Project Title: Feline liver organoid cultures for investigation of hepatic lipidosis

Principle Investigator(s): Dr. Karin Allenspach (PI), Dr. Laura Van Vertloo (Co-PI)

Collaborating Investigator(s):

Veterinary Scholar Focused Abstract: (300 words or less):

Feline hepatic lipidosis (FHL) is a complex metabolic disorder that occurs as a result of the accumulation of triglycerides within hepatocytes. Classically associated with prolonged anorexia in obese cats, FHL can lead to severe clinical illness that can be fatal. Despite the fact that this is a common disease, the pathophysiology remains poorly understood. Development of a disease model has the potential to facilitate ex vivo investigations of feline hepatic cells derived from healthy and diseased cats, as well as evaluate efficacy of therapeutic interventions. Organoids are three-dimensional structures, derived from primary cell cultures, that retain most of the functions of the organ of origin. Using previously established protocols for epithelial cell organoid cultures, the mentor’s lab has already successfully cultured canine hepatic organoids. The objective of this project is to determine if we can culture feline liver organoids. We will attempt to establish a feline liver organoid culture technique using samples of feline liver (surplus liver from necropsy specimens and liver aspirates). Liver samples will be handled as previously described (Kruitwagen et al., 2017). In brief, samples obtained from necropsy will be enzymatically digested. Supernatant will be checked for biliary duct fragments, centrifuged, and pelleted ducts mixed with cold Matrigel (BD Biosciences). The cell-matrigel suspension will then be seeded as droplets in 48- or 24-well plates and allowed to solidify before overlaying with hepatic organoid culture medium. If successful culture of feline hepatic organoids can be established, phenotypic characterization of the organoids will include bright-field imaging and assessment of organoid maturity (ie cellular differentiation) using fluorescence-based biomarkers. The student will be taught cell culture techniques, RNA-ISH staining of paraffin-embedded tissue and image analysis using Image J. Furthermore, statistical analysis of the data and writing of the manuscript will be performed by the student under the supervision of the mentors.
Project Title: Comparative Hematology – Establishing Data-based Reference Intervals and Health Status for Metarubricytosis (nRBCs) in Dogs

Principle Investigator(s): Dr. Claire B. Andreasen

Collaborating Investigator(s): Dr. Anne Barger, University of Illinois

Veterinary Scholar Focused Abstract: (300 words or less):
This is **Phase II** of a project to establish health correlations of 1-5 nRBCs/100 WBCs in non-anemic dogs (the reported reference interval for “healthy” dogs) for prognosis and health evaluation. The data is lacking to define the actual number of nRBCs/100 WBCs in this range and canine health status. Retrospective teaching hospital CBC data from 2013-2018 is sorted by year for 1700 canine patients with 1-5 nRBCs/100 WBCs. From this data, 300 patients will be randomly selected for survey analysis of 1-5 nRBCs/100 WBCs in non-anemic dogs and correlated to their medical record diagnosis. The goals are to determine if the dogs’ medical condition(s) contribute to a low level of nRBCs or if these levels are found in healthy dogs, and if there are associated morbidity or mortality outcomes. In a **phase I** (prior summer scholar project) retrospective study of canine inappropriate metarubricytosis, data analyzed from 2013-2018 for 1759 non-anemic canine patients with ≥ 1 nRBC/100 WBC and >29% hematocrit resulted in a selection of 72 patients with 119 laboratory reports based on a reported >5 nRBCs/100 WBCs. The associated syndromes were compared to results in human medicine. Analysis of that data determined that canine inappropriate metarubricytosis is associated with lead poisoning, heat stroke, primary splenic dysfunction (non-neoplastic), splenic neoplasia (with or without chemotherapy), non-splenic neoplasia (with or without chemotherapy), thromboemboli/disseminated intravascular coagulation (DIC) with a subcategory of splenic infarct, diabetes (with or without ketosis), primary hepatic dysfunction, hypoxia, sepsis/endotoxemia, intervertebral disc disease, pancreatitis, primary bone marrow dysfunction, inflammation, and miscellaneous diseases. The project will require hypothesis driven experimental design, understanding of related literature, computer skills, and the ability to utilize statistical analysis.
**Project Title:** Investigation of ceftiofur metabolism characteristics from incurred milk samples following intramammary infusion.

