reproduction in fish populations.

Research Grant: Missouri Department of Conservation, Missouri Department of Health and Senior Services, Missouri Department of Natural Resources, U.S. Fish and Wildlife Services
Student Support: IDEXX-BioAnalytics
Variation in destabilization of the medial meniscus for experimental post traumatic osteoarthritis in mice

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Destabilization of the medial meniscus (DMM) in mice to model post-traumatic osteoarthritis was first documented in 2004 and now is the most common surgical model used to induce post-traumatic osteoarthritis in mice. Despite its frequent use, difficulties in comparing results across studies have been acknowledged. The goal of this study was to better understand sources of variability by surveying investigators and research groups around the world on their laboratory animal characteristics, management, and surgical techniques used in the DMM procedure. Following Institutional Review Board approval, a comprehensive Qualtrics survey was developed, tested, and distributed to first and last authors of manuscripts published and curated in PubMed that used the DMM technique. Wide variability was reported between research groups in mouse husbandry, surgical, and post-operative factors, even when research groups cited the use of the originally published DMM model. Understanding and recording these sources of variability, together with improved reporting experimental methods, could allow improvements to be made in repeatability, reproducibility, and enhance the ability to compare across studies. Ultimately these improvements could lead to improved understanding of the pathogenesis of post-traumatic osteoarthritis.

Research Grant: None
Student Support: Boehringer Ingelheim—Purdue University College of Veterinary Medicine

Effect of hCG on days 0 and 5 of the estrous cycle on pregnancies per embryo transfer in recipient heifers

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Higher circulating concentration of progesterone (P4) during the early post-conception period (≥ 7 dpc) is associated with an increase in embryonic growth rate, interferon-tau synthesis, and pregnancy rate in cattle. In our preliminary study, heifers treated with human chorionic gonadotropin (hCG) on d0 and 5 of the estrous cycle had increased circulating concentrations of progesterone during early diestrus (≥ d5). The objective of this study was to determine the effects of hCG (3,300 IU) administered on d0, d5 or on both d0 and 5 of the estrous cycle on pregnancies per embryo transfer (P/ET) in recipient heifers. We hypothesized that hCG treatments would increase serum P4 levels during early embryonic development, resulting in more P/ET in recipient heifers. Estrus of all heifers (n = 1073) were synchronized with a 5d-CIDR protocol (d-8: CIDR inserted; d-3: CIDR removed and PGF2α). Heifers were randomly assigned a treatment group: control, hCGd0, hCGd5, and hCGd0&5. Controls were treated with GnRH on d0, while hCGd0 received hCG on d0. hCGd5 heifers received GnRH on d0 and hCG on d5, while hCGd0&5 received hCG on d0 and d5. Embryo transfers were performed on d 7 or 8. Pregnancy diagnosis was performed using ultrasound on d 32, 46, and 60 of gestation. The hCGd0 group had fewer P/ET on d60 compared with control and hCGd5, rejecting our hypothesis that hCG on d0 would improve P/ET in recipient heifers. However, hCGd0&5 had similar P/ET on d60 compared with all other groups. These findings suggest that hCG administration on d0 increased P4 levels too early, leading to detrimental effects on the maintenance of pregnancy. However, hCG treatment on d5 could mitigate some of those adverse effects of hCG treatment on d0.

Research Grant: None
Student Support: NIH T35 Training Grant: NIH T35 OD011078
Evaluating frequency and phenotype of MDSCs and Tregs in canines with Myasthenia Gravis and healthy controls

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Myasthenia Gravis (MG) is a T cell-dependent B cell-mediated autoimmune disease caused by autoantibodies against acetylcholine receptors (AChR) at neuromuscular junctions. In both dogs and humans, acquired MG occurs spontaneously suggesting that canine MG may serve as a relevant model for investigating the disease. Multiple factors such as MHC genes, breed, environment and infection play a role in breaking down immune tolerance. However, the exact cellular pathogenesis of MG remains unknown. Myeloid-derived suppressor cells (MDSCs) reduce inflammation by secreting immunosuppressive cytokines, enzymes and other molecules that block T cell activation and by recruiting regulatory T cells (Tregs) that suppress an immune response. Due to their immunosuppressive capacity, MDSCs and Tregs have the potential to play a therapeutic role in MG. In our study we compared the frequency and phenotype of MDSCs and Tregs in peripheral blood of healthy dogs, seropositive dogs (i.e. with circulating anti-AChR antibody titers greater than 0.6nmol/L) and seronegative dogs. Our preliminary data suggest there are no statistically significant differences in frequencies of MDSCs or Tregs. However, we recognized that a comparison of the activation state of myeloid and lymphoid cells could be more revealing. To determine the activation state of each cell type, we examined the expression of CD11b and MHC Class II, both of which are upregulated in inflammatory situations. Specifically, we hypothesized that dogs with MG would have more activated non-regulatory lymphocytes and less activated MDSCs as compared to healthy or seronegative dogs. We will report on our preliminary findings.

Research Grant: None.  
Student Support: NIH T35 Veterinary Summer Scholars Program.

Comparative evolutions and pathology of coronaviruses infecting animals and humans

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The pathogenesis of several coronavirus infections remains largely unknown. We analyzed coronaviruses (α, β, or γ) infecting cattle, dogs, swine, cats, chicken, turkey, ferret, mink, alpaca, dolphins, and SARS CoV-2 from human and a Tiger. Besides genome topology, multiple sequence alignments and phylogenetic trees were built for the widely expressed Spike (S), Envelope (E), Nucleocapsid (NC) and Membrane (M), proteins. Two receptors, Aminopeptidase-N (APN) and ACE2 were also compared. Results show that S, E and NC proteins segregated according to their viral genera (α, β, or γ). Poultry and dolphin γ-virus proteins or ferret and mink α-virus often co-segregated, showing distant relationships from others. As expected, swine α-viruses shared a very close common origin. Canine respiratory β-virus proteins were very closely related to bovine viruses. However, M proteins of SARS CoV-2 β-virus were very distant from other β-viruses, instead having a closer origin with poultry γ-viruses. The functions of E and M proteins in virus-host interaction are not fully known. On analysis of the receptors, the phylogeny of APN and ACE2 showed that the hosts for α-viruses shared a common APN lineage and the canine and bovine ACE2 proteins shared a common lineage. Based on these analyses, a likely interspecies crossing-point for β-viruses may be between canines and bovines, as both the viral and receptor lineages show parallel evolutionary relationships. Comparison of clinical and pathological findings among the hosts for these viruses showed that species infected with α-viruses tend to have enteric signs, β-viruses infected species show respiratory signs, and species infected with γ-viruses exhibit both enteric and respiratory signs.

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Student Support: Student support: Boehringer Ingelheim Scholars Program (Shaddox), HRSA #D34HP00001 (Ware)
Modulation of antimicrobial peptide expression as a novel approach to treating infectious keratitis

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Antimicrobial peptides (AMPs) are soluble effector molecules expressed by epithelial cells and leukocytes which exhibit broad-spectrum activity against bacteria, fungi, and viruses. In general, AMPs can either be constitutively or inducibly expressed by these cells. A recent study identified three small molecules derived from Chinese plants that had the ability to upregulate a single AMP, human beta-defensin 3 (hBD3), in human colonic epithelial cells. In particular, they demonstrated a 100-fold increase in hBD3 mRNA expression in cells treated with andrographolide when compared with controls.

Our laboratory sought to determine the ability of andrographolide to upregulate hBD3 mRNA in immortalized human corneal epithelial cells (hTCEpi cells). We found that human corneal epithelial cells treated with andrographolide resulted in a 300-fold upregulation in hBD3 mRNA expression when compared with vehicle control alone. To verify these findings at the peptide level, the current study utilized a commercially available ELISA to quantify hBD3 peptide levels in the supernatant of hTCEpi cells treated with varying concentrations of andrographolide. Indeed, hBD3 peptide expression was markedly increased in a dose-dependent manner by hTCEpi cells treated with 75 μM and 100 μM of andrographolide at 260 pg/mL and 488 pg/mL, respectively. No hBD3 peptide was detected in the supernatants of hTCEpi cells treated with lower doses of andrographolide treated cells (50, 25, 10 μM) or vehicle control (DMSO) alone. This study serves as the framework for promising translation studies investigating a novel therapeutic approach to augment host AMP expression with andrographolide in the treatment of corneal infections.

Research Grant: National Institutes of Health K08EY028199
Student Support: Summer Training in Advanced Research (STAR) from UC Davis School of Veterinary Medicine

Parainfluenza virus 5 (PIV5) expressing 3CL-pro and RdRp as novel vaccines against SARS-CoV-2

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The outbreak of COVID-19 has caused a pandemic that has spiked widespread concern and jeopardized global health security. The causative agent of COVID-19, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a newly emerged human coronavirus (HCoV) with similarities in the epidemiology, clinical features, and genetics to the SARS-CoV pandemic in 2003. This incident highlighted the importance of developing effective vaccine designs and antiviral therapies. Recently, our lab published on the generation of a PIV5-based (parainfluenza virus 5) MERS (Middle East Respiratory Syndrome) vaccine, indicating our PIV5-MERS-S as a promising vaccine candidate against MERS-CoV and potentially other emerging HCoVs, such as SARS-CoV-2. With this data, we aim to develop a PIV5-based vaccine for SARS-CoV-2 focusing on 3CL-pro (3-cysteine-like proteinase) and RNA-dependent RNA-polymerase (RdRp) of ORF1ab. Both 3CL-pro and RdRp are highly conserved among SARS CoVs and HCoVs. 3CL-pro is essential for the majority of the cleavage events during polyprotein processing and Nsp (nonstructural protein) maturation for betacoronaviruses. RdRp is the protein responsible for the formation of the replication and transcription complex, essential for viral genome replication. Furthermore, there is evidence to support the presence of T Cell epitopes in these enzymes, which have been linked to eliciting increased immune responses and long-term immunity to CoV infection when compared to B Cell responses. The high genetic and structural similarities and conservation, along with the potential to elicit T Cell responses, encourages the pursuit of engineering a PIV5-based SARS-CoV-2 vaccine expressing Nsp novel antigen targets.

Research Grant: None
Student Support: 2020 NIH T35 Summer Research Fellow (Grant Number: 5T35OD010433-14)
Hyperextension of the fetlock as a mechanism for fracture of the proximal sesamoid bones in racehorses

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Musculoskeletal injury is the leading cause of death in racehorses, with fracture of the proximal sesamoid bone (PSB) the most prevalent injury in the United States. A focal subchondral lesion located abaxially has been found in the PSBs from both limbs of horses that experience unilateral PSB fracture; the lesion precedes fracture and develops due to microdamage from repetitive fetlock hyperextension. Hyperextension may cause the PSB to extend beyond its normal articulation with the distopalmar portion of the third metacarpal bone (MC3) condylar surface, which could create high local stresses on the PSB. It is hypothesized that loading cadaveric limbs to stimulate a high-speed gallop will cause malarticulation of the PSB with the MC3 condyle in a pattern consistent with development of the subchondral lesion. The goal of this study is to describe the three-dimensional motion of the PSBs relative to the MC3 condylar articular surface and the joint contact surface regions simulated racing-speed loads. The positions of bone fixed kinematic markers were recorded with high speed video during axial limb loading to 10,500 N (racing gallop load) using a servohydraulic testing system. Intrarticular injection of methylene blue dye during limb loading will be used to assess non-contact areas of joint surfaces at limb dissection. Preliminary data indicate that the PSBs rotate in a manner consistent with increasing abaxial subchondral stress during fetlock hyperextension. Understanding the factors that contribute to PSB fracture can be used to design injury prevention strategies and improve the welfare of racehorses, jockeys, and public perception of the racehorse industry.

Research Grant: Dolly Green Endowment, JD Wheat Veterinary Orthopedic Research Laboratory, University of California, Davis
Student Support: Center for Equine Health and Student Training in Advanced Research Program

The selective BCL6 inhibitor decreases Germinal Center (GC) formation and reduces kidney in lupus induced mice

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The transcriptional repressor B cell lymphoma 6 (BCL6) is a key regulator that controls a large transcriptional network required for the formation and maintenance of germinal centers (GC) during the antibody mediated response. GCs are the main sites in which B cell go through class-switch recombination and somatic hypermutation. Since BCL6 is critical for GC formation, we hypothesize that a selective BCL6 inhibitor (BCL6i) will decrease GC formation and reduce kidney disease in NZB×NZW F1 (NZB/W) female lupus mouse model. In our studies, we examined the efficacy of BCL6i in NZB/W spontaneous mouse models of SLE. NZB/W mice were treated with a selective BCL6 inhibitor at 30 mg/kg or 100 mg/kg body weight (BW). As a positive control 2mg/kg BW Dexamethasone (Dex) was administered daily. Negative controls received Methyl Cellulose (MC) only beginning at 20 weeks of age. Treatment with both concentrations of the BCL6i significantly decreased development of proteinuria, the formation of germinal centers, and decreased anti-DNA titers. At both concentrations, the BCL6i treated mice displayed a significant decrease in GC formation in the spleen as well as a significant inhibition of both IgG and C3 in the kidney. At the 100 mg/kg BW treated mice, the total number of B cells as well as B cell subsets (immature, mature, and class switched) were decreased in the spleen of NZB/W mice. Overall, the efficacy of BCL6i in significantly ameliorating multiple pathological measures associated with lupus. Taken together these results provide a strong rationale for BCL6 inhibition as therapeutic approach for lupus nephritis.

Research Grant: Virginia Maryland College of Veterinary Medicine Office of Research and Graduate Studies
Student Support: None
Detection and species determination of nematodes in ruminant zoo populations

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Trichuris spp. are parasites infecting domestic and wild ruminants at The Wilds, a wildlife safari park in Cumberland, Ohio, as well as, at wildlife parks worldwide. These helminths cause significant morbidity and mortality in The Wilds’ giraffe (Giraffa camelopardalis) and other hoofstock. Little is known about parasitism in collection giraffes. Transmission of parasites between ruminant species is well reported, and The Wilds has an overabundant free ranging white-tailed deer (Odocoileus virginianus) population. We hypothesize that transmission of Trichuris is occurring between white-tailed deer and giraffe; meaning these hosts have the same Trichuris sp. infection. This research project will document the species of Trichuris in these host populations using PCR and genetic sequencing. Additionally, optimization of detection of fecal and environmental Trichuris eggs from different temperate areas is necessary for monitoring collections. Each species of Trichuris egg has a unique specific gravity, ranging from 1.130 to 1.150. Detection methods for soil and fecal eggs requires optimization based on specific parasite egg density and soil substrate characteristics. Previous studies have used a variety of flotation and centrifugation methods. Flotation solutions used include Sheather’s sugar (sp. grav. 1.27), sucrose (sp. grav. 1.45), ZnSO4 (sp. grav. 1.18), and MgSO4 (sp. grav. 1.24). We will test these solutions using a double centrifugation method to optimize the fecal and soil detection of Trichuris eggs from multiple semi free-ranging zoo institutions. We hypothesize that the sucrose solution (sp. grav. 1.45) will be the most sensitive.

Research Grant: T35 NIH Training Grant
Student Support: None

A call to action: exposing barriers to veterinary care in Pomona, CA

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According to the American Veterinary Medical Association and the U.S. Census Bureau, there are 38,869 households in Pomona, CA and 20,328 of those are pet-owning households. Veterinary professionals have recognized that many pets in this area do not receive regular veterinary care. The objective of this study is to uncover the specific barriers to veterinary care that are keeping Pomona pets from receiving adequate healthcare and utilize this information to improve pet health. A previous study found that financial constraints are a significant barrier to veterinary care nationwide (Blackwell et. al.). The hypothesis of this study is that there are non-financial barriers such as transportation, language, and cultural differences present in the Pomona community. We are conducting a survey of Pomona residents regarding demographics, characteristics of pets, as well as decision-making factors and barriers related to veterinary care. Results of this survey will allow us to understand the factors that impede access to care in this community. With an understanding of the financial needs, non-financial needs, and cultural identity of our local pet owners, we can work towards making veterinary care more accessible. Following our study, we may implement solutions such as subsidized costs of services, transportation, and tools for communication between veterinarians and owners. This a potential stepping stone towards the overarching goal of keeping pets in the home and off the streets or out of the shelter.

Research Grant: College of Veterinary Medicine intramural funds
Student Support: PetSmart Charities
Review on the sexual transmission of arthropod-borne viruses between vertebrate hosts

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This review was written for the purpose of studying sexual transmission of arthropod-borne viruses (arboviruses) between vertebrates. This study encompassed the three major groups of the arboviruses, the genus Flavivirus, the genus Alphavirus, and the order Bunyavirales. When previous articles and research were reviewed, the detection of virus or viral RNA in vertebrate semen or mucosa was the main factor for consideration as to whether or not the arbovirus could potentially sexually transmissible. Through searching various articles, it was discovered that four flaviviruses, three alphaviruses, and two bunyavirales may be sexually transmissible between humans or vertebrate animals. When the actual transmission of the virus was reviewed, it was surmised that male to female infection was most likely between vertebrates. It was hypothesized that male reproductive tissues, such as the testes, were excellent replication environments for arboviruses due to their trait of being immunologically privileged. Due to the growing number of studies providing evidence of sexual transmission of arboviruses between vertebrates, additional research is needed to further investigate this issue. Research has shifted to more obscure families of arboviruses where further articles are under review.

Research Grant: Iowa State University, College of Veterinary Medicine
Student Support: NIH T35 Training Grant

Exploring the molecular mechanisms of an NQO1-bioactivatable quinone for the treatment of NQO1+ breast cancers

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An estimated 1.8 million people, 6 million dogs and cats will be diagnosed with cancer in 2020. Most cancer chemotherapy treatments for companion animal species originate as drugs for human cancers. In cancer therapy, it is imperative to develop treatment modalities that selectively target tumors based on their unique molecular signatures. NAD(P)H quinone oxidoreductase 1 (NQO1), is a detoxification enzyme expressed in low amounts in normal healthy tissue, but significantly overexpressed in many solid tumors. Research from our lab has demonstrated that difficult to treat NQO1+ BRCA1/2-mutant breast tumors are selectively sensitized to the NQO1 bioactivatable quinone, isobutyl-deoxynyboquinone (IB-DNQ). NQO1 metabolizes IB-DNQ in a futile redox cycling mechanism that results in the formation of reactive oxygen species (ROS), causing cell death by DNA damage. Cytotoxicity can be prevented by inhibiting NQO1. However, the mechanism of IB-DNQ-induced cell death in NQO1+ BRCA1/2-mutant breast tumors remains unknown. Here, we analyzed RNA-seq data from BRCA1/2-mutant human breast cancer cells treated with IB-DNQ or vehicle alone at various doses and times. We uncovered a number of differentially expressed genes whose products function in ER stress responses and apoptosis that are significantly upregulated after lethal doses of IB-DNQ. Future work will validate these findings using qRT-PCR. In addition, since canine and feline tumors also overexpress NQO1, we will determine their sensitivity to IB-DNQ and other NQO1 bioactivatable quinones. The results from this study will elucidate the mechanism(s) of IB-DNQ-induced cytotoxicity for targeted treatment of NQO1+ human, canine and feline tumors.

