THE OHIO STATE UNIVERSITY

College of Veterinary Medicine

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27 April 2022

Ms. Wendy Zhan
Director of the Ohio Legislative
Service Commission
77 S High St
9th Floor
Columbus, OH 43215-6136
Wendy.Zhan@lsc.ohio.gov

RE: Annual Report for Canine Research Funds

Dear Ms. Zhan:

Please find enclosed the annual report describing the research performed by The Ohio State University College of Veterinary Medicine with the support of the Canine Research Fund. As you know, ten cents of each one year, thirty cents of each three year, and one dollar of each permanent Ohio county dog license fee is set aside in a fund to support small canine research grants, which are administered by The Ohio State University College of Veterinary Medicine. Details of the grant review process are provided in the report. Included in this annual report are 8 final and 19 interim progress and new project reports of research ranging from different types of cancer to improving techniques on joint and bone repair to microbiome medicine.

On behalf of the College, I would like to thank the members of the legislature, the Ohio County Dog Wardens' Association, and the county commissioners for their continued support in our efforts to improve canine health through the Canine Research Fund. This fund allows the College to develop advancements in the art and science of veterinary medicine in a significant way.

Sincerely,

Patrick L. Green, PhD
Professor and Associate Dean for Research and Graduate Studies
Robert H. Rainier Chair in Industrial Veterinary Medicine and Research
Director of the Center for Retrovirus Research
Associate Director for Basic Science, Comprehensive Cancer Center

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Office of Research and Graduate Studies

ANNUAL CANINE RESEARCH REPORT

FOR
2021

Submitted to:
The Ohio General Assembly

May 2022

CONTENTS OF REPORT

CANINE RESEARCH FUND

Description
The Canine Research Fund (CRF) was established by the Ohio state legislature to provide funding of research to benefit the health and welfare of dogs. The CRF is subsidized by the county dog license fee where ten cents from each one year license and kennel registration, thirty cents from each three year license, and one dollar from each permanent license is assigned to the fund. The total annual allocation from dog wardens and county commissioners is approximately $100,000-$140,000. The money in its entirety is assigned to The Ohio State University College of Veterinary Medicine for distribution as small grants to College faculty.

Canine Research Fund Grant Review
As with all intramural grants in the College of Veterinary Medicine, Canine Research Fund grants are distributed through a competitive process fashioned similar to the National Institutes of Health extramural grants program. Faculty have the opportunity to submit grant applications annually to the College of Veterinary Medicine Office of Research and Graduate Studies. The grant applications are similar to the NIH 398 form (see appendix). Application deadlines are published for the year and can be found on the College web site or requested from the Office of Research. The notice of deadlines is also e-mailed to all faculty approximately 2 months prior to the deadline.

Grant applications are reviewed by the Council for Research, ranked, and recommended for funding to the Associate Dean for Research and Graduate Studies. The Council for Research is a representative body made up of faculty from across the College. Three regular faculty members from each academic department in the College are either appointed by the department chair or elected by the regular faculty of that department. Each member serves a three year term. The Council is chaired by one of the members who is elected to that position by majority vote of the Council. The Chair is renewed annually. The CVM Associate Dean for Research and Graduate Studies is a non-voting member of the Council who will implement the Council's recommendations on grant funding.

Each grant will be reviewed by two council members. The reviewers will provide a written critique of each grant and, in open session, will share that critique with the rest of council. The critiques of each grant will be distributed to the principal investigator of each grant for their information. Council members who have a conflict of interest or who are directly involved in implementation of the grant are excused from the proceeding during that grant's review. Upon completion of the oral critique and following discussion by the entire council, each council member will assign a score of 1 to 10, where 1 is the perfect score. At the end of the proceedings, all grants will be ranked by their average score for the Councils review and recommendation on funding. Typically grants receiving a score of greater than 5 are not funded. Grant funding is capped at $30,000 per project to be distributed over a period of 1 to 2 years. No cost extensions can be requested on an as needed basis. At the end of the project, grant recipients are required to provide final reports summarizing the results of the grant. Copies of these reports are collated and distributed to the state legislature annually.