**Principle Investigator(s):** Patrick Gorden

**Collaborating Investigator(s):**

**Veterinary Scholar Focused Abstract:** (300 words or less): Ceftiofur is the most commonly used antimicrobial in lactating dairy cattle in the US. It is labeled to be used via an injectable route as well as an intramammary infusion. Following injection, there is no milk withdrawal, however intramammary infusion requires a milk withdrawal. Failure to follow the withdrawal may result in violative drug residues in milk, which are very costly to dairy farmers. The initial screening tests for antimicrobials in milk only detect the presence of a violative drug residue but do not indicate which drug is present nor the concentrations of those drugs. In order to better understand the cause of the violative residue, veterinarians often send samples to the ISU Analytical Chemistry Laboratory for liquid chromatography coupled with mass spectrometry (LC/MS) analysis.

Following injection and infusion, ceftiofur is rapidly metabolized to several metabolites. The number and rate at which the metabolites are formed is based on route of administration. When investigating violative residues, the ISU Analytical Chemistry Laboratory analyzes milk via LC/MS to determine the presence and quantity of the parent compound and metabolites. The objective of this proposal is to infuse ceftiofur into lactating dairy cow and then collect milk samples for several days. Following sample collection, milk samples will be analyzed for drug concentrations. The outcome of this work will allow diagnosticians who are performing investigations of residue violations from client submitted cases to better understand the results produced following analysis of violative samples via LC/MS.
Project Title: Evaluation of the effect of Freeze/Thaw cycles on rPCR Cts

Principle Investigator(s): Karen Harmon, Phillip Gauger

Collaborating Investigator(s):

Veterinary Scholar Focused Abstract: (300 words or less):

Real-time PCR (rPCR) for detection of bacteria and viruses has become a routine procedure in most veterinary diagnostic laboratories (VDLs). After completion of testing, samples are typically stored at -70 to -80°C. Most retesting to verify results is performed on samples that have been frozen and thawed after the initial testing. When positive results do not repeat upon retesting, the client understandably questions the validity of the original result. Such discrepancies could be caused by several different factors, but a common explanation is that the sample has undergone a freeze/thaw cycle which could adversely affect testing. However, to our knowledge, the impact of freeze/thaw cycles on PCR results has not been adequately investigated, and that is the focus of this study. Having the data to determine any effects of freeze/thaw on PCR results will assist us in more accurately responding to clients questioning retest results, and recommending the best follow-up to those clients, which may include resubmission of samples for testing if freeze/thaw is found to be deleterious to subsequent testing.

This project investigates freeze/thaw effects on rPCR and will include serum, tissue and oral fluid samples. Testing will be conducted on fresh specimens as well as specimens undergoing a defined number of freeze/thaw cycles. Results will be analyzed to determine any effects of the freeze/thaw cycles. This data will provide useful information in either supporting or contradicting the premise that freeze/thaw cycles are most likely the cause of discrepancies in initial and retest results for rPCR, for the sample types included in this research project.

The student conducting this study will obtain valuable experience related to molecular testing, both in performing the procedures and, as potential future clients of a VDL, in interpreting results and having a better appreciation for appropriate follow-up for additional testing.
D5 - Dr. Mickelson

Project Title: Phenotypic, Molecular, and Functional Characterization of Canine Urothelial Organoids

Principle Investigator(s): Megan Mickelson, Karin Allenspach

Collaborating Investigator(s): Albert Jergens, Jonathan Mochel, Chad Johannes, Meg Musser

Veterinary Scholar Focused Abstract: (300 words or less):

Urothelial carcinoma (UC) is associated with high levels of local recurrence and metastasis, in both humans and canines, oftentimes despite aggressive multimodal therapy consisting of surgery and chemotherapy.1 Canines with UC are uniquely suited for comparative analysis to humans with the most aggressive form of UC, muscle-invasive bladder cancer (MIBC), given the similarities with respect to histopathology, molecular heterogeneity, and biologic tumor behavior and treatment response. Tumor organoids are 3-dimensional cell cultures derived from patients that are useful in vitro systems for prediction of tumor mutation profiles and drug responses in cancer patients, including those with UC.2,3 We have successfully cultured canine UC organoids and performed preliminary proof-of-concept drug testing assays.