Research Grant: None
Student Support: NC State CVM Veterinary Scholars Program Fluoroscience Endowment
Alzheimer’s disease-like pathology in aged cats and cats with glaucoma

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There is ample evidence linking Glaucoma and Alzheimer’s Disease (AD), two neurodegenerative diseases that are leading causes of irreversible blindness and dementia, respectively. AD patients are more likely to develop glaucoma and vice versa but the exact mechanism responsible for this association is unknown. Neurofibrillary tangles (NFTs) of hyperphosphorylated tau proteins (pTau) and plaque aggregates of amyloid-β peptide (Aβ) are characteristic of AD neuropathology and have been found in animal models of both AD and glaucoma. Cognitive dysfunction syndrome (CDS) afflicts geriatric cats, mirroring human dementia - thus, cats may serve as a spontaneous model for AD. We hypothesize that in a feline model of chronic glaucoma, affected cats will exhibit AD-like pathology including NFTs and Aβ plaques in the brain and optic nerve at a relatively young age. Using archived tissues from young adult cats with glaucoma due to LTBP2 mutation, normal aged matched cats, and aged cats (expected to have age-related AD-like pathology), NFTs and Aβ plaques are quantified in optic nerve and in brain regions associated with visual processing and with AD pathology in humans. Cryosections immunofluorescent labeled for Aβ, pTau and Iba1 (to detect microglia), and paraffin embedded sections stained with modified Bielschowsky silver staining to detect NFTs, are examined by microscopy for manual quantification of these AD-markers. Quantification of Aβ and microglia in immunolabeled sections is ongoing. Preliminary results support our hypothesis that glaucoma is associated with an increase in NFTs in cats with glaucoma, providing further evidence to support a role for glaucoma-induced neurodegeneration in promoting AD pathology in the brain.

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Student Support: NIH T35 OD011078

Effects of exercise on intestinal tissue in an aging accelerated murine model

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Senescence Accelerated Mouse-Prone 8 (SAMP8) mice display a phenotype of accelerated aging and are used to study mechanisms and physiologic effects of aging. The aim of this study was to determine whether or not exercise benefits intestinal tissue in SAMP8 male and female mice. SAMP8 mice (8-9 weeks old) were randomly assigned to one of the following groups (n = 3-6/group): control sedentary SAMP8 male, runner SAMP8 male, age-matched wild-type (Wt) male, control sedentary SAMP8 female, runner SAMP8 female, or age-matched Wt female. Exercise consisted of wheel running on a voluntary basis seven days per week for the entire study duration of 12 weeks. The total distance ran each week was tracked. On average, female mice consistently ran further (57.516 ± 2.640 km/week, P < 0.05) than male mice (43.950 ± 2.506 km/week). SAMP8 males and females had comparable weights at the end of the study regardless of exercise training, while the age-matched controls gained significantly more weight. We are currently determining expression of key nutrient transporters SGLT1, PEPT1, GLUT2, and GLUT5 in jejunum, using standard western blot techniques. In addition, we are also assessing jejunum morphology (villi length, crypt depth, and wall thickness) using ImageJ software. Current preliminary data suggests runner SAMP8 males have an ~30% increase in villi length compared to control sedentary SAMP8 males and age-matched Wt males, with no differences noted between female groups. These findings indicate some sex-dependent benefits of exercise in the small intestine of this model of aging. [WKL1]

Research Grant: Midwestern University Arizona Alzheimer’s Consortium Grant
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Cell biology of intercellular communication probed by *Escherichia coli* enterotoxins

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Extracellular vesicles (EVs), which include exosomes and microvesicles, are nanometer to micrometer size membrane vesicles known to act as protein, lipid, and nucleic acid cargo for cell-to-cell communication. EVs are either pathogen or host-cell derived, and influence behavior of recipient cells, by their cargo of bacterial toxins. Several toxins, including lipopolysaccharide, Shiga, and Alpha, are known to mediate their toxicity on host cells, and result in either activation or inhibition of immune responses, by pathogen-derived EVs. We hypothesized that similar to other toxins, the heat-labile enterotoxin (LT) from enterotoxigenic E. coli (ETEC), and its non-toxic B subunit (LT-B), regulate the immune response by EVs. A thorough Review of the literature indicated that LT/LTB as well as the other toxins have a dual role; inhibition and activation of immune cells and their products. Thus, while LT/LTB activate B cells and CD4+ T cells, they induce apoptosis of CD8+ T cells. Moreover, LTB internalization by B cells and proliferation of CD4+ T cells are significantly delayed, compared to other antigens. Analysis of EVs in supernatant from B cell culture, incubated with LT-B or LT, revealed 50% and 25% reduction in protein concentration, respectively, compared to untreated cells, while no protein was detected in exosomes. Our investigations unraveled a possible strategy by ETEC and other pathogens, an initial disruption of communication between immune cells, followed by late activation. This strategy would be particularly important for extracellular pathogens like ETEC; their secreted toxins in EVs cause diarrhea in absence of immune reaction, thus enable them to move to infect another host.

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Identifying genes of the shikimate, isoprenoid & ubiquinone pathways in *T. gondii*: known & novel drug targets

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The protozoan parasite, *T. gondii*, is one of the leading causes of food-borne illness in humans and one of five neglected parasitic infections in the US. To date, no efficacious chemotherapeutic treatments exist against the entire spectrum of its life stages. The pathways leading to the production of ubiquinone appear to be divergent between humans and *T. gondii* and harbour known and potentially novel targets for therapeutics. In outlining these pathways, this review seeks to determine and organize the genes predicted to express the enzymes of the shikimate, polyprenoid and ubiquinone biosynthesis pathways. We analyzed the literature describing homologous pathways in humans, yeast and other apicomplexans in addition to analyzing -omic data for *T. gondii*. The aim is to postulate a roadmap of the biosynthetic pathways involved in producing ubiquinone. Using these methods, it has been possible to outline similarities and differences between *T. gondii* and other eukaryotes in an effort to identify areas where further research is needed to fill in knowledge gaps. Of particular interest is the pentafunctional AROM complex involved in the shikimate pathway and the *T. gondii* homologue of Coq2. The ultimate goal of investigating these pathways in *T. gondii* is to identify genes that may serve as novel drug targets in the search for effective treatment of toxoplasmosis.

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**Student Support:** NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-14
Calf’s immune response subsequent to bovine respiratory diseases (BRD) vaccination and infection: a review

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Bovine Respiratory Disease (BRD) is a multi-factorial and complex disease process that has major impacts on every facet of the cattle industry. These drastic effects on cattle health and industry economics drive the need for a better understanding of the inflammatory changes at the genetic level modulated by a host’s response. A review of current knowledge on the inflammatory changes as they apply to the BRD complex has not been performed. The goal of this review is to characterize inflammatory changes following a natural inoculation. Additionally, this review addresses the gaps in current knowledge of inflammatory changes following a successful vaccination and challenge. We intend to present comparisons and contrasts between natural infection and vaccination to provide future research with knowledge to develop vaccines that better mimic natural exposure. We provide information regarding the following processes: leukocyte activity and differentiation, pro- and anti-inflammatory cytokines, chemokines, and toll-like receptors.

Research Grant: Unknown
Student Support: Boehringer Ingelheim, University of Florida Research Foundation.

Expression of CD44 on equine peripheral blood eosinophils

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Equine asthma is hypothesized to be an unnecessary response to non-infectious agents, carried out by immune cells such as eosinophils. Numerous studies have shown that an increase in eosinophil concentration can be a useful marker in diagnosing human asthma and equine asthma, yet the exact pathogenesis of these diseases is undetermined. Our research aimed at discovering if the surface glycoprotein CD44 can be found on the equine eosinophil. CD44 has numerous functions, one being cell adhesion, which plays a role in eosinophil migration into the lungs. Thus, CD44 contributes to the ability of eosinophils to cause mast cell degranulation, epithelial damage, and leukocyte inhibition within the lungs of human patients with asthma. Using a discontinuous Percoll gradient and density centrifugation, our lab successfully isolated an eosinophil population with 90% purity and 78% cell viability from whole blood extracted from horses at Midwestern University’s Equine and Bovine Center. Our next steps will be staining and labeling the isolated eosinophil population with fluorescent IgG isotype monoclonal antibodies specific for the equine CD44 protein. Flow cytometry will analyze the cells for light emitted from the antibodies to determine if equine eosinophils display CD44. If equine eosinophils display CD44, further research into the role of eosinophils in equine asthma could be studied, and potentially have implications for the understanding of human asthma.

Research Grant: Midwestern University College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Evaluation of clinical signs in small and large animal patients presenting with abnormal blood glucose levels

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Glucose abnormalities among small and large animal patients are characterized by hypoglycemia and hyperglycemia. Hypoglycemia occurs when the blood glucose drops below a normal range, which varies depending on the species. Hyperglycemia occurs when the blood glucose rises above the normal range. Patients suffering from hypoglycemia or severe hyperglycemia show signs of cerebral dysfunction including behavioral changes, ataxia, seizures, stupor, and in severe cases coma. These signs occur because the brain does not store glucose on its own, therefore it relies on hepatic glycogen breakdown to supply the glucose needed for normal function. However, there are numerous other etiologies for both hypoglycemia and hyperglycemia; including excessive or decreased glucose utilization, glucose production or insulin production. The aim of this study was to examine similarities and differences in the clinical presentation of patients with abnormal blood glucose levels.

Research Grant: None
Student Support: Boehringer Ingelheim

Factors affecting health and welfare outcomes of calves in chronic pens in beef feedlots

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Care of compromised animals is increasingly of interest to stakeholders in the livestock industry. In beef feedlots, evidence-based scientific literature is lacking for calves in chronic pens, particularly timely euthanasia. The objective of this poster is to describe outcomes of beef calves placed in chronic pens, with particular focus on timely euthanasia. This poster is part of a pilot study assessing production and treatment outcomes, animal welfare, and economic impacts associated with this aspect of feedlot management. We hypothesize that limited information on effective treatment protocols and standardized procedures result in failure to provide timely euthanasia, creating potential for unassisted death and suboptimal welfare. A questionnaire was conducted at five feedlots in Iowa to survey management protocols and animal records. Animal welfare assessments of chronic pens were performed at three time points to characterize calf demographics. Four out of five feedlot managers retrospectively estimated that more calves that entered chronic pens died than were euthanized (21% vs 10%). Of calves observed during assessments, 14% died, 3% were euthanized and the remainder returned to home pens or were harvested. Decisions made regarding euthanasia also tended to coincide with marketing or shipping of other cattle. Most producers (4/5) described euthanasia plans for handling non-ambulatory cattle, but not other compromised cattle. Chronic animals present novel challenges to producers and practitioners who need to balance disease processes and unique individual animal welfare needs while maintaining a cost-effective operation. These factors will be further explored during focal group discussions with feedlot producers.

Research Grant: Iowa Veterinary Medical Association
Student Support: Iowa State College of Veterinary Medicine Summer Scholars Research Program
Dose-exposure-response of benazepril on biomarkers of the renin-angiotensin-aldosterone system in dogs

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Medications that mitigate the renin-angiotensin-aldosterone system (RAAS) are used in the pathophysiologic treatment of congestive heart failure (CHF) in dogs. Although angiotensin converting enzyme inhibits (ACEi), such as benazepril, are routinely used for treatment of CHF, the optimal dosage of ACE inhibitors for CHF treatment remains unknown. This study aimed to investigate the dose-exposure-response relationship on biomarkers of the RAAS and hemodynamic parameters in healthy dogs. Nine healthy beagle dogs were administered three different doses of oral benazepril (0.125 mg/kg every 12 hours; 0.25 mg/kg every 12 hours; or 0.5 mg/kg every 24 hours) in a randomized crossover design following induction of RAAS activation by consuming a low-sodium diet. Blood samples were collected at serial time intervals up to 24 hours after benazepril dosing for measurement of plasma benazeprilat and serum RAAS biomarkers, including angiotensin II and aldosterone. Hemodynamic parameters, including blood pressure and echocardiogram, were measured the day following each benazepril administration. A dose-exposure-response relationship will be constructed by comparing plasma benazeprilat concentration to serum RAAS biomarkers at different ACEi doses and frequencies. These results will identify the benazepril dosage that optimizes neurohormonal and hemodynamic response in a healthy dog population. The ultimate goal of this study is to revise the recommended dosage of benazepril for dogs with CHF.

Research Grant: Ceva Sante Animale
Student Support: Van Gelder Grant

Investigating potential cross-reactive antibodies to SARS-CoV-2 in BCG-vaccinated diversity outbred mice

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The Bacillus Calmette-Guerin (BCG) vaccine, composed of live attenuated Mycobacterium bovis, remains the only vaccine available for human use against tuberculosis. However, the impact of this vaccine extends beyond tuberculosis: a continuously-growing body of evidence supports non-specific cross-protective immunological benefits to BCG vaccination against a broad spectrum of pathogens unrelated to Mycobacterium. While the full mechanism of such cross-protective benefits remains to be elucidated, one proposal includes antibody cross-reactivity to a wide array of antigens. We hypothesize the BCG vaccine may deliver a humoral response against the spike protein on SARS-CoV-2. Currently, there is an urgent need to investigate potential cross-reactive antibodies to SARS-CoV-2 from two points of view: 1) Development of a vaccine to optimize the humoral immune response to the pathogen and 2) Awareness of the impact cross-reactive antibodies may have in generating false positives in serological tests from BCG-vaccinated humans. This study aims to investigate the presence of cross-reactive antibodies to SARS-CoV-2 from the sera of BCG-vaccinated Diversity Outbred (DO) mice using enzyme-linked immunosorbent assays (ELISAs). We will use the following test antigens: 1) Recombinant spike receptor binding domain (RBD) protein; 2) Recombinant spike protein; 3) Irradiated, SARS-CoV-2 coronavirus; and 4) Heat-inactivated SARS-CoV-2 coronavirus. Protocols and controls for ELISAs coated in RBD protein are currently being optimized. Absorbance values from sera of BCG-vaccinated DO mice will be compared with absorbance values from sera of adjuvant-vaccinated DO mice.

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Student Support: Cummings School of Veterinary Medicine, Tufts University
Diagnostic performance of computed tomography for diagnosis of cardiomegaly in parrot and raptor species

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The aim of this study is to evaluate and compare cardiac features of avian hearts using various measurements to differentiate between normal heart size and cardiomegaly in psittacine and raptor species with the use of Computed Tomography (CT), as it allows for visualization of fine detail of the heart. The assessment of various cardiac measurements and their ratios to adjacent structures is used to determine which structures have statistical difference in size between psittacines and raptors with cardiomegaly and those with normal hearts. As radiography, echocardiography, and electrocardiography are more routine exam procedures in avian patients with suspected cardiac disease, the use CT for this purpose is under-studied. This study aims to assess the diagnostic performance of the CT exam as a method of discriminating differences between healthy and enlarged psittacine and raptor hearts via the use of this diagnostic imaging modality. Measurements included 21 measurements of the heart and surrounding structures of 17 psittacines and 11 raptors that underwent a CT scan and necropsy at the University of Florida. Measurements were taken in dorsal, sagittal, and transverse planes. Ratios were then calculated in order to compare the size differences between differently sized birds. These ratios will be used to determine if there is a meaningful difference between birds with cardiomegaly and those with healthy hearts. Results are forthcoming.

Research Grant: University of Florida College of Veterinary Medicine
Student Support: Florida Veterinary Scholar’s Program

Identifying differences in antigen-stimulated leukocyte cytokine production in dogs with coccidioidomycosis

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Coccidioidomycosis, or Valley Fever, is a common fungal infection endemic to the southwest desert regions of the Americas and has been documented in both humans and canines following disruption of soil and inhalation of infectious arthroconidia. The infection can manifest as primary pulmonary disease or disseminated disease, with higher rates of dissemination described in canines. Previous research has shown that human subjects with a dominant cell-mediated response have adequate protection against coccidioidomycosis, as well as protection against subsequent dissemination. Our research seeks to describe immunological differences in canines by comparing constitutive and antigen-stimulated leukocyte cytokine production in healthy non-immune dogs, dogs with pulmonary restricted coccidioidomycosis, and dogs with disseminated coccidioidomycosis. Plasma samples will be collected from 10 healthy dogs, 10 dogs with primary pulmonary disease, and 10 dogs with disseminated disease to compare leukocyte cytokine concentration at baseline and after 24 hours stimulation with CTS1 protein, lipopolysaccharide, lipoteichoic acid, or phosphate-buffered saline (negative control). The results from this research will add to the immunologic knowledge of coccidioidomycosis in dogs, with the ultimate goal of aiding novel immunotherapy development to help prevent infection or abrogate dissemination.

Research Grant: Research Grant: Midwestern University College of Veterinary Medicine
Student Support: Student Support: Boehringer Ingelheim Veterinary Scholars Program
Effect of chemotherapy agents on fecal microbiome of canine lymphoma patients

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Chemotherapy-induced gastrointestinal disease (CIGD) has been associated with chemotherapy treatment resulting in symptoms of clinical nausea, vomiting and diarrhea. CIGD can decrease quality of life and is a common reason for discontinuation of potentially life-saving chemotherapy. We hypothesized that vincristine would decrease the diversity of the canine fecal microbiome and create dysbiosis of gastrointestinal bacteria in dogs with lymphoma. Nine canine lymphoma patients undergoing cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy had fecal samples collected weekly before and throughout treatment. Vincristine, cyclophosphamide, doxorubicin and prednisone were administered weekly according to CHOP protocol. 16s rRNA gene sequencing analysis was performed on DNA from fecal samples to determine the composition and relative abundance of bacterial taxa in the microbiome. From each dog, 16s sequencing data from ten longitudinal samples were analyzed using QIME2 and bacterial taxa assignments made using the SILVA database. During intervals of vincristine treatment, changes in the relative abundance of bacterial taxa were observed using heatmaps and percentage of change divergent plots. These plots provided evidence that vincristine administration caused dysbiosis within the canine gastrointestinal tract. The specific bacterial taxa affected suggested that commensal bacteria decreased and opportunistic pathogens increased. Further analysis will focus on determining if a significant association exists between the GI microbiota changes observed and clinical signs of CIGD. Future analyses will replicate a similar hypothesis with the other CHOP agents, cyclophosphamide, and doxorubicin.

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Student Support: Student Funding provided by NIH Grant 5T35OD016477-19 to Michigan State University

Investigating loss of function variants in the general equine population

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Large-scale whole genome sequencing efforts have driven huge successes in identifying and treating inherited disease. A surprising finding from these studies has been the inflated number of variants predicted to lead to loss of function (LoF) of the affected gene. We previously identified 5,027 LoF variants in whole genome sequences of 534 horses across 43 different breeds. The goal of this study was to remove false positive variants resulting from inaccurate gene annotation and genotyping errors. We are currently investigating 322 common LoF variants (minor allele frequency [MAF] ≥ 50%) and 261 rare LoF variants (MAF ≤ 5%) using hand annotation (NCBI Blast), visualization of the sequencing data (IGV), and additional computational variant effect prediction tools. Of the 322 common variants, 70% were frameshift mutations, 32% were true positives, and 31% of true positive common variants were considered evolutionarily conserved. Up to 10% of the common variants appeared to be true positives, but were at unexpected positions in the coding sequence. In contrast, 62% of the 63 rare LoF variants analyzed so far led to premature stop codons, with a lower incidence (31%) of frameshift mutations than seen in the common variants. In total, 96% of rare LoF variants were true positives, and 39% of true positive rare variants were considered evolutionarily conserved. Less than 2% of rare variants were at unexpected positions in the coding sequence. True positive LoF variants from this study will be experimentally validated to determine their likely phenotypic effects. Ultimately, this study will serve as a stepping stone into further advancement of identifying and managing genetic disease in horses.