Impact of the Canine Research Fund
The Canine Research Fund is a unique resource for the College that supports research specifically targeted for the betterment of dogs. The types of projects funded by the CRF extend across the entire breadth of basic, clinical and social research. Research projects are often for clinical studies performed by Veterinary Medical Center residents under the supervision of senior faculty. These projects are a part of the resident's Masters' degree program targeted at providing veterinarians with a research experience. Grants also go to faculty as seed money to develop projects for eventual extramural grant submission to national granting agencies. Finally, CRF grants may fund orphan projects that are important to dog welfare, but are not likely to be funded by other sources.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Perfusion Index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs
Principal Investigator (PI)	Carolina H Ricco Pereira
Co-PIs/Co-Is	Natalia Henao-Guerrero, Fernando Garcia, Turi Aarnes, Phillip Lerche, Richard Bednarski, Jonathan Dyce
Interim or Final	Final

Introduction:

Perfusion index (PI) monitoring is a noninvasive bedside monitor derived from the photoplethysmography waveform acquired by a pulse oximeter that is used to evaluate perfusion by calculating the ratio between pulsatile blood flow and static blood in peripheral tissues (Ginosar et al. 2009; Goldman et al. 2000). Studies have demonstrated decreases in PI during vasoconstriction secondary to pain and administration of vasoconstrictive agents such as epinephrine (Mowafi et al. 2009; Hasanin et al. 2017). The PI value increases with vasodilation and is an early objective monitor for detection of an effective caudal block in children (Kasthuri & Anitha 2020). One study demonstrated significantly increased PI values in the toe following pediatric caudal blocks, even during ketamine anesthesia (Xu et al. 2013). In dogs, the PI was significantly greater in the surgical limb after femoral and sciatic blocks compared with the contralateral limb (Gatson et al. 2016).

The objective of the present study was to evaluate PI as a noninvasive tool to determine effectiveness and onset of epidural anesthesia in dogs undergoing stifle surgery. We hypothesized that the PI would increase following epidural anesthesia with lidocaine. We also hypothesized that PI and hemodynamic response following a painful stimulation would be equally sensitive in identifying epidural effectiveness.

Approach:

The OSU Institutional Animal Care and Use Committee reviewed and approved the present study (no. 2016A00000108). Healthy dogs, weighing 33-100 lbs, with no contra-indications for epidural anesthesia, admitted to The OSU Veterinary Medical Center from July 2020 to November 2020 for stifle surgery were enrolled. Informed owner consent was obtained for all dogs.

Each dog was placed under general anesthesia. Parameters monitored included arterial blood pressure, heart rate and rhythm, respiratory rate, expired carbon dioxide and inhalant anesthetic, and oxygenated hemoglobin. Finally, perfusion index was measured placing the probe at the base of the tail. Following full instrumentation, baseline values were recorded. A lumbosacral epidural was performed by one of two board certified anesthesiologists (CRP, PL), unaware of group assignment.

All dogs were randomly assigned into two groups: 1. epidural morphine (EM) and 2. lidocaine and morphine (ELM). Data were recorded at 10, 20 and 30 minutes after epidural injection.

Following the data collection at 30 minutes, the dog was moved into the surgical suite. Data were recorded immediately prior to incision of the skin with a scalpel blade and at completion of the incision, immediately prior to the tibial osteotomy and following completion of the osteotomy. The % increase in HR and MAP from values prior to the skin incision and osteotomy were recorded. Combined scores of ≤ 2 were considered a lack of response and, therefore, a successful epidural. A combined score ≥ 3 initiated rescue analgesia; IV fentanyl bolus followed by a fentanyl constant rate infusion.

Results:
The values for PI did not significantly differ when comparing group EM and group ELM at baseline (T_0) or at the 10, 20 and 30 minute data collection points or within each group during these time points (Table 1).