Our aim for this summer scholar project is to phenotypically characterize the canine UC organoids. This is important, as subtype classification (especially with regards to basal versus luminal type) has been shown to correlate with outcomes in humans. The scholar will perform immunohistochemistry and RNA in situ hybridization to assess typical tumor markers for UC in canines, such as markers expressed by all UC cells (CK7 and Ck20), basal UC cells (Ck5, Ck14), luminal UC cells (Ck8, FOXA1), presumptive urothelial stem cell markers (CD44), as well as molecular classifiers (BASE47 and MDAC).4 Our long-term goal is to create a biobank of UC tumor organoids from canine UC samples collected via urinalysis, cystoscopic biopsy, and/or surgical biopsy with comparison to normal bladder organoids and phenotypically characterize the cell lines. The canine UC organoids can be compared directly to a biobank that has been established for human UC at the Mayo Clinic to eventually allow for patient-specific therapy in both canine and human UC. Preclinical testing in canine UC (precision medicine approach) could accelerate drug development in both human and veterinary medicine.

**D6 - Dr. Minion**

**Project Title:** Construction of mutants of Mycoplasma hyopneumoniae

**Principle Investigator(s):** Chris Minion

**Collaborating Investigator(s):** Melissa Madsen

**Veterinary Scholar Focused Abstract: (300 words or less):**

*Mycoplasma hyopneumoniae* (Mhyo) is the causative agent of enzootic pneumonia (EP), a world-wide problem in the pig industry. EP is characterized by a dry, non-productive cough, labored breathing, and pneumonia. Despite years of research, vaccines are marginally effective and none fully protect pigs in a production environment. A better understanding of the host-pathogen interactions of the Mhyo-pig disease, which are complex and involve both host and pathogen components, is required. Among the surface proteins involved in virulence are members of two gene families called P97 and P102. These proteins are the adhesins directing attachment of the organism to the swine respiratory epithelium. Unexpectedly, these proteins undergo proteolytic cleavage events during their translocation to the surface. The proteases involved in this processing are not known, but genome sequencing has identified 6-7 potential candidates. The goal of this project is to construct mutants of these genes by recombination and characterize them in terms of the loss of adhesin processing activity. This project will involve construction of plasmids for the recombination event, transformation of *M. hyopneumoniae*, picking and the characterization of mutants. Characterization of the mutants will be performed by PCR analysis of the mutant chromosome to confirm loss of the target gene and analysis of the effect of the mutation on the processing of specific adhesins by SDS-PAGE electrophoresis and immunoblots using specific antisera. In this way, we hope to gain a better understanding of the processing of these important surface proteins and their role in pathogenesis.
Title: “Application of bupivacaine liposome injectable suspension for sustained analgesia from disbudding pain in calves.”

Co-Summer Scholar Faculty Advisors
Dr. Joe S. Smith, DVM, MPS, PhD, DACVIM, DACVCP (Food Animal Medicine)
Dr. Dane M. Tatarniuk, DVM, MS, DACVS-LA (Equine Surgery)

Veterinary Scholar Focused Abstract:

Cautery disbudding of calves is a common production practice to limit horn formation later in life as a means to improve animal welfare. Current means to manage pain from cautery disbudding include:

1. A corneal nerve block with the local anesthetic lidocaine, which provides short-term analgesia of approximately 60-90 minutes of duration.

2. Administration of a Nonsteroidal Anti-inflammatory Drug (NSAID) to decrease nociceptive windup as well as prevent the production of mediators of inflammation.

An exciting new product (Exaparel for humans; Nocita for veterinary use) has taken the local anesthetic bupivacaine, and suspended it in a liposome. This allows for extended release, with the potential of administering local anesthesia for a total of 72-96 hours (figure 1).

Currently, a lack of knowledge exists regarding which application of liposomal bupivacaine in food animal species.

A cohort of research calves will be cautery disbudded. Multiple variables will be assessed including the biomarkers cortisol and substance P. Tactile measurements of pinprick testing and pressure algometry will also be assessed. Finally calves will also be assessed for heart rate variability. Several plasma concentrations of bupivacaine will be collected to determine the elution profile of the bupivacaine formulations. Twenty four Holstein dairy calves will be cautery disbudded after randomly being assigned to one of three treatments groups (8 per group).

1. Liposomal bupivacaine
2. Standard formulation of bupivacaine
3. Standard formulation of lidocaine

To mimic production settings calves will be housed at the Iowa State University Dairy.

Skills in basic statistics will be taught and applied as part of the project. Supervision of manuscript preparation for submission as a peer-reviewed publication and with the summer scholar student as a co-author will conclude the project.