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Student Support: University of Minnesota College of Veterinary Medicine Summer Scholars Program
A dose escalation and drug interaction study of cannabidiol administration in dogs with intractable epilepsy

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CSU Clinical Sciences Department

Idiopathic epilepsy affects nearly 5% of the canine population, with nearly a third of those afflicted demonstrating uncontrolled seizures on the standard drugs available. Cannabidiol (CBD) has demonstrated antiepileptic properties in humans and canine patients recently and could therefore present itself to be an effective adjunctive medication to standard antiepileptic drugs (AEDs). The purpose of this clinical trial is to discover the effective oral dose at which CBD, in adjunct to the patient’s standard AED protocol, will reduce seizures by 50%. We will also be looking at the pharmacokinetic and pharmacodynamic properties of CBD. Through this study we expect to find the effective oral dose of CBD to reduce monthly seizure activity by 50%. We also expect that CBD will result in measurable and predictable plasma concentrations. We will be enrolling patients that are having two or more seizures a month for at least 3 months while receiving standard AEDs. Patients will be randomly assigned to one of 3 dosing groups for 12 weeks with CBD doses given BID (twice a day) as follows; 5mg/kg, 10mg/kg, and 20mg/kg. Clients will be recording seizure activity daily and we will monitor the animal’s health every 4 weeks through physical exams, CBC, and chemistry panels. CBD plasma levels will be collected every 4 weeks throughout the study period. With this study we expect to be able to determine the effective oral dose of CBD that will reduce average monthly seizure activity by 50% or more in dogs with uncontrolled idiopathic epilepsy when used in adjunct to standard anticonvulsive therapy.

Research Grant: Internal Funding
Student Support: Boehringer Ingelheim Veterinary Scholar

Here’s the tea on T cells: equine supporting limb laminitis (SLL) is associated with CD3+ T cell infiltration

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Supporting limb laminitis (SLL) is a common, debilitating, and often fatal complication of equine lameness. Altered weight-bearing disrupts the epidermal and dermal lamellae resulting in failure of the suspensory apparatus of the digit within the hoof capsule. The tissue damage triggers inflammation and lesions similar to human psoriasis. As reported, the interleukin (IL)-17 pathway, the major pro-inflammatory effector cytokine in psoriasis, is also activated in SLL. Th17 cells, a subset of CD3+ T cells, produce IL-17 in psoriasis. We tested the hypothesis that lamellar CD3+ T cell infiltrates correlate with laminitis histopathological severity using immunohistochemistry (IHC) and image analysis to identify and quantify T cells using an anti-equine CD3 primary antibody (a pan T cell marker) in archived lamellar tissue from SLL cases and controls. Positive cells were counted as a percentage of all cells in three separate locations along the primary epidermal lamella, axial, middle, and abaxial relative to the limb’s axis, using digital image analysis (QuPath). Samples were assigned to histopathological subgroups (N = 7 to 9) based on severity and stage of laminitis: Control, Developmental, Moderate Acute, Severe Acute (SA), or Severe Chronic (SC). ANOVA on Ranks and Dunn’s test for multiple comparisons were used to demonstrate significantly higher proportions of CD3+ T cells in SA and SC cases vs controls. Scoring is in progress to investigate differences in CD3+ T cell tissue distribution between controls (mostly perivascular) and SA or SC cases (more diffuse). These data support the hypothesis that CD3+ T cells are positively correlated with SLL severity and IL-17 pathway activation.

Research Grant: School of Veterinary Medicine intramural funds (Raker/Tulleners Fund)
Student Support: NIH/BI Summer Veterinary Scholars Program (T35 Training Grant 5T35OD10919-23)
Pharmacokinetics of Simbadol™ in laboratory rats

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Buprenorphine is a common postoperative analgesic in rodents, however it fails to maintain proper therapeutic levels between dosing intervals and as a result inadequate pain management. As an alternative, Simbadol is an FDA approved veterinary drug labeled for 24 hr analgesic in cats and has been used in preliminary studies in mice to demonstrate behaviors indicative of prolonged and effective pain control. Little is known about Simbadol's effect on rats. The purpose of this study was to evaluate the safety and pharmacokinetics of Simbadol in a total of 18 male and female outbred Sprague Dawley rats ranging from 6-8 weeks of age. The rats were assigned an identification letter, weighed and dosed with 0.5 mg/kg of Simbadol subcutaneously in the interscapular region. Blood was drawn from the jugular vein from 3 rats of each sex prior to and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, and 72 hr time points following Simbadol injection in order to assess plasma concentrations. Plasma was separated, stored in -80° freezers, and further analyzed. Daily observations were recorded for any adverse side effects such as pica, injection site reactions, or weight loss. Rats exhibited mild signs of pica behavior and mild sedation for the first few hours following Simbadol dosing. No other adverse effects were seen. We hypothesize that therapeutic levels of Simbadol (1.0 ng/ml) are maintained up to 12 hrs in the rats, T_max ranges from 1-2 hrs, and the half-life is 4 hrs. The use of Simbadol as an analgesic for post-operative pain management enhances the recovery of rat surgical models by providing a safe and prolonged therapeutic level with minimal side effects.

Research Grant: ACLAM Foundation Greg Boivin Memorial Award
Student Support: ASLAP Summer Fellowship Program

Wild parrots in the city: a case study of parrots and parakeets in Point Loma, San Diego

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Worldwide, many parrot and parakeet populations are declining in their natural ranges. Several species of parrots and parakeets that are vulnerable, threatened, or endangered in their native habitats have established breeding populations in Southern California. However, due to their non-native status, these parrots and parakeets are not monitored. Beyond a few reports documenting species presence and population size estimates at particular roosting sites, little is known about the ecology of these birds. Specifically, no recent studies exist describing how parrots and parakeets interact with the highly urbanized environment they inhabit or the threats that this environment might pose to their health. The purpose of this study was to take a first step in filling this void by systematically documenting wild parrots and parakeets in their non-native environments in a Southern California urban coastal community. Wild parrots and parakeets were observed daily for seven weeks at a single location and along transects in Point Loma, San Diego. Parrot and parakeet species, numbers, behaviors, perching sites, and food sources were documented, and potential threats associated with their behaviors and locations were noted. A survey of local residents will collect data to complement these observations, identify additional health threats, and gauge residents’ attitudes and opinions towards the wild parrots and parakeets in their neighborhood. Threatened and endangered species breeding and apparently thriving in a highly urban environment far from their native ranges presents a unique conservation dilemma and an opportunity to study the role that these birds play in their new ecosystem.

Research Grant: None
Student Support: Western University of Health Sciences Office for Research and Biotechnology, and CVM
An unbiased O-GlcNAc transferase interactome confirms a role for OGT in X-linked intellectual disability

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The O-GlcNAc Transferase (OGT) is an essential and ubiquitous nucleocytoplasmic glycosyltransferase with roles in many diseases common to humans and animals, including cancer, diabetes, and neurodegeneration. OGT modifies thousands of proteins with β-N-Acetyl-Glucosamine (O-GlcNAc) and is involved in many cellular processes, including nutrient sensing, transcriptional regulation, and modulation of signaling pathways. O-GlcNAc is analogous to protein phosphorylation, but in contrast to the hundreds of known protein kinases, OGT is the only enzyme responsible for the O-GlcNAc modification in mammals. Therefore, the mechanism of OGT substrate selectivity is a major area of interest. It is thought that the tetratricopeptide repeat (TPR) domain of OGT, rather than the catalytic domain, is responsible for OGT substrate selection, in part by the recruitment of partner proteins that target OGT to specific substrates. However, very few partner proteins for OGT have been identified. In order to understand OGT’s cellular functions and its role in disease, it is essential to identify OGT interactors. To this end, we have defined an unbiased OGT TPR interactome using the proximity proteomic method BioID in HeLa cells. This interactome consists of 115 interactors enriched in nuclear localization and chromatin remodeling functions. Many of the interactors are also linked to developmental disorders that feature intellectual disability. Several mutations in OGT have recently been identified to cause X-Linked Intellectual Disability (XLID), but the mechanism of this disorder is currently unknown. Therefore, these interactors represent a set of candidate proteins whose interaction with OGT may underlie the mechanism of OGT-linked XLID.

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Student Support: Glycoscience Training Program (T32GM107004), NICHD (F30HD098828)

Investigating the effects of tadalafil and antifibrotic agent IPW-5371 on cardiac function in irradiated mice

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Currently therapeutic options for fibrotic diseases such as liver cirrhosis and idiopathic pulmonary fibrosis are very limited. Tadalafil, a phosphodiesterase 5 inhibitor commonly prescribed for erectile dysfunction and has some antifibrotic actions, and the investigational anti-fibrotic drug IPW-5371 were examined for therapeutic fibrosis therapy in an irradiated mouse model. The combination of agents unexpectedly resulted in increased mortality in C57 B6/N male mice, but not in female mice. This incidental finding indicates the lack of information regarding the possible relative contraindications between these two drugs in both mouse and human models. Death appeared to result from gross hemorrhage into body cavities and this study aimed to evaluate the pathological findings relating to these deaths. We investigated the effects of tadalafil and IPW-5371 administration on the cardiac and left ventricular outflow tract tissue in 134 irradiated C57 B6/N male and female mice. Tissues were collected, fixed, and sectioned and then evaluated and scored using a 0-3 scale to indicate the severity of the lesions using hematoxylin and eosin, and Masson’s trichrome stained serial sections. We hypothesized an increase in inflammation, hemorrhage, degeneration, as well as various other lesions would be noted more commonly in mice that had the combination treatment compared to other mice.

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Student Support: NIH T35 Training Grant T35OD10946
Development of a novel goat kid health scoring system

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The dairy goat industry is growing in Minnesota, with a 17% increase in animal inventory in 2018 (USDA, 2018). Gastrointestinal and respiratory diseases are common in pre-weaned goat kids, contributing significantly to compromised young-stock welfare and farm economic loss. If identified early in the disease process, incidence of morbidity and mortality due to these common diseases can be greatly decreased both on a population and an individual kid level. Currently, the dairy goat industry lacks a standardized way to efficiently assess the health of goat kid populations. The practice of routine kid health screening currently varies widely between operations, and often relies on subjective measures of health. The implementation of routine health scoring utilizing a standardized scoring system has been found to be effective in identifying respiratory disease early in dairy calves (McGuirk and Peek, 2014). Early disease identification and treatment is essential on dairy goat farms to promote kid health and welfare. We aim to develop a health scoring system for pre-weaned goat kids. The objectives of this system are to efficiently and economically assess the overall health of an entire kid herd, while also individually identifying kids in need of veterinary intervention. This health scoring system will allow veterinarians and producers to easily implement routine health screenings within a herd. This will promote active disease identification and management in pre-weaned goat kids within the dairy goat industry, leading to a reduction in goat kid morbidity, mortality, and farm economic loss.

Research Grant: None
Student Support: University of Minnesota, College of Veterinary Medicine, Office of Graduate Programs

The role of reptiles in tick-borne bacterial zoonoses worldwide

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Ticks are obligate hematophagous ectoparasites that serve as one of the most important vectors for human and veterinary pathogens worldwide. Tick-borne disease is increasing globally, including tick-borne bacterial zoonoses such as Lyme borreliosis caused by *Borrelia burgdorferi s.l.* and rickettsial diseases caused by *Rickettsia* spp., *Anaplasma* spp. and *Ehrlichia* spp. Reptiles are important hosts for ticks, and evidence exists that they may play a crucial role in the transmission of certain zoonotic pathogens such as *Borrelia lusitaniae* and *Rickettsia honei*. Many other zoonotic pathogens have been detected in ticks feeding on reptiles and in reptile tissue samples in recent years. Despite this, the reptile’s role in tick-borne disease remains largely understudied and unrecognized. In this review, we compile every record of *Borrelia, Rickettsia, Anaplasma*, and *Ehrlichia* in reptiles and ticks feeding on reptiles worldwide in order to synthesize current information on tick-borne bacterial zoonoses in reptiles, uncover patterns in disease ecology, inform public health and direct future research.

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Student Support: Woodmansee/Lider endowment funds at the UC Davis School of Veterinary Medicine
Identifying and characterizing behavioral phenotypes in captive gibbons

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Novel enrichment is the main staple of maintaining the overall mental and physical health of captive apes, including gibbons. Finding devices that can be categorized as successful forms of enrichment is especially difficult as gibbons exhibit preference for certain types of enrichment over other types. The main goal of this study is to classify several categories of behavioral phenotypes within the normal captive gibbon environment. Following the documentation of this functioning library, classified behavioral phenotypes can be more easily identified and quantified in captive settings. Three different species of Hylobatidae (Eastern Hoolock, Javan, and Pileated) are currently being observed for distinct behavioral phenotypes ranging from feeding and grooming behaviors to interaction with a variety of enrichment devices. From this data, we hope to better understand and identify behavioral phenotypes seen in these gibbons and ultimately relate certain behavioral phenotypes in these primates to something equivalent to human emotion that can serve to provide insight into successful enrichment devices.

Research Grant: Western University Intramural Student Summer Research
Student Support: None

Circadian rhythms effect on the efficacy of optogenetic seizure

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Epilepsy develops in roughly one in twenty-seven people during their lifetimes (England et al. 2012). Unfortunately, a high portion of these individuals do not receive adequate seizure relief with current treatment options. Other therapeutic treatments are being explored in order to combat the problem of no seizure relief. On-demand optogenetic stimulation of long-range inhibitory neuronal nitric oxide synthase expressing cells (LINCs) is being studied as a potential treatment in the Krook-Magnuson lab. The expectation is to decrease seizure duration, by stimulating hippocampal LINCs with light when a seizure is detected. However, other innate factors, such as circadian rhythms (Jin et al. 2020), may influence the pathophysiology of a seizure and ultimately affect therapeutic outcomes. The goal of my project is to investigate if circadian rhythm changes the efficaciousness of seizure interventions, using two different conditions: one with light (optogenetic intervention) and one with no light (as a control). We conducted on-demand optogenetic intervention over a 7-day period in three animals and gathered electroencephalogram (EEG) recordings that were analyzed in 2-hour intervals across each day to examine the duration of seizures with and without intervention. Preliminary data suggests a possible circadian rhythm effect. More specifically, optogenetic intervention appears to be more successful during the animals’ sleep cycle. Ongoing efforts include increasing our sample size to ensure the robustness of our findings and completing statistical analysis.

Research Grant: none
Student Support: Office of Graduate Programs
Pharmacokinetics, adverse effects, and effect on thermal nociception of codeine in horses

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Increased analgesic options are needed for the continued improvement of equine patient care. Although potent analgesics, the use of opioids in horses has been limited due to significant adverse effects including neuroexcitation and decreased GI motility. Preliminary studies with oral codeine in horses demonstrated similar morphine and morphine-6-glucoronide concentrations to that observed following IV administration of the reported analgesic dose of morphine, without the adverse effects. This study aims to describe the pharmacokinetics of codeine and metabolites, as well as describe the pharmacodynamics including anti-nociceptive and adverse effects. Seven horses received three doses of oral codeine (0.3 mg/kg, 0.6 mg/kg, and 1.2 mg/kg), a negative control of oral saline, and a positive control of 0.2 mg/kg IV morphine in a randomized, balanced 5-way crossover design. Liquid chromatography-mass spectrometry was performed on blood samples collected at various times up to 72hrs post-drug administration for concentration determination and pharmacokinetic analysis. Pharmacodynamic data obtained included effect on thermal threshold, step counts as an assessment of excitation, heart rate and rhythm, gastrointestinal borborygmi, and defection incidence and consistency. No significant adverse behavioral effects were observed with administration of the three codeine and saline doses. The results of the current study warrant further research to continue to explore the analgesic properties of codeine in horses and its potential use in equine patients.

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Student Support: National Institutes of Health T35 OD010956

Evaluation of potential reverse zoonosis of SARS-CoV-2 and establishment of reservoirs in domesticated animals

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Current COVID-19 pandemic is caused by SARS-CoV-2, which uses angiotensin-converting enzyme 2 (ACE2) as a receptor to enter cells. Widespread, extended infections of SARS-CoV-2 in human population could lead to potential reverse-zoonosis to animal species that come in close contact with humans (e.g. cats, dogs and other domesticated animals). Better understanding of the molecular interactions between the Spike (S) glycoprotein and ACE2, and how ACE2 differs between different species could provide insights into the potential for establishment of animal reservoirs and how easily the virus could be transmitted between humans and animals. Based on the published cocrystal structures of human ACE2 and the receptor-binding domain (RBD) of S glycoprotein, and based on sequence alignment analyses of ACE2 of different animal species and humans, we have estimated number of mutations that might allow SARS-CoV-2 to become able to infect other animal species. From our analyses, we hypothesize that different number of mutations are likely needed to infect different animal species. During the remaining research period, we hope to test our hypotheses experimentally. We are planning to do this biochemically using recombinant RBD and ACE2, as well as virologically using mutant pseudoviruses and cells expressing ACE2 of different animal species.

Research Grant: Unknown
Student Support: NIH T35
Immune effects of carboxylesterase inactivation in the neonatal murine lung

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Carboxylesterases (Ces) are enzymes that metabolize xenobiotics and anti-inflammatory lipid mediators. Chlorpyrifos (CPF) is an organophosphate pesticide (OP) that inhibits Ces activity at doses that do not inhibit acetylcholinesterase, the canonical toxic mechanism of action. Our lab’s recent data showed that chronic low-dose CPF inhibited lung Ces1c and Ces1d in neonatal mice. We hypothesized that pretreatment of neonatal mice with CPF would attenuate LPS-induced lung inflammation because of the diminished catabolism of anti-inflammatory lipids. Neonatal mice were treated with CPF (2.5 mg/kg, p.o.) or corn oil for 7 days from post-natal days 10 to 16, followed by LPS (1.25 mg/kg, i.p.) or saline. Tissues were harvested 6 hours after the LPS treatment. Lung IL-6, TNF-α, and IL-1β mRNA levels in response to LPS were unaltered by CPF exposure. CPF also did not alter the innate and adaptive immunophenotype of lung tissue in response to LPS. On the other hand, CPF decreased lung 2-arachidonoylglycerol (2-AG) levels independent of LPS treatment, but it did not alter the levels of other lipid mediators including prostaglandins. Contrary to our hypothesis, Ces inhibition by CPF did not modulate LPS-induced lung inflammation, and 2-AG levels were decreased rather than increased. These results suggest that Ces1c and 1d are bio-scavenging enzymes that have a protective role against CPF exposure in neonatal lung.

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Student Support: NIH R15 GM128206

Review of current diagnostic tools and recent clinical trials in canine hemangiosarcoma

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Canine hemangiosarcoma (HSA) is a highly aggressive cancer of vascular endothelial cell origin often arising in the spleen, heart, and skin. Splenic HSA is the most common form of this disease and it is frequently observed in older, large breed dogs. Dogs with splenic HSA often present at the clinics in an emergency setting with hemoabdomen, due to rupture of the tumor. Although splenectomy, followed by adjuvant chemotherapy can prolong survival time, most dogs will die from metastatic disease within 1 year following diagnosis. Current diagnostic tools for HSA include clinical staging and histological grading, however, new research is focussed on investigating biomarkers such as microRNAs. Emerging clinical trials testing novel chemotherapies and immunotherapies hope to improve the quality of life and survival time of HSA patients. The goal of this review is to analyze limitations in current research and evaluate the potential efficacy of promising new treatments with the potential to improve the standard of care for this disease.