Table 1 Median (interquartile range) perfusion index (PI) values in isoflurane-anesthetized dogs administered epidural injections of morphine (group EM; 7 dogs) or lidocaine and morphine (group ELM; 14 dogs) before surgery. Values recorded immediately prior to epidural injection (Time 0) and then at 10 minute intervals following the injection. Values of $p \leq 0.05$ were considered significant.

Group	Time points (minutes)				*p* (within group)
	T_0	T_{10}	T_{20}	T_{30}	
EM	4.10 (1.40 – 6.80	3.00 (1.00 – 4.70)	3.80 (0.98 – 4.60)	4.40 (1.00 – 4.50)	0.24
ELM	1.85 (0.80 – 2.80)	1.70 (1.00 – 2.00)	1.65 (1.10 – 2.00)	1.70 (1.10 – 2.40)	0.92
p (between groups)	0.08	0.37	0.39	0.42	

There was no significant difference when comparing the PI values before and after skin incision or before and after the osteotomy within each group or between groups at each time point.

There was no significant difference before or after skin incision or before and after the osteotomy when comparing group EM and ELM, or within group EM and ELM for the skin incision for any additional physiological variables including HR, SAP, DAP, MAP, PE'CO2 or f_R (Table 3). Within each group, values were significantly different before and after the osteotomy for HR ($p = 0.0001$, $p = 0.04$), MAP ($p = 0.03$, $p = 0.05$) and DAP ($p = 0.01$, $p = 0.01$), in groups EM and ELM respectively (Table 3). F_E'Iso was significantly different between groups before skin incision ($p = 0.04$) and after skin incision ($p = 0.05$) but not within each group.

Relevance & Impact to Canine Health:
Unfortunately, we were unable to demonstrate that perfusion index is a valuable tool in identifying epidural onset and effectiveness. In great part, this could be due to the vascular effects of some of the sedatives and/or general anesthesia itself. However, it is not feasible to perform an epidural injection in a canine patient with the animal awake and doing so would inflict unnecessary distress.

Conclusions:
No significant difference in PI was recorded in isoflurane-anesthetized dogs after epidural administration of morphine or lidocaine and morphine during TPLO surgery. The PI did not provide an objective means for determining the onset or the effectiveness of epidural anesthesia. The PI did not increase following the expected vasodilation secondary to lidocaine administered via epidural injection and it did not decrease as expected in correlation to an increase in physiological variables following a painful stimulus. Additional studies are recommended to determine alternative noninvasive methods to determine epidural onset and effectiveness in anesthetized dogs.

Publications/Presentations/Grant Submissions:

- The research results were presented at the 2021 American College of Veterinary Anesthesia and Analgesia Annual Meeting, held in Nashville, TN on September 12, 2021 as an oral presentation by the PI (Dr. Carolina Ricco Pereira). The peer-reviewed abstract was published on *Veterinary Anesthesia and Analgesia* volume 48, issue, page S999 November 2021 (https://doi.org/10.1016/j.vaa.2021.08.040)
- The peer-reviewed manuscript was published on *Veterinary Anesthesia and Analgesia* volume 48, issue 5, pages 782-788, September 2021. (https://doi.org/10.1016/j.vaa.2021.06.011)
- This project fulfilled the research requirements for a graduate student (Dr. Crystal Doyle), who defended her Master's Degree and graduated in Spring 2021. Dr. Doyle is currently an officer with the US Army Veterinary Corps and is serving our country overseas.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Assessment of regional intestinal perfusion by thermal imaging during foreign body surgery
Principal Investigator (PI)	Dr. Ed Cooper
Co-PIs/Co-Is	Dr. Finstad, Dr. Yaxley, Dr. Mcloughlin, Dr Guillaumin
Interim or Final	Final

Introduction:
Dogs with intestinal obstruction secondary to foreign body ingestion commonly present with signs including inappetence and vomiting. Once diagnosed, surgical intervention is often required. During surgery, the surgeon must assess the area of intestine for signs of injury such a leakage and lack of blood flow. This is a difficult task as many of the changes are subjective and may not be readily visible. Infrared thermal imaging can be used to assess the intestinal surface temperature and can highlight colder areas in blue while warmer areas are red. Areas that are colder would raise concerns for compromised blood flow and would more likely need to be removed. It therefore has potential as a non-invasive, fast and easy to use way to assess intestinal viability. Improved intraoperative assessment of intestinal viability could lead to fewer post-operative complications, less need for revision procedures and shorter hospital stays with lower mortality rates.