During non-intensive study times the student will have the ability to assist with other research projects currently undertaken by Drs. Smith and Tatarniuk.
Project Title: Hematologic Findings in Cats and Dogs PCR positive for *Bartonella sp.*

Principle Investigator(s): Dr. Laura Van Vertloo (PI), Dr. Claire Andreasen (co-PI)

Veterinary Scholar Focused Abstract: (300 words or less):

Goals of this project are to survey if there are specific CBC hematologic findings in cats and dogs that are PCR positive for strains of *Bartonella sp.* (a gram negative bacteria), and if these findings differ among strains of *Bartonella sp.*. There especially is a lack of knowledge regarding leukograms in this disease. The retrospective study will collect data via the teaching hospital electronic medical records for dog and cat *Bartonella sp.* and CBC reports (~ 68 patients).

*Bartonella sp.* continues to be an emerging zoonotic disease in many species, including humans, dogs, and cats, that usually results in fever. Additionally, it is currently thought that bartonellosis is under-diagnosed in cats (possible stealth pathogen) and dogs due to the long list of causes of “fever of unknown origin”. It would be significant for patients with fever of unknown origin to have hematologic finding(s) that correlate with bartonellosis; therefore, indicating the need for confirmatory testing.

“Cat scratch disease” in humans is caused by *Bartonella henselae* characterized by fever, and *B. henselae* bacteremia was documented in a dog and 3 cats with fever of unknown origin. In these few case reports, there were no consistent hematological, biochemical or urinalysis abnormalities, despite the acute onset of febrile illness. Changes have been noted such as anemia, thrombocytopenia, and in 2 cats, neutrophilia progressing to neutropenia, which may have been related to *B. henselae*, known to infect neutrophils *in vitro*. Bartonellosis is a complex disease and the agent is sometimes difficult to confirm via PCR, Western blot, or cell culture. Treatment can resolve clinical signs in dogs and cats, but it is unknown if they remain as life-long carriers after treatment. This project will require hypothesis driven experimental design, understanding of related literature, computer skills, and the ability to utilize statistical analysis.
D9 - Dr. Ward

Project Title: **Dose-exposure-response of benazepril on biomarkers of the renin-angiotensin-aldosterone system in dogs**

Principle Investigator(s): **Dr. Jessica Ward**

Collaborating Investigator(s): **Dr. Jonathan Mochel**

Veterinary Scholar Focused Abstract: (300 words or less):

Activation of the renin-angiotensin-aldosterone system (RAAS) is involved in the pathophysiology of congestive heart failure (CHF). Angiotensin converting enzyme inhibitors (ACEi) such as benazepril are therefore recommended for the treatment of dogs with CHF; however, little is known about the optimal dosing of ACE inhibitors in dogs. Initial canine studies showed that the RAAS-blocking action of benazepril was relatively independent of doses > 0.2 mg/kg, providing rationale for the label dose of benazepril in Europe (0.25mg/kg PO q24hr). However, other studies suggest that the hemodynamic (blood pressure lowering) effects of ACEi are indeed dose-dependent, and ACEi doses up to 0.5 mg/kg PO q12hr benazepril are frequently used in the United States for the treatment of canine CHF. Additionally, previous studies of benazepril pharmacodynamics have relied sub-optimal endpoints (such as ACE activity) to characterize the effect of benazepril on the RAAS.

The objectives of this study are to characterize the dose-exposure-response relationship of benazepril on (1) biomarkers of the RAAS (including angiotensins and aldosterone) and (2) hemodynamic parameters (including systemic blood pressure). This study involves healthy beagle dogs (n=10) fed a low-sodium diet to experimentally induce RAAS activation. Two doses of benazepril (0.25 mg/kg PO and 0.5 mg/kg PO) will be studied in a cross-over fashion. Blood samples will be collected for pharmacokinetic (benazeprilat) and pharmacodynamic (angiotensin and aldosterone) determination at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 10 hours after administration of a single dose of benazepril. Echocardiography and Doppler blood pressure measurements will be performed at 0, 12 and 24 hours after dosing. Pharmacokinetic analyses will be performed by the Iowa State PhAST Laboratory, and pharmacodynamic analyses (RAAS biomarkers) will be performed by Attoquant Diagnostic Laboratories (Vienna, Austria). Pharmacokinetic, pharmacodynamic, and hemodynamic variables will be compared between doses and across timepoints to assess dose-exposure-response relationships.