Research Grant: None
Student Support: Cornell Leadership Program
Evaluating the suspected etiology of aprosencephaly with otocephaly in a stillborn goat

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A fetal goat presented with lack of facial structures and conjoining of the ears near the midline of the ventrocranial aspect of the cranium, consistent with otocephaly. The intracranial central nervous system was greatly reduced in size and appeared to consist of the cerebellum, pons and more distal brainstem and the absence of the cerebral hemispheres (grossly interpreted as aprosencephalic microcephaly). It was hypothesized that the defect present in this animal is otocephaly with aprosencephaly, which is agenesis of facial features and the diencephalon and telencephalon, or forebrain, respectively. Microscopic examination of this fetus confirmed the presence of aprosencephaly as evidenced by the absence of cerebral hemispheres or other telencephalic or diencephalic structures. The most cranial aspect of the brain consisted of structures present at the midbrain-hindbrain junction. It is further hypothesized that the congenital abnormalities are due to a mutation of the OTX2 gene, which has been shown to result in similar congenital malformations in mice, though this has not been previously reported in the goat. Evaluation of this gene is still in progress and its role in the congenital malformations of this animal have yet to be elucidated.

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Surrogate outcomes are prevalent in randomized controlled trials in horses

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Outcomes are one of the most important aspects and the end goal of scientific articles. Outcomes can be classified as either “hard” or “surrogate”. Hard outcomes are associated with proven benefits of the patient, whereas soft outcomes are not associated with proven benefits of the patient, and more so focused on laboratory findings. In human scientific literature, it has been proven that the primary use of soft outcomes in literature can result in flawed results. The objective of the present study is to determine the prevalence of surrogate and hard outcomes in randomized controlled trials (RCTs) performed on horses.Materials and methods: RCTs published from 19 journals in 2017 and 2018 were extracted into a data excel sheet. The RCTs were evaluated for outcome name, classification of outcome (hard/surrogate), how outcome was measured, and where outcome was mentioned (materials and methods/results/both). All procedures were performed by 2 operators independently and then compared. We extracted a total of 1129 outcomes from 145 RCTs. The vast majority of outcomes (981/1129; 86.9%, 95%CI: 84.8 to 88.7%) were surrogate outcomes. Seventy-three (73/1129; 6.5%, 95%CI: 5.2 to 8.1%) were hard outcomes. A number of RCTs did not include any hard outcomes. Veterinary clinicians should be aware about the prevalence of soft outcomes when changing clinical practice on the basis of results of a RCT. Researchers should design RCTs in order to include hard outcomes. Editors should prioritize research articles including hard outcomes and highlight research articles in which conclusions are solely based on surrogate outcomes so clinical decisions from information of these papers will not be made.

Research Grant: None
Student Support: Boehringer Ingelheim
Physiological response to water contamination by strip mined lands measured by leukograms in two fish species

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Coal mining has previously been shown to pollute surrounding water systems by leaching in metal contaminants that impact water quality and build up in the tissues of aquatic animals. Currently a study is underway comparing the leukograms of fish caught in two water sheds. The first is located within The Wilds, currently a conservation area in Cumberland, Ohio with a previous history of land use for strip mining, while the second, a control site, is located in Port Clinton, Ohio. Two species of fish have been selected for this study: bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*). This is because of the contrasting niches they inhabit and their differing lifespans. The fish have been collected by rod and reel, weighted, measured, euthanized, and necropsied. Additionally, blood was drawn for smears and age was being determined by otolith analysis. White blood cell counts have been determined by manually reading the blood smears and compared between both sites, while controlling for possible variations in fish age. This study will hopefully demonstrate how metal contaminants remaining from past mining may influence the immune systems of aquatic animals within the affected region.

Research Grant: Unknown
Student Support: Monahan Summer Research Fellow

The role of the retrobiome in aging of dogs: a preliminary analysis of T cell phenotypes in retired sled dogs

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Aging is the process of becoming frailer and more susceptible to disease and deterioration. Retroelements, collectively known as the retrobiome, generate DNA damage and therefore contribute to aging. Retired sled dogs at Cornell University provide a model for aging as (1) the canine genome is susceptible to retrobiome activation, and (2) there is an interest to extend the lifespan of these companion animals. We hypothesize that inhibiting the retrobiome will result in longer life and delay physiological aging effects. The aging process, retrobiome, and reversal strategies were reviewed in existing literature. In a placebo-controlled clinical study, half of the dogs are treated with lamivudine, which hinders the retrobiome. Baseline T cell phenotypic data were compared across multiple demographic groups including age, sex, and presence of disease, and generally revealed no difference (p values > 0.05). However, a statistical difference was found among treatment randomization groups within CD4+CD25+FoxP3+ (p-value < 0.0001), CD4+CD28+ (p-value 0.019), CD8+CD28+ (p-value 0.0004), and CD8+CD28- (p-value 0.0004); (lamivudine n = 31; control n = 30). Data collected preceded treatment; therefore, though results show a significance between treatment groups, this must be due to chance or other factors. Data collection is ongoing and analysis at future timepoints may show more contrasting results. The substantial heterogeneity observed in proportions of the T cell populations among dogs in this cohort warrants further investigation. Aging in dogs remains an enigma, as most methods that slow aging have not yet been proven in dogs.

Research Grant: Vaika Foundation
Student Support: Veterinary Investigator Program T35 OD010941- NIH
Measures of passive maternal immunity and the occurrence of negative health events in pre-weaned beef calves

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The objective of this study was to determine the relationship between measures of transfer of passive maternal immunity on the post-test probability of negative health outcomes in pre-weaned beef calves. Blood was collected from 370 calves, between 2 and 7 days of age, from a ranch with 4 herds. Negative health events were recorded by the producers prior to weaning. Sera were analyzed for IgG using a commercial radial immunodiffusion and 3 refractometry scales: Brix percentage (Brix), total protein (STP), and specific gravity (SG). Immunological values were categorized as low, medium, and high, for each immunological measure with the criteria that each category had to have at least 5 individuals with a negative health. Categories for IgG were < 1800 mg/dL, 1800-4300 mg/dL, and > 4300mg/dL. Categories for STP were < 5.5 g/dL, 5.5-7.5 g/dL, and > 7.5 g/dL. Categories for Brix were < 8.5%, 8.5-10%, and > 10%. Categories for SG were < 1.035, 1.035-1.040, and > 1.040. A negative health event occurred in 7% (26/370) of calves. Positive likelihood ratios (LR+) were 2.6 (2.6, 2.7), 3.9 (3.9, 4.0), 2.9 (2.8, 2.9), and 2.2 (2.1, 2.2) for the lowest categories of IgG, STP, Brix, and SG, respectively. LR+ for other categories were less than 1. Positive predictive values for the lowest categories were only marginally better than pre-test probabilities. Negative predictive values were non-informative. Immunological measures of passive maternal immunity are only marginally better than using clinical judgment alone to predict which calves will develop negative health outcomes in the pre-weaning period.

Research Grant: None
Student Support: Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Population

Bordetella pseudohinzii’s pertussis toxin-like genes contribute to persistence in otitis media infections

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Infections causing otitis media (OM) are a major health concern in young children that can result in multiple doctor visits and prescriptions for antimicrobials. Bordetella bronchiseptica and Bordetella pseudohinzii are murine pathogens that mimic the natural infection process of OM in humans; B. bronchiseptica causes acute infections and B. pseudohinzii causes chronic infections. Here, the genome sequences of B. bronchiseptica and B. pseudohinzii were compared for dissimilarities in the sequence to identify putative candidate genes contributing to the persistence of B. pseudohinzii in OM based on a similarity score (H-value) ranging from 0-1. Guided by previously studied virulence factors of the Bordetellae species, we identified four genes that appear to encode the ability to assemble a pertussis toxin-like toxin. Subunit A (psxA) is the catalytic domain and has the highest similarity score (H = 0.5), while subunits BC, D, and E had a lower similarity to B. bronchiseptica genes. Bordetella pertussis produces and secretes pertussis toxins, which contribute to the colonization and pathogenesis of the disease whooping cough through immune modulation. We hypothesize that in the case of B. pseudohinzii, the pertussis toxin-like genes will contribute to its persistence in OM. In vivo experiments showed that when the psxA gene is knocked out, B. pseudohinzii is no longer able to persist at high levels in the lungs, trachea, and middle ears, compared to wild type B. pseudohinzii. This gene analysis is a starting point for future studies analyzing the function of the pertussis toxin-like genes in B. pseudohinzii and the role these genes may play in immune evasion, resulting in persistent OM.

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Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-14
Evaluation of capromorelin and mirtazapine on food intake in budgerigars (*Melospittaundulatus*)

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Psittacines are frequently kept as companion birds and often require veterinary care in a hospital setting. In the unknown and restrictive environment of an animal hospital, handling by unfamiliar people, administration of medications, and medical procedures results in stress, frequently leading to reduced food intake. Furthermore, hyporexia and anorexia also occurs with most systemic diseases in pet birds, and is detrimental to the health of small, highly metabolic birds and may cause death. Nutritional support in dogs and cats reduced duration of hospitalization, and appetite stimulation improves nutritional status and recovery from disease or injury in animals. Crop gavage, a common method to provide nutritional support in pet birds, is considered stressful and may result in morbidity and rarely mortality. Appetite stimulation to increase feeding in birds would decrease the need for crop gavage and reduce the associated stress response, however, little to no research has been conducted to test drugs that could safely increase appetite in psittacine birds. The aim of this study is to address the efficacy and duration of mirtazapine, a tetracyclic antidepressant and capromorelin oral solution, a ghrelin agonist which has been FDA approved for use in dogs to stimulate appetite. Food intake will be measured in randomized, blinded, placebo-controlled, complete crossover studies at 2-hour intervals over 12 hours post-administration in cohorts of 12 budgerigars. We hypothesize that the administration of capromorelin and mirtazapine will cause a dose-dependent increase in food intake without inducing adverse effects and that capromorelin will have a longer duration of action compared to mirtazapine.

**Research Grant:** Association of Avian Veterinarians

**Student Support:** Morris Animal Foundation Fellowship

Dynamic changes in chromatin accessibility and Runx transcription factor interactions in T cell development

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During T cell development, hematopoietic precursors migrate to the thymus where they acquire T cell identity. In “Phase1” of this process, progenitor cells retain multipotentiality; they progress to “Phase2” as they commit to the T lineage. Expressed throughout development, Runx1 and Runx3 play a crucial role in T lineage specification by activating the T cell program and repressing alternative lineage potentials in a stage-specific manner. Our goal is to explore changes in chromatin accessibility and Runx-DNA binding during T development through ChIP/ATAC-seq analysis. ATAC-seq data suggested dynamic changes in chromatin accessibility, with more open regions in Phase1, and a decrease in accessibility after commitment. Runx factors mainly interacted with accessible regions in Phase1, but bound to both open and closed chromatin in Phase2. Motif analysis implied that Runx binding to closed sites was at least as specific as to open sites. We also examined whether genes associated with sites of interest were correlated with differentially expressed genes defined by deletion of Runx proteins in pro-T cells. Runx dependent genes, including genes critical to T cell identity (eg. *Bcl11b*, *Cd3*, and *Tcf7*) were highly correlated with regions of Runx binding found in both open and closed chromatin states. However, many Runx repressed genes were correlated with Runx binding regions that were open in Phase1 but closed in Phase2, suggesting Runx may be repressing genes through a mechanism that closes the associated chromatin. Together, our results suggest that, despite constant expression throughout T development, Runx mediates phase specific roles by interacting with genomic loci undergoing dynamic changes in chromatin accessibility.

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**Student Support:** NIH T35 OD010956
The pathology and incidence of building-caused bird mortality on Midwestern University’s Glendale campus

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Window collisions are a major cause of mortality among wild birds. To understand window-caused mortality and increase successful treatment outcomes, we determined the pathology from window collisions and the incidence of building-caused bird mortality on Midwestern University’s Glendale campus. We found that window-killed birds have characteristic gross pathology, most of which resulted in death. Eighty-two window-killed birds, from 17 species, were collected over 16 weeks yielding 70 reliable postmortem examinations to record sustained lesions and determine cause of death. We found that birds die from injuries of blunt force trauma sustained by and/or translated through their pectoral girdle/muscle area. Specifically, 77.1% had lower airway injury, 68.6% had hepatic/splenic injury, and 40% had cardiovascular pathology. Skeletal fractures were also common, including pectoral fractures (58.6%), pelvic fractures (8.6%), and long bone fractures (4.3%). In contrast, only 17.1% of birds had cranial injuries, suggesting that cranial injury is less common. Forty-eight (68.6%) of the examined birds had fatal pathologies from two or more of the aforementioned categories and, in 23 cases (32.9%), it was primary skeletal pathology that caused the fatal visceral injuries. Understanding the cause of death of window-killed birds will improve treatment of injured birds admitted to wildlife rehabilitation centers, as not all window collisions are immediately fatal. The incidence of window collisions to identify troublesome areas among large buildings will also provide further insight into future remediation. Thus, subsequent work will investigate the role and design of large buildings and their effect on avian mortality.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Screening native fishes for infection with KHV in a lake exposed to a known host species, Cyprinus carpio

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Koi Herpes Virus (KHV, Cyprinid herpesvirus 3) is the etiological agent of a viral disease (KHVD) that can cause high morbidity and mortality in cultured and wild populations of common carp (Cyprinus carpio), hereafter referred to as carp. KHVD in wild carp occurs primarily during spawning aggregations in the spring. Contradicting literature and incomplete testing has led to uncertainty about the susceptibility of other species. Furthermore, it remains unclear if fishes native to North American lakes, where common carp is an invasive species, are susceptible to KHV infection. The goal of this study was to determine the prevalence of KHV in native fishes collected from a Minnesota lake with a history of KHVD in carp. Big mouth buffalo (Ictiobus cyprinellus) in particular was considered at risk for KHV exposure due to its shared habitat and spawning behavior which brings it into close proximity with breeding carp. Gill tissue, the primary site of KHVD, was collected from samples of 240 big mouth buffalo and 240 randomly samples native fishes, sampled at three time-points during permissive temperatures for KHV infection (N = 60 fish per sample period for a 5% design prevalence). Gill tissues were first screened for KHV DNA using Taqman probe-based qPCR for detection of the KHV genome. Species which tested positive for KHV DNA were then tested for the presence of KHV mRNA using RTPCR for confirmation (replicating virus). Results of this study are pending.

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Student Support: Summer Scholars, College of Veterinary Medicine, University of Minnesota, St. Paul, MN
This means [inflammatory] war: inflammasome recognition of the bacterium *Brucella abortus*

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*Brucella abortus* is a bacterium that leads to chronic inflammatory conditions in the zoonotic disease brucellosis. Once transmitted from infected cattle to humans, the bacterium’s modified LPS layer allows it to hide from many aspects of the immune system, making treating the disease difficult. Despite its virulence, immune recognition of *B. abortus* has been relatively understudied. In the immune system, pattern recognition receptors, such as NOD-like receptors (NLRs), play a key role by recognizing harmful pathogens. After recognition, some NLRs serve as scaffolding to form an inflammatory signalling structure called the inflammasome. Once formed, the inflammasome activates signaling proteins to promote inflammation and initiates inflammatory cell death. This indicates that the inflammasome could be a possible defender against *B. abortus* if it becomes activated. However, the activation of the inflammasome in response to *B. abortus* has not been fully characterized. Therefore, we used wild type (WT) and genetically-modified Asc–/– mice that cannot form the inflammasome to understand what signaling proteins are expressed in response to *B. abortus* infections. In Asc–/– mice, we saw decreased inflammatory signaling and bacterial growth *in vitro*, as well as increased bacterial load in the liver. Additionally, these mice had higher mortality and morbidity rates than WT mice. This may indicate that *B. abortus* is detected by NLRs of the inflammasome and that the inflammasome subsequently protects against this infection by inflammatory protein signaling and cell death. This work contributes to a greater understanding of *B. abortus* immune recognition and further contributes to our development of a novel vaccine for brucellosis.

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**Inhibition of experimental autoimmune uveitis by intravitreal gene therapy using AAV-Eq IL10**

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Uveitis is a recurrent, chronic inflammation of the uveal tract that can often lead to blindness in many mammalian species, notably humans and horses. Current treatment consists of frequent daily treatment with non-specific anti-inflammatory medications, such as corticosteroids. Consequently, the use of a single intravitreal (IVT) injection of gene therapy that induces immunosuppression and prevents recurrence of uveitis would be advantageous. This study used an adeno-associated viral (AAV) vector to deliver the equine Interleukin 10 (Eq IL10) gene, a cytokine that is naturally immunosuppressive and contributes to the normal ocular immune privilege. Naive Lewis rats were given an IVT injection of either a high or low dose of AAV8 Eq IL10, while the controls were given an IVT injection of balanced salt solution (BSS). One week later, experimental autoimmune uveitis (EAU) was induced using subcutaneous injection of interphotoreceptor retinoid binding protein. Eyes treated with AAV Eq IL10 had significantly lower clinical and histologic inflammatory scores and cellular infiltrates following high and low doses of Equine IL-10 compared to the BSS treated EAU eyes. These results provide clinical and histological evidence that a single IVT injection of AAV8-Eq IL10 inhibits EAU and supports further evaluation of this innovative therapy for equine recurrent uveitis.

**Research Grant:** NC TraCS Institute

**Student Support:** Veterinary Scholars Program at North Carolina State College of Veterinary Medicine
The role of selected genetic variants in canine morphology

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Variation in body size within the domestic dog, *canis familiaris*, is greater than any other single species. Body size is also intimately associated with health and longevity since there are differences in lifespan and healthspan between sizes of dogs. The difference in body size is likely due to a number of genetic variants, as demonstrated in several across-breed studies. The purpose of this study is to determine how size variants can predict size differences within breeds. The hypothesis is that a number of these variants will predict a large portion of body weight variation, both within and across breeds. Samples collected from the UC Davis Veterinary Medical Teaching Hospital were genotyped using the Illumina canine HD SNP array (N = 511). Some previously identified variants for body size are included on the array (IGF1R, GHR1, GHR2, IGF1, IGSF1, FTSJ3, GH1, and PAN2). Additional SNPs in linkage disequilibrium with size markers were also included (LCORL, SMAD2, HMG2A2, and STC2). Descriptive statistics were performed by genotype for 12 body size markers. A quantitative genome-wide association study for body weight was performed within breeds in order to identify novel size variants. Two breeds were selected for initial analysis. The Poodle was chosen since it comes in three different varieties which differ in weight 10-fold within one breeding population (N = 78). The Golden Retriever was selected as there is a robust sample size for this breed (N = 100). The study will analyze the individual and cumulative effects of these variants with respect to body weight within each breed. A broader understanding of within-breed size variants could offer insight into conditions that typically correspond to body size.