Approach:
Client owned dogs that are presented to the OSU-VCM and diagnosed with a small intestinal foreign body obstruction undergoing exploratory laparotomies were eligible for enrollment. Dogs had to be excluded if the foreign body was not located in the small intestine or patients had concurrent major surgical emergencies. Ten client owned dogs were also included as controls, to image the normal gastrointestinal tract. Patients underwent a physical examination and a blood pressure was obtained using noninvasive blood pressure techniques followed by venipuncture and collection of blood for full bloodwork as a systemic health check. Initial stabilization and supportive care were provided by the admitting clinician, at their discretion. Anesthetic protocols and surgical decisions were not influenced by this study.

Using the thermal imaging camera, the pre-surgery image was taken, centered on the foreign body. Surgeons were asked to point to the oral side as a reference point. The data was saved on the device's memory card. Sublingual microcirculation data was collected concurrently using the Microscan™ to obtain five, 20-sec videos, which were stored for later quantitative vascular analysis. The patient's vital parameters and ambient room temperature were also record at the time of imaging. Surgery location, type and foreign-body type were recorded. Post enterotomy or resection anastomosis, the affected intestinal loop, centered on the surgical incision site, was imaged using the thermal imaging camera. Sublingual microcirculation data, vital patient parameters and ambient room temperature were recorded as described above, at the time of imaging.

Results:
The study data showed that there is a decrease in intestinal temperature associated with mechanical obstruction secondary to small intestinal foreign body compared to healthy control patients undergoing a laparotomy. Overall, this improved after the obstruction was relieved. There was not a specific association between intestinal temperature and systemic vitals or microcirculatory data, suggesting that it is a function of local changes in blood flow. Not enough patients experienced the need for resection and anastomosis (rather than enterotomy), so a comparison could not be made.

Relevance & Impact to Canine Health:
Intestinal foreign bodies are a common diagnosis for canine patients presenting to veterinary emergency centers. The non-specific presenting signs and the time to diagnosis can be highly variable. Canine patients undergoing surgical explore are at risk of significant post-operative complications including intestinal ischemia and dehiscence of intestinal segments, which can lead to peritonitis, septic shock and death. Rapid stabilization, recognition and accurate diagnosis is important to initiate appropriate treatment and improve survival. Dogs, specifically, have a greater risk of developing intestinal leakage following resection anastomosis surgery in comparison to cats.

This study proposed a more objective intestinal viability assessment technique, which can be utilized to help with intraoperative determination of intestinal perfusion and viability. To date, the use of thermal imaging for assessment of intestinal perfusion has not been done in dogs with naturally occurring GI foreign bodies.

Conclusions:
The results of this study suggest there may be utility in using thermal imaging to provide objective, real-time information about local perfusion and tissue viability. Thermal imaging is an easy and non-invasive imaging modality that can be used during foreign body surgeries and has the potential to provide additional objective information. The combination of subjective and objective assessment can strengthen the decision making for the most appropriate surgical technique and hopefully lower the risks of intestinal surgical site dehiscence, reducing the number of surgical complications. Further investigation is warranted.

Publications/Presentations/Grant Submissions:
Manuscript preparation has been initiated and will be completed once the data has been finalized. Expected submission Fall 2022.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Effects of antimicrobial therapy on virulence and antimicrobial resistance of canine UPEC UTIs
Principal Investigator (PI)	Thomas E. Wittum
Co-PIs/Co-Is	Gregory Ballash, Dubraska Diaz-Campos
Interim or Final	Final

Introduction:

Urinary tract infections (UTI) will affect 15-20% of dogs at least once in their lifetime. Escherichia coli, referred to uropathogenic *E. coli* (UPEC), is the most frequent cause of UTI causing upwards of 80-85% of cases in some canine populations. In typical, uncomplicated UPEC UTI, empirical antimicrobial therapy with first-line antibiotics is the standard practice and typically resolves the infection. However, two observed trends in UTI epidemiology are causing for concern for treatment and prognosis of UTI. First, recurrent UTI are becoming increasingly more frequent. Among dogs with recurrent infections, *E. coli* is significantly more likely to cause recurrence compared to other UTI bacteria. Second, today's UPEC UTI isolates are at greater odds of harboring resistant mechanisms to one or more antimicrobial therapies. Antimicrobial therapy is consider a risk factor for the development of antimicrobial resistant bacterial infections, but its role in UPEC UTI needs further evaluation. In addition, antimicrobial therapy may promote the development of recurrent UTI by creating reservoirs of antimicrobial resistant bacteria that later infect the urinary bladder. Finally, antimicrobial therapy may influence the recurrent state by selecting for UPEC that are more successful at colonizing the bladder and causing infection. Here we aim to evaluate and characterize the role antimicrobials play in developing antimicrobial/multidrug resistant UPEC UTI, if antimicrobial resistant isolates are more likely in recurrent UTI and how antimicrobial use influences virulence traits that result in UTI, including those that are critical for establishing recurrent UTI.

Approach:

Uropathogenic *E. coli* samples were collected from diagnostic submissions to The Ohio State University College of Veterinary Medicine Clinical Microbiology Diagnostic lab from 2018-2020. Each UPEC was tested against a standard set of 23 antimicrobials at differing concentrations to determine susceptibility profiles using the Clinical and Microbiology Standard Institute (CLSI) testing and interpretation protocol. UPEC isolates underwent whole genome sequencing to determine the presence of antimicrobial resistance and virulence genes. For each patient with a positive UPEC UTI we collected a standard set of variables including: age, sex (spayed female, intact female, castrated male, intact male), breed (small vs. large), comorbidity status, historically or currently diagnosed with a recurrent UTI, currently diagnosed with pyelonephritis, presence of clinical signs attributable to a UTI, currently taking a immunosuppressive medication, currently taking a non-steroidal anti-inflammatory medication and current antimicrobial use for any reason within the past 72 hours and 30 days. Logistic and Poisson regression models were generated to determine associations between antimicrobial use and antimicrobial resistant UPEC, multidrug-resistant UPEC and virulence factors.

Results:

We collected 121 UPEC isolates from 110 unique dogs over the course of the study period. Of this sample, 88 unique dogs (80%) representing 99 unique UPEC isolates (81%) had a detailed history that allowed for retrospectively analysis of previous antimicrobial use in the past 30 days. Demographically, our sample consisted of 24.1% small breed dogs, 20.8% large breed dogs and 15.4% mixed breeds, 19% spayed females, 26.7% intact females, 19% castrated males and 16.7% intact males, with an average and median age of 7.8 and 8 years, respectively. Using phenotypic data we found that current antimicrobial use and a history of antimicrobial use were the strongest predictors of a dog having an antimicrobial resistant UPEC infection. Dogs with multidrug resistant UPEC UTI were more likely to have a history of current or previous antimicrobial use. Within our sample, we found 44 dogs that we could estimate the number of antimicrobial prescribed in the past thirty days. If dogs were prescribed greater than 1 antibiotic in the past thirty days their odds of having a multidrug resistant UPEC UTI were increased with marginal significance. Eighty-eight unique E. coli (73%) were submitted for whole genome sequencing. Antimicrobial use significantly increased the incidence of acquired resistance genes, but did not influence the incidence of virulence genes. Despite this, antimicrobial use was

associated with the presence of specific virulence genes, mainly those promoting attachment and colonization. The UPEC virulotype was negatively associated with antimicrobial use and maintaining antimicrobial resistance genes, but positively associated with harboring virulence genes.