Research Grant: Maxine Adler Endowed Chair funds
Student Support: National Institutes of Health T35 OD010956

RNAseq-directed discovery of cancer-testis antigens in canine hematologic cancers

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T-cell-based immunotherapies could eradicate tiny burdens of chemoresistant cells that cause treatment failure in hematologic cancers, such as T-cell lymphomas (TCLs) and histiocytic sarcomas (HS). Both are largely incurable in dogs and humans. An important focus in immunotherapy development is finding proteins made exclusively by these cancers. These tumor-specific antigens (TSAs) let anti-cancer T cells attack malignant cells but spare normal ones, avoiding autoimmunity. An important TSA category are cancer-testis antigens (CTAs), proteins made in germ cells, silenced in somatic tissues and sometimes re-expressed in tumors. Since rare in hematologic cancers, their discovery is challenging, but rewarding. We hypothesized that CTAs could be found using RNA-seq, by comparing transcriptomes of 4 representative canine tissues (testis; normal brain; TCL; HS). Top candidates would be assessed by reverse-transcription (RT) PCR of archived normal, HS and TCL samples to find those with the idealized profile: expression in multiple patients, but not normal tissues. We constructed an algorithm to search > 40,000 transcripts for those with testis, but not brain, expression, yielding 27 hits. The list was pared by stepwise, hierarchical filtering. We first culled transcripts expressed in TCL at levels < 1% of testis or with brain expression in variants. Thos in HS were prioritized. Secondly, we prioritized those where human orthologs had idealized expression patterns in public databases, or with literature ties to cancer. The winning candidates were IZUMO2, ODF4, PASD1, MAGEB10, CT83 and FATE1. All but IZUMO2 are established human CTAs; the latter 4 are X-linked. RT-PCR analysis of archived tissues is ongoing.

Research Grant: Shadow Whatley Research Fund
Student Support: Herbert Benjamin Endowment
Developing a translational tool to detect and track laryngeal dysfunction

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Certain age-related neurodegenerative diseases, such as amyotrophic lateral sclerosis and Parkinson’s disease, cause laryngeal dysfunction that may lead to aspiration pneumonia, a life-threatening pulmonary infection. Laryngeal dysfunction is also highly prevalent in horses, in what is known as recurrent laryngeal neuropathy (RLN). RLN is an idiopathic, progressive distal axonopathy that commonly leads to paresis or paralysis of the left side of the larynx. Resultant airway obstruction interferes with athletic performance and affected horses are retired from work prematurely. Unfortunately, for humans and horses, clinical detection and monitoring of laryngeal dysfunction is largely subjective, relying on inconsistent questionnaires and Likert scales that do not provide predictive information. Further, effective preventive or curative treatments for laryngeal dysfunction do not exist. To address this unmet clinical need, our research group recently developed a prototype diagnostic tool to objectively evaluate the laryngeal adductor reflex (LAR), an airway protective reflex that exists in all mammals. We have successfully used this innovative tool in healthy human adults to establish normative LAR parameters. The purpose of this study is to establish normative LAR parameters for healthy adult rodents and horses, paving the way for subsequent investigations with rodent models of neurodegenerative diseases and horses at high risk for RLN. Ultimately, this translational research may accelerate the discovery of novel and effective treatments for laryngeal pathology, particularly for disease conditions associated with fatal aspiration pneumonia.

Research Grant: USDA Animal Health Formula Funds
Student Support: USDA National Institute of Food and Agriculture, Animal Health project 1021569

Culturally appropriate tick bite prevention recommendations for Amish communities

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In 2010, blacklegged ticks (Ixodes scapularis), the primary vector for Lyme disease in the eastern United States, were determined to be established in Tiverton Township, Ohio, and in subsequent years, there has been an increase in the reported number of cases of Lyme disease and other tick-borne zoonoses in Ohio. Tiverton Township is in Coshocton County, one of several counties in Ohio that are home to over 76,000 Amish members. The Amish community are a high-risk group due to their heavy use of outdoor spaces for recreation, farming, and other outdoor occupations. Due to the large population of Amish in Ohio and their increased risk, culturally appropriate tick bite prevention methods are important to help protect this community. After reviewing the CDC’s “Tick Management Handbook” for recommended tick bite and Lyme disease prevention methods, and literature on Amish history and culture, we compiled prevention methods understood to align with Amish cultural practices. These recommendations will be reviewed by Amish families in Ohio to assure their cultural acceptability within Amish communities. While some methods such as tick checks, landscape modifications, and discouraging rodent activity were deemed culturally appropriate other common methods such as deer management, tick habitat avoidance, and protective clothing were not. In particular, Amish are unlikely to use methods requiring synthetic chemicals such as repellents and acaricides but may be receptive to the use of EPA-approved, plant-based products. Our culturally appropriate tick bite prevention methods, in conjunction with tick education, may increase compliance and reduce tick-borne disease among Amish communities.

Research Grant: None
Student Support: NIH T35 Training Grant - 5T35OD010977-12
Investigation of the Psittacine syrinx using microCT imaging and three-dimensional reconstruction

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Birds have captured our fascination for centuries because they have evolved numerous unique features. For example, they have a vocal organ at the caudal end of the trachea called the syrinx. Furthermore, three major groups of birds (songbirds, *Passerines*; hummingbirds, *Apodiformes*; and parrots, *Psittaciformes*) share the ability for vocal learning, i.e. sometime after hatching they acquire new vocal behavior through learning. In all three groups, such vocal behavior is produced during exhalation and by the regulation of abduction, adduction, and membrane tension within the syrinx. Previous research suggests that only the distantly related songbirds and hummingbirds have converged on a similar morphology, while parrots employ a different cartilaginous syringeal framework to facilitate their phonation. Our goal was to investigate the anatomy of the parrot syrinx in order to evaluate previous statements about its relation to the passerine and hummingbird vocal organs. We generate an equivalent data set for parrots to allow for comparison of syrinx anatomical structures between the three vocal learners. The syrinx organs of male and female Bourki’s parrots (*Neopsephotus bourkii*) and Elegant parakeets (*Neophema elegans*) (2 females and 2 males from each species) were excised. We used either contrast-enhanced microCT or histology to image the syrinx. Syringeal cartilages, musculature, membranes, and the airway were traced manually using AVIZO software. Qualitative and quantitative analysis of three-dimensional reconstructions of the parrot syrinx is now being used for comparison with hummingbirds and songbirds

**Research Grant:** Midwestern University Start-Up Funds
**Student Support:** Boehringer Ingelheim Veterinary Scholars Program

Systematic review on published canine microbiome research

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Findings from human and animal microbiome studies have highlighted the importance of standardization. Unfortunately, variability in the conduct of microbiome studies hampers reproducibility. The objective of this review was to promote standardization by characterizing currently published canine gastrointestinal microbiome studies. PubMed was used to identify relevant articles using the keywords: “gastrointestinal microbiome”, “gastrointestinal tract”, “dogs”, and “canine”. Articles were excluded if they did not contain canine microbiome sequence data or were not in English. Factors related to study design and methods were extracted from each article. From PubMed, 166 articles published from 2013-2020 were retrieved. Of 46 articles reviewed to-date, 15 met the selection criteria. Thirteen articles evaluated the microbiome in relation to a specific disease or diet. Preliminary results indicate feces as the most common sample type (N = 14) and 16s rRNA amplicons as the most common data type (N = 13). Preliminary results indicate that many canine microbiome studies do not report critical details including breed or sampling device. For studies that do report these details, populations and methods are highly variable. The variability and lack of detail in the current canine microbiome literature limits our ability to compare across studies and reproduce important results. As we collate the results of our review, our goal is to identify areas requiring standardization, such as in storage methods and 16S rRNA hypervariable region. By identifying such factors, our review can contribute to reproducibility of canine microbiome science, and thus promote microbiome-based discovery for canine health.

**Research Grant:** None
**Student Support:** National Veterinary Summer Scholars Program
Preliminary review of literature on the methods and success rates of in vitro maturation of canine oocytes

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Reproductive technologies such as in vitro fertilization (IVF) and embryo transfer provide opportunities to propagate rare breeds and produce offspring from sub fertile mammalian species; however, in dogs, the unique reproductive biology has made it difficult to apply many aspects of reproductive technologies. The objective of this study was to review the literature and present data on common and best practices of in vitro maturation (IVM) of canine oocytes. Most of the oocytes used for IVM are collected from spay and neuter clinics. Incubation temperatures range from 37.0 to 38.5 degrees Celsius. Basic media used for IVM include CMRL1066, SOF and TCM-199. Among the base media, TCM-199 medium seems to give better results with maturation rates of up to 34%. Studies indicate the usefulness of supplements such as LH, FSH, and 9-cis retinoic acid; however, pyruvate and glutamine do not seem to have beneficial effects. The optimum IVM incubation duration is no longer than 48 hours. Oocytes recovered from 8 mm follicles yield better results than those recovered from smaller follicles. In conclusion, various methods and supplements have been investigated for their effects on the IVM of canine oocytes; nevertheless, only a third of the oocytes complete maturation leaving a big room for improvement.

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Student Support: HRSA #D34HP00001, TU-CBR/RCMI #U54MD007585, and USDA-NIFA Award Number 2015-38821-24347

Bioinformatic investigation to characterize the secretome of Toxocara canis larvae

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The canine roundworm, Toxocara canis is a well-known agent of zoonotic infection in human hosts, which oftentimes causes visceral and ocular larva migrans (VLM and OLM), as well as neurotoxocarosis. This is a neglected tropical disease prevalent in many countries worldwide; impoverished areas of North America can also be affected. Millions of people in the United States are exposed to T. canis, while larva migrans is reported especially in young children and senior citizens. The parasite is only infectious to humans during the L3 larval stage, and never matures to adulthood in human tissues. Proteins secreted by T. canis larvae facilitate the parasite’s migration through host tissues and help evade immune capture. The aim of this study was to analyze the secretome of the parasite’s L3 stage using bioinformatics tools. The presence of a signal sequence identified 1147 secretory proteins in the L3 stage. These were categorized to predict their functions during the migratory phase. Many belonged to various protein families involved in functions such as transmembrane signaling, carbohydrate/lipid-binding, neuropeptide signaling pathways, and endopeptidase inhibition. Another 313 proteins were hypothetical proteins of unknown function, most likely shared between T. canis and other closely related nematode species. Thirteen secretory proteins were categorized as proteases, which potentially play a role in parasite-host interactions such as host connective tissue and mucus plug degradation, parasite molting, pro-inflammatory response, immune evasion etc. These proteases could also be potential candidates for vaccine development or anthelmintic drug discovery.

Research Grant: none
Student Support: Purdue University College of Veterinary Medicine- Summer Research Fellowship
Comparison of biofilm production by *S. pseudintermedius* isolates from canine superficial pyoderma

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*Staphylococcus pseudintermedius* is a common skin commensal isolated from canine superficial pyoderma. Recently, multi-drug resistant (MDR) *S. pseudintermedius* has emerged as a major therapeutic challenge in veterinary medicine and is now recognized as a zoonotic agent. Additionally, the presence of biofilm may contribute to antibiotic resistance. There are limited studies investigating the ability of *S. pseudintermedius* to produce biofilm along with evaluating the potential relationship to MDR. This study focused on determining the ability of isolates to produce biofilm and compare the biofilm-forming ability of MDR to non-MDR strains. *S. pseudintermedius* isolates were assessed for biofilm production using a crystal violet microtiter plate assay. Biofilm production in tryptic soy broth (TSB) was assessed at 48h of incubation at 30°C. All isolates tested (n = 35) were classified as biofilm formers. Biofilms were formed by 16/35 (46%) multi-drug resistant (MDR) strains and 19/35 (54%) non-MDR strains. Further studies are needed to assess factors increasing formation of biofilm and investigate biofilm-disrupting compounds for successful treatment of canine superficial pyoderma.

**Research Grant:** None  
**Student Support:** UW School of Veterinary Medicine, Dean’s Office

Investigating the vascular effects of Clopidogrel: a review of current literature

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Clopidogrel is an antiplatelet drug used to treat acute coronary syndromes (ACS). Clopidogrel, and other P2Y12 antagonists, work by inhibiting the P2Y12 receptor on the surface of platelets. Preventing the activation of P2Y12 decreases platelet aggregation, thereby preserving blood flow in patients at risk for heart attacks. Though effective at treating ACS, there are adverse side effects associated with clopidogrel. One adverse effect is cerebral microbleeds. This effect cannot be explained by platelet P2Y12 inhibition as even in animals without the receptor, bleeding is prolonged with clopidogrel treatment. As the bleeding mechanism is not well understood, a look at the drug’s impact on the vasculature, beyond platelet inhibition, must be evaluated. We hypothesize that clopidogrel has off-target effects on other purinergic receptors within the vasculature to impair vessel response to changes in pressure and blood flow. Furthermore, clopidogrel efficacy is reduced in patients suffering with type II diabetes (T2D). The metabolism of clopidogrel in patients with T2D was investigated to determine antiplatelet resistance. We hypothesize that patients with T2D have altered expression of cytochrome P450 enzymes, thereby affecting the metabolism of clopidogrel. To investigate these hypotheses, literature searches were performed using PubMed database. The literature was assessed for information on clopidogrel’s impact on vascular functioning, including possible interactions with other purinergic receptors, and clopidogrel’s impact on inflammation. These searches were done with the goal of creating a targeted hypothesis that provides a mechanism for clopidogrel-induced cerebral microbleeds and clopidogrel resistance in T2D.

**Research Grant:** Michigan State University Undergraduate Research  
**Student Support:** Michigan State University Undergraduate Research
Evaluation of SARS-2 infection and transmission in domestic livestock

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SARS-CoV-2 (COVID-19) has spread to nearly every country worldwide and concerns regarding the impacts of this virus continue to rise as human case numbers increase. SARS-CoV-2 likely originated through spillover from an animal reservoir, but the exact mechanism remains unknown. Thus, it is important to understand whether other species can serve as reservoir hosts or become clinically infected. Livestock play a crucial role in the human food supply and are frequently in close contact with the human population. Therefore, infection of livestock could cause major ramifications to animal and human health, food security, the economy and trade if SARS-CoV-2 becomes established in livestock populations. Due to these concerns, this study evaluated susceptibility of goats and cattle to SARS-CoV-2 infection. We found that goats and calves do not become clinically ill when intranasally inoculated with SARS-CoV-2 and infectious virus could not be detected from nasal or rectal swabs. However, SARS-CoV-2 was isolated from the trachea of a calf three days post-inoculation, which indicates some level of susceptibility to infection. Further studies involving serology and viral tissue loads are ongoing and will be crucial to evaluate to what extent livestock are susceptible to SARS-CoV-2 and what role they may play, if any, as reservoirs for the virus.

Research Grant: This study is being supported by the Animal Models Core at Colorado State University
Student Support: USDA

Co-infection of uropathogenic Escherichia coli and Enterococcus in canine UTIs: a 5-year retrospective study

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Urinary tract infections (UTI) are a leading cause of morbidity among canine patients and frequently require antimicrobial therapy. Recurrent UTI infections especially challenge clinicians because treatment with commonly prescribed drugs often fails. The most common etiologies of recurrent canine UTI are uropathogenic Escherichia coli (UPEC) followed by Enterococcus spp. (ENT). Interestingly, these two agents are frequently co-isolated from canine patients with a polybacterial UTI, which further complicates treatment. Potential synergies between UPEC and ENT may be an unrecognized contributor to canine UTI and may affect the clinical severity of the infection. However, clinical outcomes of co-infections are mostly unknown. Medical records were inspected to identify 50 patients with UTI caused by UPEC and ENT co-infections, and clinical outcomes as well as antimicrobial resistance among isolates were compared to those of patients with single agent UTI. UTI recurred more frequently in patients with UPEC/ENT co-infections compared to those with ENT mono-infections. Frequency of recurrence was not different when co-infection patients were compared to patients with UPEC mono-infections. UPEC isolates from UPEC/ENT co-infections exhibited increased resistance to doxycycline, enrofloxacin, and gentamicin while UPEC isolates from mono-infections were more resistant to amoxicillin/clavulanic acid. The increased reoccurrence of UPEC/ENT co-infections and the rise of antimicrobial resistance (AMR) in both UPEC and ENT emphasize the need for novel UTI treatment strategies and implicate new clinical management strategies for UTI caused by UPEC and ENT polybacterial infection.

Research Grant: Morris Animal Foundation Pilot Study Grant
Student Support: None
Modeling avian influenza virus transmission dynamics in migratory waterfowl to assess risk to pig populations

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Infectious avian influenza has the potential to influence more than just bird health; this disease can have rippling effects on biodiversity, livestock, public health, and create significant economic losses worldwide. Migratory waterfowl (MWF) are the wildlife reservoir for avian influenza virus and the source of spillover events that can infect other wildlife and susceptible livestock. However, influenza dynamics in MWF remain unquantified and transmission to livestock remain unidentified. Here, we use a long-term dataset that describes influenza seroprevalence in avian wildlife in Ohio from 1976 to 2015 to quantify transmission among mallards (Anas platyrhynchos) and other MWF. We also quantify the risk of transmission from avian wildlife to swine. We fit age-structured catalytic and reverse catalytic transmission models to serological data to estimate the time-varying force of infection and the rate of waning immunity. The models indicated varying transmission rates based on the sample year, sample month, and mallard age (mature or immature). Additionally, they suggest that immunity can wain, allowing for potential re-infection of avian influenza within the same year. From this, we simulated a model to determine the probability of swine influenza infection from MWF during the fall and validated our final model with data that describes swine influenza infections from 2015 to 2019. Our computational tools, calibrated with timeseries prevalence data, quantify reservoir dynamics of influenza and identify how migration contributes to cross-species transmission. This work can improve disease surveillance in both bird and swine populations and contribute to more accurate disease risk assessment among swine production.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Summer Scholar

Developmental timeline of neuropathological, behavioral, and electrophysiological abnormalities in 5xFAD mice

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Alzheimer’s disease (AD) is a devastating neurological disease, affecting over 5 million Americans. The disease is always fatal and developing a cure has been unsuccessful, likely because irreversible brain pathology like plaque deposition develops for years before memory related deficits are seen clinically. The 5xFAD mouse, which has 5 AD-linked mutations, is used as a model organism to investigate the pathology of AD. This review paper aims to create a timeline for when changes are first seen in these transgenic mice, which utilize either a C57BL6 or B6SJL background. In particular, neuropathology such as plaque deposition and neuronal degeneration, deficits in working and spatial memory, and electrophysiological disturbances will be investigated. The focus will be on the earliest of these changes, which occur at or before 2-4 months of age, because early identification of AD is key to developing a therapeutic treatment. Additionally, there will be analysis of behavioral data from long term memory, associative memory, and cognitive flexibility tasks involving 4-month 5xFAD mice fed ibuprofen in their diet. This data tests the hypothesis that NSAIDs could alleviate previously identified N-methyl-D-aspartate receptor hyperexcitability and potentially be a useful way to prevent the progression of AD.

Research Grant: Oregon Alzheimer’s Disease Center Pilot Program
Student Support: Boehringer Ingelheim Veterinary Scholars Program
Histopathologic findings in necropsy cases with *Coccidioides* spp. infection

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Fungal infection with *Coccidioides* spp. is a prevalent problem in endemic regions, such as Arizona and California, and is commonly referred to as Valley Fever. Humans and animals alike are exposed to infectious arthroconidia in the environment, and treatment of symptomatic patients is lengthy and requires frequent retesting of titers. Although the clinical course usually begins with respiratory symptoms, the *Coccidioides* organism often disseminates to other tissues during disease progression, causing widespread inflammation. While *Coccidioides* may spread to any tissue, a recent comprehensive investigation and review of *Coccidioides* tissue tropism in veterinary patients has not been performed. For this retrospective study, we searched the Midwestern University Diagnostic Pathology Center case database for necropsies that contained *Coccidioides*-specific search terms, including “coccidioidomycosis” and “Valley Fever.” This search resulted in 115 necropsies performed between July 2016 and June 2020. Species breakdown indicated the majority of patients were canine (~70%), with the remainder of cases split almost equally between feline, equine, ruminant, and other species. Cases are currently being reviewed and tissue distribution of *Coccidioides* lesions recorded, along with other comorbidities and whether *Coccidioides* infection was the main cause of mortality. Results from this study will help add to the current knowledge base of *Coccidioides* pathology in veterinary medicine.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Canine bite wounds in the ER: evaluating opportunities to reduce incidence

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Canine bite wounds are one of the most common forms of trauma presented to veterinary emergency rooms. Many studies have been done evaluating location of the wounds and subsequent treatments; however, the cause of biting incidents is rarely mentioned. The specific aim of this exploratory study is to further understand how biting incidents take place and determine if there is a pattern in the cause. A questionnaire based study is used for recording data on all dogs with bite wounds presented to Tufts emergency room. Data to be collected include: breed, severity of wounds, treatments, relationship between the dogs, and events that lead to the biting incident. Upon receiving the patient, the severity of the wound is determined by the doctor on the case on a 6-level basis. The relationship between the patient and the other dog involved, as well as details on the biting incident are recorded when the patient history is collected. Following data collection, we will examine the correlation between each factor. Our hypothesis is that most biting incidents occur between dogs that have bitten each other before. With the analyzed data, we hope to form injury prevention plans to reduce risks of biting incidents between dogs.