Relevance & Impact to Canine Health:

UPEC UTI are one of the most frequent causes of veterinary visits. More concerning is the increased frequency of antimicrobial resistance and recurrent infections seen among UTI pathogens, most notably UPEC isolates. Here we provide evidence that current antimicrobial use at the time of infection can increase the frequency of antimicrobial resistant and multidrug resistant UPEC UTI. In addition, antimicrobial use for any reason within the past 30 days is also a significant risk factor for developing an antimicrobial resistant and multidrug resistant UPEC UTI. Based on the whole genome sequencing data, we observed that the use of antimicrobials promotes UPEC UTI with uncommon UPEC phylogroups that maintain virulence factors that permit colonization and infection while maintaining a more robust resistome. This data suggests that antimicrobial use, for any reason, promotes an environment for developing antimicrobial resistant and multidrug resistant infection that can promote recurrence states. These resistant infections and recurrent states can lead to treatment failure, protracted clinical signs and disease states affecting the patient, increased treatment costs and more owner suffering. This data also supports the numerous calls by international and national societies, including the AVMA, for the judicious use of antimicrobials through antimicrobial stewardship programs. Implementing and practicing standard diagnosis and treatment protocols will reduce unnecessary antimicrobial use and reduce antimicrobial resistance.

Conclusions:

Current and previous antimicrobial use for any reason can result in the development of antimicrobial resistant UPEC, including multidrug resistant UPEC. Moreover, if a dog is treated with more than one antibiotic the risk of developing a multidrug resistant infection marginally increase. These resistant and multidrug resistant UPEC may promote the development of recurrent UPEC UTI states. The whole genome sequencing data supports our phenotypic antimicrobial resistance findings. In addition, it identifies a novel occurrence of atypical UTI phylotypes that use maintain uncommon UPEC virulence profiles. This data suggests antimicrobial use may shift the gut microbiome to a more resistant, commensal-enteric pathotype that maintains the ability to cause UTIs. Our data support the call for judicious use of antimicrobials in any situation and promotes the establishment of antimicrobial stewardship programs in veterinary medicine.

Publications/Presentations/Grant Submissions:

We plan to submit this data as preliminary data for Morris Animal Fund and American Kennel Club funds during their next funding cycles. We have presented this data as part of the CRWAD 2020 conference and at OSU's IDI ARIG meeting and anticipate presenting these finding at the OSU-CVM Research Day 2021, the UTI Global Alliance "UTI Hour" 2021 and the NIAMRRE Annual Conference 2021. Five publications are in progress using portions of this work:

1. **Comparative phylogenetics and pathogenomics of Uropathogenic *E. coli* in humans and dogs identify distinct phenotypes and molecular genotypes.**
2. **Current and previous antimicrobial use drives alterations in uropathogenic *E. coli* phylotype, resistome and virulome independent of patient epidemiology.**
3. **Genome-wide pathogenomics and patient epidemiology associated with recurrent uropathogenic *E. coli* UTI in dogs.**
4. **Leveraging big data for comparative analysis and predictive modeling of urinary tract associated *E. coli* phylogenetics and virulomics among humans, dogs and commensal strains.**
5. **Understanding the influence of pathogenomics and patient epidemiology on the biofilm forming capacity of uropathogenic *E. coli* isolated from canine urinary tract infections.**

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms
Principal Investigator (PI)	Laura Selmic
Co-PIs/Co-Is	Brittany Abrams, Josephine Dornbusch, Ryan Jennings, Vincent Wavreille
Interim or Final	Final

Introduction:

Surgery is the primary treatment for many skin tumors affecting dogs. The best chance of cure is if the surgeon can fully remove all traces of the tumor. Unfortunately, to assess this we rely on traditional methods that assess <1% of the sample, providing results several days later. Other more accurate, rapid and complete methods are critically needed. Especially as missing incomplete tumor removal for dogs represents missed treatment opportunities and can result in devastating tumor recurrence. Optical coherence tomography is an emerging diagnostic imaging tool that uses light waves to generate real-time, high-resolution microscopic images of tissue. These images can be used to look for residual tumor during surgery. This study focused on validating this technology for the imaging of skin tumor surgical margins. If successful, this could benefit patients by guiding accurate treatment recommendations and attempting to reduce the need for other additional treatments.