Research Grant: None
Student Support: None
Histopathologic and immunohistochemical analysis of acute microwave thermal ablation zones in lung and liver

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Microwave thermal ablation (MTA) is an emerging therapy technique for various tumor types. It is critical to define the boundary between viable and non-viable cells for evaluation of the safety and efficacy of MTA; however, regular H&E staining has limited use when cell morphology is relatively conserved after acute MTA. In this study, we investigated the use of immunohistochemistry (IHC), Transmission Electron Microscope (TEM) imaging and Nitro Blue Tetrazolium (NBT) staining in analyzing the margin of cell viability after acute in vivo MTA in normal swine lung and liver. IHC was performed on formalin fixed paraffin embedded sections of swine lung and liver collected from an acute in vivo MTA using antibody markers for vimentin, HMGB-1 and HSP-70. TEM imaging and NBT staining were performed on ex vivo bovine liver to confirm cell viability. Immunohistochemical staining for vimentin and HMGB-1 was reduced in the ablation center, and there was a gradient change towards normal staining intensity through the transition or hemorrhagic zone. The translocation of HMGB-1 from nucleus to cytoplasm indicates the ongoing degenerative and inflammatory process in the transition zone and peripheral. The staining pattern change of selected proteins in the ablation center suggests direct cellular alteration and injury caused by extreme heat. The combination of H&E, IHC, TEM and NBT staining provides a promising approach to analyze cellular responses to acute MTA ablation.

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Microenvironmental immune effects of stereotactic radiotherapy and immunotherapy in canine solid tumors

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Purpose: Combining radiotherapy with immunotherapy is a novel therapeutic approach to treating cancer. In this study, we characterize the microenvironmental immune effects of canine tumors treated with stereotactic body radiation therapy (SBRT) and local injection of agonistic OX40/TLR9 immunotherapy (SBRTi) compared to SBRT+saline control (SBRTC).Methods: A randomized case-matched prospective study was performed in dogs with solid tumors. Tumor samples were obtained pre- and 2 weeks post-treatment. Immunohistochemistry (IHC) analysis of immune cell density was performed with antibodies against CD3 (T cells), CD79a (B cells), CD204 (macrophages), and FoxP3 (Tregs) using VIS image analysis software. Nanostring gene expression analysis was performed using a customized 48 gene immune panel. Results: Tissue samples from the first 5 dogs of the study were analyzed: SBRTC (n = 2, melanomas), SBRTi (n = 3; melanoma, carcinoma, sarcoma). From IHC analysis, the mean percentage of intratumoral Tregs increased following SBRTC (+111.5%; range = 67.4-155.5%) and decreased following SBRTi (-78.7%; range = -71.4 to -86.1%). The mean percentage of T cells increased following SBRTC (+68.2%; range = 3.4-133.3%) and decreased following SBRTi (-57.1%; range = -37.4 to -76.7%). The mean percentage of macrophages decreased post-treatment for both SBRTC (-51.3%; range = -32.3 to -70.4%) and SBRTi cases (-39.8%; range = -15.3 to -64.2%). No pattern was found for intratumoral B cells. There is an emerging trend where expression of TReg-related genes (CTLA4, FoxP3, GATA3, Lag3) is increased post-SBRTC and decreased post-SBRTi. Conclusion: These results suggest that SBRTi suppresses immunosuppressive Tregs in the tumor microenvironment compared to SBRTC.

Research Grant: Eldred Foundation
Student Support: College of Veterinary and Biomedical Sciences, Colorado State University
Healthy children, healthy chimps: reducing human respiratory disease transmission to chimpanzees in Uganda

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Reverse zoonotic respiratory diseases threaten wild chimpanzees across Sub-Saharan Africa. In the Kanyawara chimpanzee community in Kibale National Park, Uganda, respiratory disease has caused 59% of deaths over the past 30 years, with mortality rates of up to 10% during outbreaks. Our studies of the Kanyawara and nearby Ngogo communities have identified the causative agents as “common cold” pediatric human pathogens. We hypothesize that respiratory viruses circulate in children living near chimpanzee habitats, and that adults in those villages become asymptotically infected and can carry the pathogens into the forest and infect chimpanzees. Our objectives are to characterize respiratory pathogens in local children, forest workers, and chimpanzees using comprehensive molecular diagnostics and metagenomic DNA sequencing, and to examine the reverse zoonotic transmission risk that varies with pathogen type, season, environment, and the individual characteristics of humans and chimpanzees. To date, we have collected respiratory disease symptoms data and 1,444 nasopharyngeal swabs from 280 human study participants and 492 fecal samples from roughly 120 chimpanzees. Initial data show that children exhibit high frequencies and severities of symptoms while adults are largely asymptomatic. Fortuitously, our project is the only cohort study of respiratory disease transmission in chimpanzees and humans in Africa prior to and during the COVID-19 pandemic, making it of paramount importance for safeguarding vulnerable apes during a critical juncture in global public health. Our data will inform evidence-based strategies to reduce transmission to the approximately 1,500 chimpanzees of Kibale and, by extension, to the entire species.

Research Grant: Disney Conservation Fund, NIH R01 AG049395, University of Wisconsin-Madison Global Health Institute Graduate Student Research Award
Student Support: University of Wisconsin School of Veterinary Medicine intramural funds

Characterization of IL-4+ basophils in equine neonatal PBMC

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Culicoides hypersensitivity is the most common IgE-mediated seasonal skin allergy in horses. Multiple immune cell types are involved in the development of the disease. Basophils are critical to the allergic mechanism by releasing IL-4, a regulatory cytokine that induces immunoglobulin class-switch recombination to IgE and guides naive T-cells to the type 2 (Th2) phenotype. The purpose of this study was to phenotype the IL-4+ cell population within the equine neonatal PBMC. Peripheral blood mononuclear cells from 0-6 day old foals were collected, and basophils were isolated using magnetic cell sorting. IL-4 production was characterized by intracellular staining and flow cytometry. IL-4+ cells in equine PBMC were IgE+ and MHCIIlow, but did not express cell surface markers representative of T- or B-cells. After cell sorting, IgE+ cells in equine PBMC were divided into MHCIIhigh (monocytes) and MHCIIlow (basophils) populations. Only IgE+ MHCIIlow basophils produced IL-4 after IgE crosslinking. These findings show that the IL-4-producing cells in equine neonatal PBMC are IgE+ MHCIIlow basophils.

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Student Support: Boehringer-Ingelheim
Concurrent \textit{Pten} loss and \textit{Kras} activation in embryonic mouse germ cells induce chemosensitive testicular cancer

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Testicular germ cell tumors (TGCTs) are among the most curable malignancies owing to their exceptional sensitivity to genotoxic chemotherapeutics. The molecular mechanisms underlying TGCT chemosensitivity have yet to be completely determined, in part due to the lack of an accurate animal model representing human malignant TGCT. To tackle this, we developed a genetically-engineered mouse model of malignant TGCTs, called gPAK, for germ-cell specific \textit{Pten} inactivation and \textit{Kras} activation. gPAK mice developed metastatic mixed germ cell tumors that bore resemblance to human TGCTs as they 1) contained both teratoma components and a substantial population of pluripotent OCT4+ embryonal carcinoma (EC) cells, 2) originated in utero during a restricted period of embryonic development and 3) exhibited marked responsiveness to genotoxic chemotherapy. Chemotherapeutic treatment of TGCT bearing gPAK mice resulted in significantly reduced tumor size and prolonged lifespan, accompanied by depletion of OCT4+ EC cells and loss of tumor propagating activity in transplantation assays. These findings demonstrate that the inherent sensitivity of EC to genotoxic chemotherapy likely underlies the chemosensitivity of TGCTs. Using the gPAK model, we can further investigate the initiation, progression and treatment of both chemosensitive and chemoresistant TGCT and eventually gain insights that will aid the development of more effective therapies for other cancers.

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Meta-analysis of clinical efficacy of GLP-1-based therapeutics in dogs and cats

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Glucagon-like peptide-1 (GLP-1) is a highly conserved, potent incretin hormone that enhances glucose stimulated insulin secretion and promotes weight loss by decreasing appetite. GLP-1-based therapeutics are widely used in humans for management of diabetes mellitus (DM). However, it is not well understood how effective GLP-1-based therapeutics may be in treating and preventing DM in dogs and cats. Therefore, we performed a meta-analysis of pre-clinical and clinical studies assessing the safety and efficacy of GLP-1-based therapeutics in dogs and cats. Studies performed in dogs and cats with and without DM, reveal that GLP-1-based therapeutics are safe, have minimal side-effects, and can improve islet function. Furthermore, all studies performed in cats (overweight or obese, with or without concurrent DM), and one study in dogs with pre-diabetes, suggest that GLP-1 analogs may be an effective weight loss modality. While there is a paucity of work testing the efficacy of GLP-1-based drugs in dogs and cats, studies thus far suggest that GLP-1-based therapeutics may be beneficial. For example, one study conducted in dogs with DM reports that a single dose of a GLP-1 analog given with insulin decreased blood glucose levels by 46% over 12hr following a meal. Furthermore, another study reports that cats with DM receiving a combination of insulin glargine and a GLP-1 analog experienced significant weight loss and a decrease in exogenous insulin required. Overall, the existing research shows that GLP-1-based therapeutics hold much promise as adjunctive therapies in the treatment of DM in dogs and cats, but more clinical studies are needed to assess the effects of long-term therapy on DM management and patient outcomes.

\textbf{Research Grant:} None

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Effects of postnatal prebiotic supplementation on intestinal barrier restitution in an ischemic injury model

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The integrity of the intestinal barrier is vital to neonatal survival, however epithelial restitution following ischemic intestinal injury is diminished in the early postnatal period. Preliminary work in our pig model has shown that the development of the enteric glial cell (EGC) network, which is immature at birth, may be a novel regulator of epithelial barrier restitution. Postnatal development of this network is known to be driven by the microbial colonization of the gut. Therefore, we propose that dietary prebiotic oligosaccharides in neonates will accelerate microbial colonization and maturation of the EGC network, enhancing mucosal restitution following ischemic injury. Sixteen one-day-old pigs were fed either a standard or oligosaccharide-supplemented milk diet for 14 days. Ischemic jejunal and colonic injury was surgically induced, and both uninjured and injured tissues were collected for ex vivo recovery on Ussing Chambers and immunofluorescent (IF) histological analysis. Epithelial restitution response will be visualized and compared between standard and prebiotic-supplemented groups using IF for Annexin A2, Integrin B1, and Focal Adhesion Kinase, which mediate cell migration. We expect IF to show increased density and organization of these protein targets in migrating jejunal and colonic enterocytes in neonates fed a prebiotic-supplemented diet. Defining a link between prebiotic supplementation and improved epithelial barrier restitution in neonatal pigs may lead to potential dietary interventions to improve outcomes in human infants affected by intestinal disease.

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Student Support: NIH T35 Training Grant 2T35OD011070-11

Assessment of plasma 25-hydroxyvitamin D concentrations in two collections of captive Magellanic penguins

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Vitamin D is essential for the maintenance of calcium and bone metabolism in vertebrates, and deficiency can contribute to a variety of forms of metabolic bone disease (MBD) including osteomalacia/rickets, osteoporosis, and fibrous osteodystrophy. MBD has been documented in various species of birds, including penguins managed under human care. The objective of this study was to evaluate whether plasma 25-hydroxyvitamin D and ionized calcium (iCa) concentrations differ between indoor housed Magellanic penguins with access to UVB lights and outdoor housed penguins exposed to natural sunlight. Frozen banked plasma samples from 16 healthy, Magellanic penguins (n = 10 outdoor, n = 6 indoor) with similar husbandry practices were evaluated. Mean ± SD plasma 25-hydroxyvitamin D concentrations did not differ significantly (P = 0.372) between the penguins housed indoors (48.9 ± 16.7 nmol/L) and those housed outdoors (50.9 ± 26.5 nmol/L), however plasma iCa concentrations differed significantly (P = 0.02) between indoor (1.04 ± 0.09; n = 5) and outdoor (1.21 ± 0.02, n = 3) penguins. The results in this preliminary study suggests that indoor housed penguins with access to UVB have comparable levels of vitamin D to outdoor housed penguins. To evaluate if plasma could be used to analyze vitamin D and iCa in penguins, tandem serum and plasma samples were submitted from one zoo population. iCa concentrations in plasma (1.21 ± 0.02 mmol/L, n = 3) and serum (1.29 ± 0.34, n = 6) differed significantly (P = 0.01), however the obtained 25-hydroxyvitamin D values were not significantly different (P = 0.429, n = 6). These results suggest that banked plasma samples may provide an accurate representation of vitamin D levels in Magellanic penguins.

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Student Support: None
Sublethal effects on motility seen in *Amblyomma americanum* treated with lotilaner (Credelio®)

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Isoxazolines are a novel class of acaricides with a rapid speed of kill. However, ticks must feed on the host to ingest the acaricide, which raises the question of whether the host is exposed to pathogens during that time. We expect the sublethal effects of the isoxazoline lotilaner (Credelio®) to shut down normal physiological processes, which will result in reduced speed and duration of movement. To test the sublethal effects of lotilaner, six purpose-bred beagles were treated according to labeled dose bands and six were left untreated. *Amblyomma americanum* ticks were applied and groups of five ticks were removed after 2, 4, and 8 hrs. Upon removal, movement of each tick was recorded and total distance, mean velocity, and maximum velocity were analyzed in EthoVision XT software. A significant difference was observed at all three time points between treated and untreated ticks for mean total distance traveled ($P < 0.0413$). Significant differences were observed between treated and untreated ticks for average mean velocity and mean maximum velocity only at 4 and 8 hrs ($P < 0.0001$). Within the treated group, motility of the 2 hr ticks was significantly different from 4 and 8 hr ticks for all three parameters ($P < 0.05$), but the 4 hr ticks were not significantly different from the 8 hr ticks ($P > 0.08$). The untreated ticks showed no significant motility differences between the time points ($P > 0.31$). Ticks treated with lotilaner showed a time-dependent decrease in motility as compared to untreated ticks. These data exhibit evidence that, while still attached to the animal, the normal processes of the tick appear to be shutting down, potentially reducing the risk of pathogen transmission to the host.

Research Grant: Elanco Animal Health
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Improving rat ultrasonic vocalization classification, deep learning versus traditional experimenter analyses

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Rats are highly social animals that communicate using a wide range of auditory calls. These auditory vocalizations fall outside the range of human hearing and as such are known as ultrasonic vocalizations (USVs). Adult rat USVs fall broadly into two categories based on frequency; 50 kHz and 22 kHz calls. 50 kHz USVs are referred to as “positive” calls and are associated with positive affective states and appetitive stimulus. Inversely, 22 kHz calls are generally referred to as “negative” and are associated with painful and noxious stimuli. Wright et. al (2010) identified 14 different patterns in 50 kHz rat USVs. These categories represent a significant step forward in understanding the nuance in rat auditory communication. However, identifying and classifying individual rat USVs is difficult, time consuming, and subjective work. The ideal solution to this problem would be an automatic USV detector, one that is fast, accurate, and consistent. In the past this kind of detector was impossible to implement, but newer machine learning approaches are promising. DeepSqueak (Coffey et. al 2018) was designed as a deep learning approach to mouse and rat USV analysis. This project utilizes DeepSqueak to analyze multiple recordings of rat USVs. Unsupervised clustering is used to filter identified calls into categories, either the 14 patterns published by Wright et. al, or new categories generated de novo out of the available data. The machine identified USVs and the categories the machine placed each USV into are compared to manually identified calls and labels from the same recordings. This project explores the efficacy and limits of machine learning based USV analysis to maximize the efficiency of future USV analysis pipelines.

Research Grant: NIH GRANT12520615—Chronic deficits in auditory communication following early life seizures
Student Support: AVC Veterinary Summer Research Award (VetSRA)
Diversity and ubiquity of *Pseudomonas aeruginosa* specialized metabolites

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Over the course of the summer I will be working with Dr. Robert Quinn on a project that is focused on the human gut and cystic fibrosis. Cystic fibrosis (CF) is an inherited disorder that causes severe damage to the lungs, digestive system and other organs in the body. CF affects the cells that produce mucus, sweat and digestive enzymes. Secondary infections of the lungs by pathogens such as *Pseudomonas aeruginosa* are a major complication of cystic fibrosis. *P. aeruginosa* produces a number of small molecule virulence factors including phenazines, quinolones, rhamnolipid, and pyochelin, that contribute to CF lung disease. For this project we will use tandem mass spectrometry and computational analysis to search for these compounds in public data. Tandem mass spectrometry is a common technique that produces spectral fingerprints (MS/MS spectra) of molecules based on their mass. In the public database GNPS, thousands of datasets are available and millions and millions of MS/MS spectra that can be searched with an algorithm called MASST. We will search for all known quinolones, phenazines, rhamnolipids and pyochelins to identify where these compounds are found in nature and describe their diversity and ubiquity in different sample types. A better understanding of which of these metabolites the bacterium produces will help us better understand the biology of *P. aeruginosa* infections in cystic fibrosis and develop MS/MS methods for their diagnosis.

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Effective dose 50 of oral and intramuscular midazolam and its effects on corticosterone in zebra finches

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While routine care is essential in companion avian species, birds can become easily distressed when handled, leading to an increased risk of injury. Intranasal (IN) midazolam has become increasingly popular in avian medicine to induce sedation for minor procedures. While IN midazolam is a convenient mode of administration, the total volume administered intranasally to small species like zebra finches can make drug administration challenging, and could require prolonged handling when compared to other routes. Therefore, we examined optimal sedation doses of oral and intramuscular (IM) routes of midazolam administration and will assess the clinical practicality of these different sedation routes in small birds, as compared to IN midazolam. The effective dose 50 (ED$_{50}$) of oral and IM midazolam was determined using Dixon’s Up-and-Down method. Briefly, randomly ordered birds (n = 14) were treated with a starting dose of 6 mg/kg of midazolam, and sedation score was determined at 20 min post-administration. A decrease or increase of 2 mg/kg from the previous dose was then administered to the following bird based on the preceding sedation score. Similar to what was found for IN midazolam (1.9 mg/kg; concurrent study), the ED$_{50}$ of oral midazolam was 1.7 mg/kg. In contrast, the ED$_{50}$ for IM midazolam was substantially lower, at 1.08 mg/kg. Currently, the impact of midazolam administration route (oral ED$_{50}$ vs. IM ED$_{50}$ vs. placebo) on stress response in zebra finches is being assessed by measuring changes in levels of circulating corticosterone. Results of this study will provide more accurate dosing of midazolam and will determine if either oral or IM midazolam administration will improve sedation protocols in small avian species.

**Research Grant:** OSU Veterinary Clinical Sciences departmental funds and the OSU Dr. Kristie Plunkett Exotic Animal Fund  
**Student Support:** Morris Animal Foundation Veterinary Student Scholar Grant
**The pharmacokinetics of transdermal flunixin in dairy goats**

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Flunixin meglumine is an injectable nonsteroidal anti-inflammatory drug (NSAID) approved for use in dairy cows to manage pyrexia associated with bovine respiratory disease, mastitis, and endotoxemia. In the United States, no NSAIDs are approved for use in goats, but there is a need to treat painful conditions to improve animal welfare. Therefore, flunixin is occasionally administered extralabel in dairy goats to manage pain associated with lameness, mastitis, and other conditions. This study will evaluate the pharmacokinetics of transdermal flunixin in dairy goats to determine a withdrawal interval (WDI), the period of time an animal must wait after receiving a drug before its products can enter the food supply. Six female dairy goats will receive 3.3 mg/kg of transdermal flunixin. Milk, interstitial fluid, and plasma samples will be collected at various times for 21 days. The samples will be analyzed using UPLC-MS/MS to detect the flunixin marker metabolite, 5-hydroxy-flunixin (5-OH-FLU). Data will be plotted to see how long it takes for concentrations to drop below 2 ppb, the US tolerance level for 5-OH-FLU in dairy cow milk. However, there is zero tolerance for 5-OH-FLU in goat milk. It is hypothesized that transdermal flunixin will be absorbed and eliminated slower in dairy goats than it is in dairy cows, therefore dairy goats will have a longer WDI. Based on data from previous studies evaluating the pharmacokinetics of transdermal flunixin in meat goats and dairy cows, a projected WDI was estimated to be 14 days for dairy goats. The findings of this study will provide a more reliable WDI for transdermal flunixin, thus improving animal welfare and providing a safe food supply.

**Research Grant:** Food Animal Residue Avoidance Databank  
**Student Support:** Center for Chemical Toxicology Research and Pharmacokinetics

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**Characterization of subcellular localization of chlamydial antigens translocated using connexin 43**

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*Chlamydia muridarum* is the mouse analog to the human pathogen *Chlamydia trachomatis* and is used to study the pathogenesis of infection and guide therapies for human *Chlamydia* genital infections. In *C. muridarum* infected mouse genital tract, chlamydial antigens were observed in uninfected cells adjacent to *Chlamydia*-infected cells. Among the various mechanisms available for transport of chlamydial antigens from infected to uninfected cells, gap junction-mediated antigen transport provides the most direct route without crossing multiple membranes. HeLa S3 cells engineered to express connexin 43 (Cx43), an important gap junction protein, and infected with *C. muridarum*, translocated bacterial antigens to adjacent uninfected cells. Within these uninfected cells, the chlamydial antigens appear to be confined within distinct membrane-bound compartments. The goal of this study is to determine the subcellular compartmentalization of *C. muridarum* antigens translocated to uninfected HeLa S3 cells. We investigated early endosomes (EEA1), late endosomes (RAB7A), the Golgi apparatus (GOLGA1), and the endoplasmic reticulum (CANX) using immunocytochemistry followed by fluorescent microscopy. HeLa S3 cells were stained with primary antibodies for each of the respective compartments and secondary fluorescent antibodies. The conditions for staining EEA1, RAB7A, and GOLGA1 were standardized, and are now being evaluated in *C. muridarum*-infected cells. Identifying the compartmentalization of *C. muridarum* antigens in uninfected cells will provide insights into pathogenesis, and likely identify targets for reduction of clinically relevant pathologies caused by *Chlamydia* genital tract infection.

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**Student Support:** Boehringer Ingelheim Veterinary Scholars Program
Assessing the value of hematologic and biochemistry results on disease diagnosis and outcome in exotic pets?

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Hematologic and biochemical data are routinely used to assess the health status of exotic pets. This information is considered an important component of baseline testing and is used to guide requests for additional diagnostics and treatment. Because of the stoic nature of exotic pets, hematologic and biochemical testing are also recommended as part of an annual examination. However, there has been much debate regarding the value of hematologic and biochemical testing in these species, as there are cases with fulminant disease and no abnormalities, sedation is required for some species, and patients can die during blood sampling. To determine the value of hematologic and biochemistry data in exotic pets, research is needed to investigate whether the results impact the outcome. The goal of this study is to determine the predictive value of hematologic and biochemistry data on disease diagnosis and outcome of exotic pets presented to the LSU Veterinary Teaching Hospital. The specific hypotheses of this study are that: 1) hematologic data will be predictive of diagnosis and outcome in bird and exotic small mammal patients, but not reptiles, 2) tissue enzyme data will not be predictive of diagnosis and outcome in any group, and 3) electrolyte and mineral data will be predictive of diagnosis and outcome. A cross-sectional study will be performed reviewing signalment, hematologic, biochemistry, diagnosis, and outcome data from exotic pets presented to the LSU VTH from 2018-2020. Univariate and multivariate statistics will be used to determine whether blood results are predictive of diagnosis and outcome.

Research Grant: National Institute of Health, Louisiana State University School of Veterinary Medicine
Student Support: National Institute of Health B18, Louisiana State University School of Veterinary Medicine

Evaluating the efficacy of flunixin meglumine or oral meloxicam at reducing post-surgical pain in sheep

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Common livestock procedures like castration cause pain, and lack of analgesics for species like sheep impacts the ability to manage pain. Chronic pain leads to hyperalgesia and allodynia, neuropathic disorders impacting animal health and welfare. This study evaluated the efficacy of flunixin meglumine (FLU) or meloxicam (MEL) at providing pain-relief post-soft-tissue surgery in sheep. It is hypothesized both analgesics will be effective in pain management. Thirty ewes (Hampshire, Rambouillet, Polypay; n = 10/breed) were randomly assigned to one of three treatments where sheep received either: FLU: pre-laparotomy, at 24h and 48h post-surgery, 2.2 mg/kg intravenously; MEL: pre-laparotomy, 2.0 mg/kg orally and at 24h and 48h post-surgery 1.0 mg/kg orally; or control (CON): sedation only. Outcome variables were recorded at -24h, 4h, 6h, 24h, 30h, and 48h post-surgery. Video recordings collected behavior data of sheep in their pens and facial, vocalization and gait score data as they crossed a pressure mat. Average temperature/inflammation and mechanical nociceptive threshold (MNT) around the incision site were collected using an infrared thermography camera and an algometer respectively. CON sheep spent more time walking and chewing at their body and had higher MNT at all sites near the incision than FLU and MEL sheep (P ≤ 0.0014 and P < 0.001 respectively). There was no difference in behavior or MNT between MEL and FLU sheep (P > 0.05). Preliminary results suggest FLU and MEL provide some pain relief with no efficacy difference. Further analysis of outcome variables will be used to confirm these findings. These results direct future research in effective analgesia for sheep helping prevent neuropathic disease and welfare deficits.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program
Risk factor analysis of blaNDM-5 carbapenem-resistant *Enterobacteriaceae* at a Philadelphia veterinary hospital

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Carbapenem resistant *Enterobacteriaceae* (CRE) infections have been sporadically reported from both humans and companion animals in the United States, Europe, and China. Since July 2018, the CRE strain *bla*NDM-5 has been identified in over 40 animal patients in the Ryan Veterinary Hospital at the University of Pennsylvania (Ryan VHUP), a teaching veterinary hospital in Philadelphia. A period prevalence study was conducted by fecal culture on all companion animals present in Ryan VHUP from June 24 to September 24, 2019 on weekly sampling days. Medical records the patients were used to investigate hospital services and patient factors and their potential associations to *bla*NDM-5 colonization. The 18 identified positively colonized animals were compared to 255 animals with negative cultures through simple logistic regression. So far, results have shown that increased antibiotic, steroid, and immunosuppressant use were significantly associated with an increased likelihood of colonization, as were longer stays in the hospital and greater numbers of prior visits to VHUP. Patient-specific factors, such as age, weight, and purebred status were not significantly associated with increased risk of *bla*NDM-5 CRE colonization. This indicates that the risk of colonization is more related to exposure and veterinary care than to an inherent quality of the animal, and that more research needs to be done to minimize the healthcare-associated risk of colonization.

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Down under in the land down under: monotreme reproduction

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Monotremes (Monotremata) are one of the oldest living taxon of the class Mammalia that branched off from the lineage two-hundred million years ago. These mammals exist today as one of five extant species. This includes four distinct species of echidnas (Tachyglossidae), three of which are long-beaked (Zaglossus) and one of which is short-beaked (Tachyglossus); and the famous amphibious duck-billed platypus (Ornithorhynchidae). Incredibly enough, all five of these species are native to “the land down under”, Australia. Australia as a continent has a very diverse system of mega-habitats, ranging from tropical forests and deserts, to grasslands and tundra. The survival, conservation, and reproduction of these species in this varied environment begs the question of why these mammals lay eggs and does this provide some evolutionary benefit in a highly varied continent like Australia? One of the main reasons for their survival is credited to the complex reproductive tract that both male and females utilize. The reproductive tracts in monotremes are seen to correlate with aspects of reptilian, avian, and even mammalian reproduction. Nevertheless, the focus of this review article is to dissect the intricacies of reproductive anatomy of the three subgroups of monotremes, both male and female. In addition, this information might provide information on what conservation endeavors can be implemented to better understand the impact these organisms have on the natural world and how we can add to the populations and knowledge of these iconic species.

**Research Grant:** None  
**Student Support:** Student Funding provided by NIH Grant 5T35OD016477-19 to Michigan State University.
Meningoencephalitis in addra gazelle (*Nanger dama ruficollis*) from zebra associated equine herpes-virus 1 and 9

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Equine herpes-virus (EHV) infection in domestic and wild equids ranges from asymptomatic carriers to clinical disease including respiratory signs, abortions, and myeloencephalopathy. In zoos zebras are the typical reservoir host for EHV responsible for causing fatal myeloencephalopathy in a variety of species including; giraffe (*Giraffa camelopardalis*), Thomson’s gazelle (*Eudorcas thomsonii*), and polar bears (*Ursus maritimus*). In 2020 an outbreak of EHV-1 at Binder Park Zoo affected 50% of the addra gazelle (*Nanger dama ruficollis*) herd (herd size n = 4). A 9-mon-old male presented with pyrexia, head tilt, eyelid paresis, circling, and exertional myopathy. Animal initially responded to supportive treatment, but was euthanized one week later due to vision deficits from EHV associated retinitis. This animal’s dam presented recumbent with convulsions, and died 72 hours after the male’s presentation. Both animals were diagnosed with multifocal histiocytic to lymphoplasmacytic meningoencephalitis with intranuclear inclusion bodies. Polymerase chain reaction on brain tissue was negative for the male and positive for the female; zebra associated EHV-1 strain T-616 and isolate B6 404 was identified with bidirectional sequencing. Immunohistochemistry of the male’s retinal tissue was positive for EHV-1. Banked serum samples from the surviving herd members and a subset of the zebra herd will be evaluated for EHV-1 titers via serum virus neutralization. A case of acute death in an addra gazelle due to EHV-9 was also identified at another zoological institution. These cases reinforce the risks of mixed species exhibits at zoos and further investigation into the effects of EHV vaccination on viral shedding in zebra is warranted.

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Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

Establishing regulatory thresholds for endemic pathogens in shellfish transported across state jurisdictions

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Outbreaks of disease in the marine environment occur over massive spatial scales. Habitats located hundreds of miles apart are connected by ocean currents that carry and spread viruses, bacteria, and other infectious agents. One such agent is the protozoan parasite *Perkinsus marinus*, which causes Dermo disease in the eastern oyster, *Crassostrea virginica*. In the mid 1980s and early 1990s, this parasite devastated the oyster aquaculture industry across the mid-Atlantic, and it continues to be a major cause of mortality on shellfish farms. While Dermo disease transcends state boundaries, biosecurity is implemented at the state level, creating a unique management challenge. We are creating an Integral Projection Model (IPM) to understand the disease outcomes resulting from the importation of infected oysters. IPMs are apt at describing diseases in which transmissibility and survival depend both on prevalence (number of hosts infected) and intensity (pathogen burden within an individual), and have previously been used to describe facial tumor disease in Tasmanian devils, white-nose syndrome in bats, and aspergillosis in sea fans. Our IPM will test how prevalence and intensity of Dermo disease in imported oysters influences disease-related mortality and resulting parasite burden in a local population. We hypothesize that intensity is a stronger driver of disease outcomes than prevalence, such that a small number of heavily infected hosts will create more significant disease impacts than a larger number of lightly infected hosts. With this model, we hope identify a tolerable threshold of infection in imported stock, and guide regulatory practices governing the importation of oysters across state boundaries.

Research Grant: NOAA Sea Grant Aquaculture Initiative
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070
Comparative description of the rostral muscles in the giraffe (Giraffa camelopardalis)

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The masseter muscle of the giraffe (Giraffa camelopardalis) have been thoroughly described in previous research by Saki, et., al (2001). In contrast, there is little muscular description involving the rostral portion of the giraffe skull. Early reports of giraffe limb muscle structure have indicated more similarity to equines, despite their closer relation to bovines. The giraffe skull, however, appears to share similarities to that of both bovines and equines. The giraffe and bovine skulls possess ornamentation on the top and have no rostral dentition, while the former share the narrow and elongated rostrum of equines. We dissected the facial muscles of a giraffe and found they share structures like those observed in bovines, while possessing their own unique muscle distribution. For instance, the elongated snout isolates the facial muscle to the rostral portion and do not extend far caudally past the maxillary molars, unlike in bovines, where the muscles have a greater overlap and completely cover the skull. One major difference between the species lies within the buccinator and the orbicularis oris. The bovine buccinator covers the cheek and extends towards the lips, ending right before the caudal edge of the o. oris. The o. oris of the giraffe covers the entire upper lip, but only about one-fourth of the lower lip, whereas the bovine o. oris covers the entire lower lip. The remainder of the giraffe’s o. oris is covered in cutaneous tissue. These differential findings could be due to giraffe variations in diet and skull structure, as giraffes tend to graze on taller trees, requiring a longer snout and more muscle focus rostrally.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Canine hemangiosarcoma establishes a unique immune niche to promote tumor growth

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Hemangiosarcoma (HSA) is a highly metastatic and lethal cancer that is prevalent in dogs. HSA is a vasoformative tumor that tends to occur frequently in organs with well-developed vascular networks. Histological morphology of abnormal, destructive vascular tissue is a symbolic feature of HSA: this tumor is characterized by disorganized, irregular vascular spaces filled with blood. The complex, dynamic behavior of HSA cells, particularly their interactions with other cells in the tissue microenvironment remains poorly understood. In this study, we aimed to determine distinct gene signatures between tumor and stromal cells using xenograft of canine HSA. We found that canine HSA enriched genes associated with pro-inflammatory cytokines but minimally expressed genes encoding their receptor proteins. The mRNA expression profile showed highly expressed IL-6, IL-10, IL-15 in HSA samples. Our xenograft model of canine HSA established species-specific transcriptomic profiling and we identified distinct expression pattern distinguishing sources of cytokines and the receptors in the tumor tissues. We found that canine HSA cells increased canine-specific CCL2 gene but reduced COL1A1 expression, and tumor stroma enriched mouse-specific Ccr2 in abundant Col1a1 gene. Our data suggest that the molecular mechanisms that control cellular behavior of HSA cells associated with immune regulation and their interactions with the local tumor microenvironment are necessary for tumor development and progression.

Research Grant: National Canine Cancer Foundation
Student Support: CVM Summer Scholars, Skardon Family Fund
The effect of berry extracts on EGFR expression and hypoxic response in feline oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is a locally invasive tumor that is commonly diagnosed in cats. Feline OSCC (FOSCC) progresses rapidly with poor prognosis and limited treatment options. Berry extracts are of interest as potential FOSCC therapy due to their antineoplastic properties, which include inhibition of cancer cell survival, proliferation, differentiation and migration in a variety of cancer studies. Furthermore, cats possess unique differences in drug metabolism that may result in higher bioavailability of berry-derived compounds. The purpose of this paper is to review the current knowledge of berry extracts as a possible therapy for FOSCC, with emphasis on their ability to target epidermal growth factor receptor (EGFR), one of the most studied molecular targets of cancer therapy. This literature review is based predominantly on human and rodent studies published after 2010. Feline studies were examined in detail whenever possible, but were limited due to the number of studies available and small sample sizes. This review provides an overview of the anticarcinogenic effects of berry compounds in relation to EGFR expression and activity in human and feline OSCC. Controversies around the mechanism and significance of EGFR overexpression, and the efficacy of berry flavonoids as EGFR inhibitors are also discussed. Localized hypoxia (low oxygen levels) is an important feature of tumours that can contribute to altered EGFR activity, malignant behaviour and treatment resistance. Therefore, evidence related to the effects of berry compounds on EGFR signaling and OSCC behaviour under hypoxic conditions is also presented, along with recommendations for future studies of berry phytochemicals as part of FOSCC treatment.

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Student Support: NSERC Undergraduate Student Research Award (S.Xie)

Effects of GNAS inactivation mutation on differentiation potential of Dpp4+ progenitor cell

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Progressive Osseous Heteroplasia (POH) is a human genetic disorder of mesenchymal cell mis-differentiation that causes progressive extra-skeletal ossification in cutaneous, subcutaneous and deep connective tissues including skin and fat. Most cases of POH are caused by heterozygous inactivation of the GNAS gene that encodes the α-subunit of the G stimulatory protein. We have observed significant changes of subcutaneous adipose tissue in response to loss of GNAS, however, the specific identity of the progenitor cells that undergo osteogenesis in response to GNAS mutation remains unidentified. The purpose of this study is to investigate the effects of GNAS inactivation on inguinal white adipose tissue (iWAT) through single cell RNA sequencing (scRNAseq). Data analysis was conducted on an existing scRNAseq dataset from developing iWAT of wild-type mice. We successfully replicated the expected expression patterns of adipogenic progenitor cell markers including Dpp4, ICAM1, PPARγ, and PDGFR-α in normal iWAT. Cells expressing these markers are hypothesized to be cells that are mis-directed to ectopic bone formation. We therefore also investigated expression patterns of osteogenic markers, such as Runx2, alkaline phosphatase and collagen in these cells. Results show that these osteogenic markers were absent in normal iWAT, confirming that cell fates are committed towards adipogenesis instead of osteogenesis in normal Dpp4+ cell and preadipocyte populations. The next steps of this study will be to generate, analyze and compare scRNAseq data from iWAT of GNAS knockout mouse model with control group to determine whether the GNAS inactivation alters the abundance, localization, and/or lineage hierarchy of Dpp4+ progenitor cells.