Approach:

Forty dogs with skin and subcutaneous tumors had their tumors surgically removed, and after removal the tumor sample was imaged using a spectral domain optical coherence tomography (OCT) system.

The *purpose of the proposed study* was to assess the diagnostic accuracy of OCT for assessment of surgical margins for resected canine skin and subcutaneous tumors. The specific aims were as follows:
Aim 1: Correlate the normal and abnormal features in OCT images of surgical margins with corresponding histology to create an image training set for observers. 10 dogs were utilized for this aim.
Aim 2: (a) Determine the diagnostic accuracy of OCT for assessment of surgical margins. (b) Determine the frequency that OCT and standard pathology assessment detect incomplete margins. 30 dogs (expanded to 80 dogs total with additional AKC funding) were utilized for this aim.

The OCT imaging was compared to the gold standard of histopathology in the first part of the study to create a training set of images to train clinicians. In the second part of the study, clinicians were trained to read OCT images to determine if the tumor extended to the surgical margins. The results of their interpretation was used to determine the accuracy of the technique.

Results:

Aim 1: We accrued 10 cases for aim 1. Five dogs had mast cell tumor, two dogs had a soft tissue sarcoma, one dog had a fibroepithelial polyp, one dog had an undifferentiated carcinoma and one dog had a fibroma. We were able to image different normal and abnormal tissue types at the surgical margins throughout the imaged specimens. We have correlated OCT images of these tissues at the surgical margins to histopathology sections for all tumors.

In evaluating these images, we have been assessing the optical characteristics of these tissues. The following series of images illustrate these correlations and characteristics for tumor tissues (**Figures 1 and 2**). **Figures 3 and 4** are examples of correlations and characteristics of normal tissues.

Figure 1: *Appearance of two different mast cell tumors with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the mast cell tumor are high scattering and heterogeneity in scattering.*