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Student Support: Boehringer Ingelheim Summer Veterinary Scholars Program
Intrinsic traits and extrinsic factors predictive of RNA viral spillover between wildlife and livestock

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As environmental change brings wild and domestic animals in increasingly close contact, disease spillover at the wildlife-livestock interface is an emerging threat to food production systems. This study aims to identify critical extrinsic factors and intrinsic traits of host-viral interactions which are predictive of viral spillover between wildlife and livestock. Following the PRISMA 2009 systemic review guide, we searched PubMed using designed search keywords to retrieve RNA viral spillover and relevant topics published between 1968 and 2020. Titles and abstracts of articles were screened by the metagear package in R. Article content was examined, with intrinsic traits and extrinsic factors identified in each spillover event. In total, we retrieved 1,950 literature records, and among those 266 (13.6%) were classified as relevant, 670 (34.4%) were potentially relevant and 1,014 (52.0%) were non-relevant. To date, we examined 55 relevant literature and obtained 67 recorded RNA viral spillover events. Our preliminary results (additional data pending) showed 21 RNA virus species, including 21 (31.3%) zoonotic, 48 (71.6%) enveloped, 18 (26.9%) segmented as important intrinsic traits associated with spillover events. Key extrinsic factors identified include, 25 (37.3%) human-animal contact, 33 (49.2%) livestock- and domestic-wildlife interface, 30 (44.8%) local livestock production. The host range of viral pathogens that can spillover from wildlife to domestic livestock is determined by viral and host ecology, genetic traits, and pathogenesis within a host. Preliminary results show that livestock management system scale, and frequency of animal contact appear to be critical determinants of spillover likelihood.

Research Grant: Foundation for Food and Agriculture Research Vet Fellowship
Student Support: Foundation for Food and Agriculture Research Vet Fellowship, UGA College of Veterinary Medicine

A survey of the veterinary community’s opinion of venipuncture on snakes

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Current textbooks identify 4 snake venipuncture locations: coccygeal vein, heart, sphenopalatine vein and jugular vein. There are conflicting opinions on the safety of cardiocentesis in snakes. Topics such as pain perception and analgesia in snakes have not been fully investigated. This study will survey the views of the veterinary community on snake venipuncture and their opinions about pain perception in snakes. It is hypothesized that if a veterinarian believes snakes feel pain, they would be less inclined to use cardiocentesis on an awake patient and would consider the coccygeal vein in place of the heart as the primary site of venipuncture for humane reasons. Out of 206 participants, 39% of the participants self-reported 20+ years of exotic animal experience and 81% of participants rated themselves as either moderately or extremely experienced in reptile medicine. 89% of the participants believe that snakes feel pain in a graded potential. In the clinical scenario questions, 91% of participants would select coccygeal vein as their first site compared to 9% for the heart. 40% of participants believed that sedation or anesthesia is required for cardiocentesis compared to only 4% for sedation and 1% for anesthesia for the coccygeal vein. The coccygeal vein consistently was the highest rated site in terms of humaneness for an awake, sedated or an anesthetized patient. Though further statistical analysis is pending, inferential statistics suggests that the veterinary community believes snakes feel pain similarly to mammals and will preferably use the coccygeal vein in many clinical scenarios. This study should encourage veterinarians to make a community-conscious decision in their clinical practice of snake venipuncture.

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Student Support: None
Safety and efficacy of lidocaine continuous rate infusions for the treatment of ileus in ruminants

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Lidocaine is used routinely to treat postoperative ileus (POI) in horses, but it has not been commonly used for treatment of ileus in ruminants due to a lack of safety and efficacy data. Research into prokinetics in ruminants has focused on antibiotics and their impact on abomasal motility, demonstrating the need for a non-antibiotic option. The purpose of this retrospective case series is to determine if lidocaine is a safe and effective treatment for ileus in ruminants. A search was conducted using the online UVIS databases of the Oklahoma State University and North Carolina State University using keywords to identify ruminants that were treated with lidocaine as well as those with certain diagnoses to provide a comprehensive search of those receiving lidocaine as a treatment for ileus. Nine ruminant case records from both institutions met the inclusion criteria of first having a diagnosis of ileus and then starting treatment with a continuous rate infusion (CRI) of lidocaine. The clinical signs observed after the start of the lidocaine treatments were the primary indicator of the outcome of the treatment. The patient demographic consisted of mostly cattle (7/9) with a median age of one year. Ileus was the most common stated diagnosis, and 7/9 of the animals received an abdominal explore surgery. All animals received a loading dose of 1.3 mg/kg and a CRI rate of 0.05 mg/kg/minute. Treatment ranged from 1-3 days with a median of two days. Seven of nine animals had documented improvement in intestinal motility after treatment with lidocaine, and they survived to discharge. This suggests that a lidocaine CRI at this dose is safe in ruminants, as well as its potential as an effective treatment for ileus in ruminants.

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Student Support: North Carolina State University Veterinary Scholars Program Herbert Benjamin Endowment

Risk factors of gastrointestinal parasites in Central Ohio dogs, a retrospective study

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Infections with endoparasites, especially gastrointestinal helminths, are a common finding in client-owned dogs. The Community Practice (CP) section at the Ohio State University College of Veterinary Medicine (OSU-CVM) follows Companion Animal Parasite Council (CAPC), American Animal Hospital Association (AAHA), and American Veterinary Medical Association (AVMA) guidelines for parasitology by recommending annual fecal analyses of dogs and prescribing year-round, broad-spectrum parasite preventatives. There is increasing interest in determining if drug resistant helminths are present in canine populations as there are reports of greyhounds with drug resistant hookworms. Our 2019 study retrospectively analyzed the fecal analysis results from varying dog breeds. We report 891 total canine fecal samples submitted during 2019 from 681 dogs of varying breeds. Of the 891 canine fecal samples, 190 (21.32%) of these samples had a positive fecal analysis for gastrointestinal parasites. We included in this analysis 130 fecal samples derived from 63 greyhounds. To determine if COVID-associated hospital restrictions impacted the fecal examinations performed, data from January to July, 2020, was compared to the same period in 2019. The age of dog, time of year, reproductive status, purpose of fecal examination, gastrointestinal signs, and type of parasite preventives were assessed as potential risk factors of gastrointestinal parasite infection in dogs. Identifying such risk factors in dogs will guide veterinarians to advise annual fecal examinations more strongly to clients with high risk dogs or when routine health visits are postponed for an extended period.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program
The role of spontaneously occurring canine glioma in preclinical therapeutic studies

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Gliomas are the most abundant type of primary brain tumor in humans. In particular, glioblastoma carries a poor prognosis with a median survival time of less than 15 months after standard of care treatment. Preclinical rodent glioma models have shown a lack of reliability in predicting the efficacy of investigational treatments, as very few clinical trials have resulted in new drugs or devices that prolong survival times in human patients despite preclinical success in rodent models. Companion dogs also spontaneously develop gliomas with similar frequency and biologic features to human gliomas. Having a larger brain size than mice, comparable environmental conditions to humans, and an intact immune system, dogs with spontaneously occurring gliomas are now being recognized as a preclinical model for therapeutic studies. Recent efforts to characterize the molecular features of canine gliomas have provided substantial advancements in our understanding of these tumors. This review discusses the similarities and differences between human and canine gliomas, highlighting the importance of this information for potential translational studies. This analysis also describes examples of therapeutic studies utilizing data from canine glioma patients to inform human clinical trials. Taking into account the advantages and limitations of this model, pet dogs diagnosed with gliomas may be a reliable preclinical model to evaluate specific glioma therapeutics and thus provide valuable knowledge to improve both human and veterinary patient care.

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Student Support: Students Training in Advanced Research Fellowship

Transcriptomic alterations in microglia from rat offspring of a model of maternal sleep apnea

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Maternal sleep apnea, characterized by gestational intermittent hypoxia (GIH) is prevalent during pregnancy, but its adverse effects on offspring neurodevelopment remain largely unknown. We find that juvenile and adult offspring of a rat GIH model have significant impairments in cognitive and social behavior, with males being more severely affected than females. The medial prefrontal cortex (mPFC) is critical for these behavioral deficits, and mPFC pyramidal neurons in male GIH offspring have increased dendritic spine density. Since microglia, brain resident immune cells, are critical for synaptic pruning in brain development, we investigated transcriptomic differences in mPFC microglia isolated from adult offspring of dams exposed to gestational normoxia (GNX) or GIH, following an immune challenge with lipopolysaccharide (LPS) or saline vehicle. While there were few GIH-related sexual dimorphisms in quiescent microglia, following LPS challenge, male GIH microglia exhibited greater downregulation of inflammatory genes than female GIH microglia. Further bioinformatics analyses identified STAT and IRF transcription factors as major drivers of these downregulated genes, suggesting that male GIH microglia may have impaired function. Genes differentially downregulated in activated male GIH microglia were associated with the Rho-protein cytoskeleton remodeling pathway, providing a potential mechanism for reduced microglial phagocytosis or migration in GIH microglia. Together, these data suggest that impaired cytoskeletal remodeling in male GIH mPFC microglia may underlie their dysfunction in synaptic pruning, leading to the neural deficits observed in male GIH offspring.

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Student Support: UW School of Veterinary Medicine, Dean’s Office
Coronavirus through time and species: analysis of coronaviruses in relation to the Covid-19 pandemic

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Coronaviruses, positive-sense, single stranded, RNA viruses, are a large group of viruses in the Coronaviridae family that cause a wide variety of diseases in humans and animals. From the avian IBV, the first coronavirus isolated from poultry with respiratory disease during 1930s to current SARS-CoV-2, coronaviruses continue to present challenges to animals and humans. Animal coronaviruses can cause asymptomatic to fatal infections in the respective target host including avian, porcine, bovine, canine, feline, or murine species. Cross-species transmissions are common for coronaviruses and could cause large outbreaks until herd immunity is established. Typical human coronaviruses such as 229E and OC43 are associated with common cold causing mild upper respiratory infection. More recently found human coronavirus including NL63 and HKU1 have an increasing tendency to cause lower respiratory disease. Since 2003, highly virulent coronaviruses including SARS-CoV, MERS-CoV, and SARS-CoV-2 have emerged with severe lower respiratory disease with systemic signs. Coronavirus S protein is a major structural protein and is responsible for viral attachment and entry to target cells. This protein is involved in cross-species transmission, tissue tropism, and virulence and can serve as a vaccine component and potential therapeutic target. In this literature review, various animal and human coronaviruses are analyzed with focus on S protein including interactions with the cellular receptor, subdomain arrangement, functional mutations and alterations in virulence, and receptor affinity. Understanding this fundamental biology should help find solutions for preventive and therapeutic measures for current and future coronavirus pandemics.

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Student Support: AVMA/AVMF 2nd Opportunity Research Scholarship

Baseline cognitive screening using a puzzle feeder across four species of Hylobatidae

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Hylobatidae, also referred to as lesser apes or gibbons, are divided into four extant genera and further specified into 20 species. Gibbon cognition has yet to be studied thoroughly likely due to the small number of captive gibbons and their species-specific statuses ranging from vulnerable to critically endangered. Current knowledge supports that gibbons are highly intelligent with a generally strong interest in novel objects. The Gibbon Conservation Center located in Santa Clarita, California has housed various research studies and provides the ideal environment for enrichment-based projects. This research focuses on male-female pairs spanning four species: the Hoolock leuconedys (Eastern Hoolock), Hylobates pileatus (Pileated), Nomascus leucogenys (Northern White-Cheeked), and Hylobates moloch (Javan) and their interactions with a novel puzzle feeder. This study uses an adjustable puzzle feeder to allow for the formatting of nine different levels of difficulty. Our intention is to introduce the puzzle feeder as a tool for baseline cognitive screening for a food reward. Measurements include frequency, duration, solving capacity and behavioral observations relating to the introduction of the puzzle feeder. We hypothesize that providing the puzzle feeder as a problem-solving based enrichment tool will help to create a baseline understanding of gibbon cognition highlighting differences in species. The ability to better understand the problem-solving capacity and how it varies between species will be a step in creating a library to understand gibbon cognition. Preliminary data suggests a difference in solving ability and time taken to solve between those in our study.

Research Grant: Western University of Health Sciences Summer Fellowship Program; and Western University of Health Sciences CVM Small Grants for Projects Involving Students
Student Support: Western University of Health Sciences Office for Research & Biotechnology
Exploring African swine fever virus protective antigens

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African swine fever virus (ASFV) causes a fatal disease in its swine hosts and has large economic effects in the swine industry. Currently, no vaccine is available for ASFV and vaccine development has been hindered by the lack of understanding of the virion proteins responsible for inducing the effector immune response. ASFV is a DNA virus with multiple viral proteins that make it difficult to understand the immune response the virus generates in its host. In this proposed study, the role of ASFV proteins in inducing immune response including effector antibody responses and effector T cell responses in swine hosts will be investigated. To investigate the response, 20 ASFV virion proteins will be expressed using a poxvirus-ASFV library generated from an attenuated Orf virus and injected into swine. In this proposed study, antigens will be designed, and in silico construction strategies will be developed to create the poxvirus-ASFV library. This library will be used in future studies to immunize swine. Host antibody and T cell responses will then be assessed in future studies. By increasing knowledge on host immune responses to ASFV virion proteins, the results from this study may be help in the development of vaccine candidates for ASFV.

Research Grant: D.G. Diel Start-up package
Student Support: NIH T35 Training Grant 5T35AI007227-32

An exploratory study of stress behavior in dogs

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The stressors that dogs are exposed to in shelters can impact their behavior and welfare. Results from studies tend to show decreased welfare and reduced adoptability due to stress. Because of this, it is important for animal caretakers to be capable of identifying signs of stress in dogs at the shelter in an easy and non-invasive way. To establish which stress behavior patterns can be easily identified by observers, this exploratory study will examine existing videos of kenneled and isolated dogs to look for stress behaviors. These behavior patterns will ultimately be used as the basis for a canine behavioral stress assessment. This will be accomplished by coding 100 videos from past research using Noldus Observer XT. In each video I will code for 33 behaviors described in an ethogram and uploaded into the software for either occurrence (count) or duration (number of seconds) or both. A second rater will code 20% of the videos so inter-rater reliability may be calculated. The data analysis will include descriptive statistics and principal component analysis to analyze the behavioral data. Through use of more advanced coding software, a greater volume of behavior data can be analyzed at a deeper level than in many previous studies. This analysis could identify subtle but important stress behaviors that may not be identified as frequently as well-agreed upon stress signals. This would allow for progress toward the use of a behavioral tool that can be used in a variety of settings, which could be utilized by the animal care industry to improve the welfare of shelter animals.

Research Grant: NIH Student Scholar Program
Student Support: None
Biomechanical risk factors of pacing and trotting on the incidence of osteochondrosis in Standardbred foals

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Osteochondrosis (OC) is a common developmental orthopedic disease that occurs when endochondral ossification is delayed. Both genetic and environmental risk factors contribute to OC. It was previously reported that pacers and trotters are susceptible to developing OC lesions at different locations in the tarsocrural joint (hock). This observation may be due to the biomechanical differences between pacing and trotting that could affect which OC lesions heal and which become permanent. The goal of this study was to quantify activity in Standardbred foals in their typical farm environment and correlate these data with the development and locations of OC lesions. We hypothesized that the amount of time foals spend pacing or trotting influences the distribution of OC lesions. Standardbred foals (n = 137) on three farms were prospectively followed from 2-12 months of age. Radiographs were taken of their hocks every two months from 2-12 months of age to look for OC lesions. Foals were also video recorded in their normal paddock/pasture turnout for at least two consecutive hours per week. Based on the video recordings, activity was assessed in 30-second time intervals and categorized as nursing/eating, standing quietly, lying down, walking, trotting/pacing, and running. Foals spent ~82% of their time standing or eating with < 18% of their time in active movement. Less than 1% of their time was spent pacing or trotting. Permanent OC lesions developed in 26% of foals; the majority of these were at the distal intermediate ridge of the tibia. Lesion distribution did not differ between pacers and trotters. More work is needed to elucidate the potential role of biomechanics on OC lesions.

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Student Support: Office of the Director, NIH, T35 OD011145

Spatial distribution of microplastics and zoonotic pathogens along coastlines in North America

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Plastics are widely recognized as pervasive marine pollutants. Microplastics (plastics < 5 mm) have been garnering increasing attention due to reports documenting their ingestion by fish and invertebrates, including shellfish intended for human consumption and commonly consumed by wildlife. Microplastic contamination has thus raised concerns for both human and wildlife health. Last summer we investigated an unexplored area of research targeting the interaction between plastic and pathogen pollution in coastal ecosystems. We found that microplastic beads and fibers can associate with the zoonotic parasites Toxoplasma gondii, Cryptosporidium parvum, and Giardia enterica in seawater and may act as vectors for pathogen transport. This project aims to develop a spatial model that evaluates the distribution and overlap of microplastics and these zoonotic pathogens in nearshore habitats. A literature review was conducted to compile data from field studies documenting the presence of microplastics and parasites in coastal environments along North America. We developed a spatial map using R and preliminary analysis revealed that both microplastics and zoonotic parasites were found along the central California coast, where several fisheries and the endangered California sea otter are present. The observation that microplastics and zoonotic pathogens contaminate the same nearshore waters, supports our hypothesis that anthropogenic pollutants such as plastics may alter the transmission behavior of zoonotic parasites in nearshore waters. Future research by our team will test whether pathogens that are associated with microplastics are more likely to be ingested by shellfish that are commonly consumed by both humans and wildlife.

Research Grant: Ocean Protection Council and California Sea Grant (Project # 19-0592), National Oceanic and Atmospheric Administration
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Sensitivity of *E. affinis* and *P. forbesi* to bifenthrin, copper, and chlorpyrifos in acute toxicity testing

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Within the last two decades in California, there has been a regime shift in pesticide application. The use of pyrethroid insecticides in agriculture and structural pest control is increasing while the use of organophosphate insecticides is decreasing. State agencies made continuous efforts to limit the use of organophosphates due to high toxicity on non-target organisms. New compounds such as pyrethroids that target specific organisms have thus been synthesized. While pyrethroids are effective against terrestrial insects, they may cause harm to non-target aquatic invertebrates via entry into aquatic environments through agricultural runoffs and wastewater effluents. Toxic effects of pyrethroids in calanoid copepods have not been recorded in literature before. However, calanoids are known to be integral food source for fish in higher trophic levels. This study evaluates the sensitivity of calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* to bifenthrin - a pyrethroid, using LC50s generated from 96hr acute toxicity exposure tests. Sensitivity to bifenthrin will be compared with sensitivity to two other prevalently used pesticides chlorpyrifos - an organophosphate and copper fungicide. We will discuss whether the results will help inform us about the potential toxicity of the aforementioned pesticides on the copepod species. Study findings may also be helpful in evaluation of any unintended effects from pesticide use as the California regime continues to shift toward pyrethroid use.

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Review of the physiology of pain, assessment techniques, and painful conditions in dairy cows

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Assessment of pain in dairy cattle is limited to measurements of physiologic, biochemical, and behavioral changes. The aim of the present review is to compile and integrate current methods of pain evaluation in dairy cattle using bovine-specific indicators. Special emphasis was placed on three common painful disease conditions: lameness, metritis, and mastitis. The database used for this literature review was PubMed, and the primary keywords used in the search were “dairy cow” and “pain.” An advanced search was performed to target each specific disease condition. To be eligible for inclusion, articles required full-text availability in English with a publication date after 1998 to limit search results to more current research studies. Research studies cited in articles initially selected through this search process were also included. Validated pain detection measures are a prerequisite for evidence-based pain mitigation. Although there are established guidelines for preemptive and post-procedural analgesia based on such measures for painful procedures, there remains a scarcity of validated guidelines for painful disease conditions, especially those causing visceral pain. This review underscores the need for additional research to validate pain response measures to guide effective pain management and secure dairy cattle welfare in diseased animals. This review further highlights the barriers to widespread analgesic use, including misconceptions regarding pain in production animals, food safety, FDA approval, and cost-benefit analysis.

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