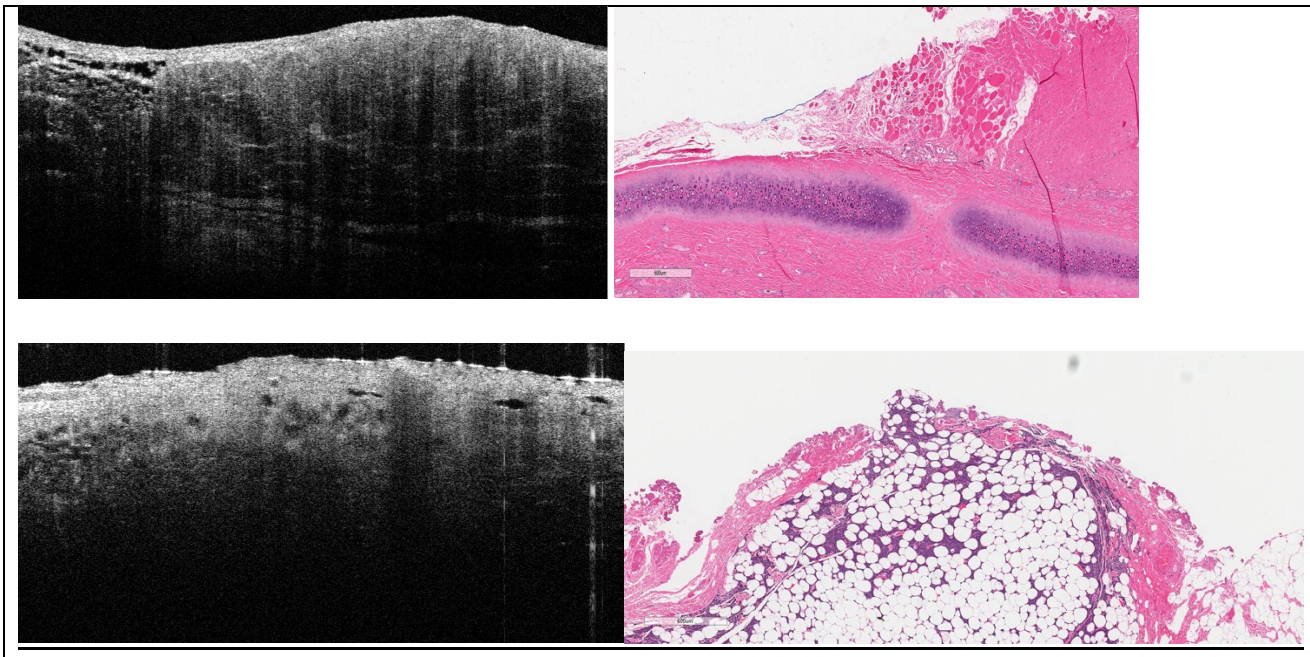


Figure 2: *Appearance of a fibroma with narrow excision with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the fibroma are heterogenous with generally high scattering.*

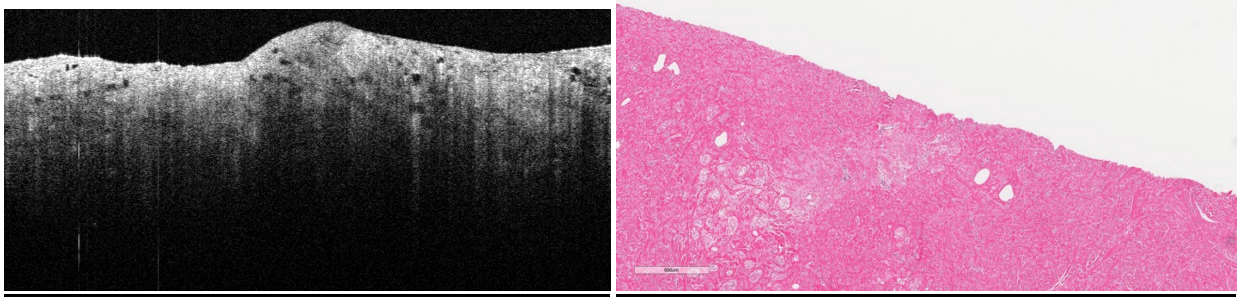


Figure 3: *Appearance of normal tissues of fascia and fat with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the fascia are heterogenous high scattering thin layer of tissue with underlying honey comb structure showing mature adipocyte cells.*

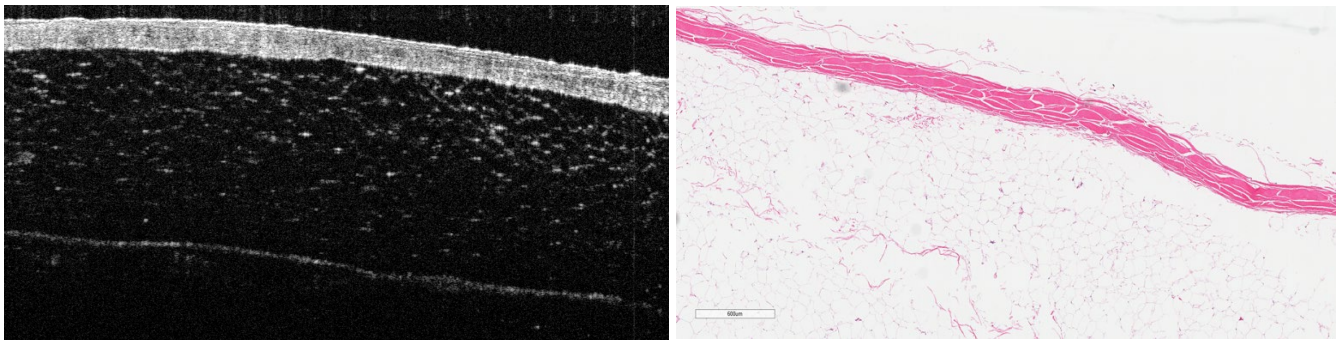
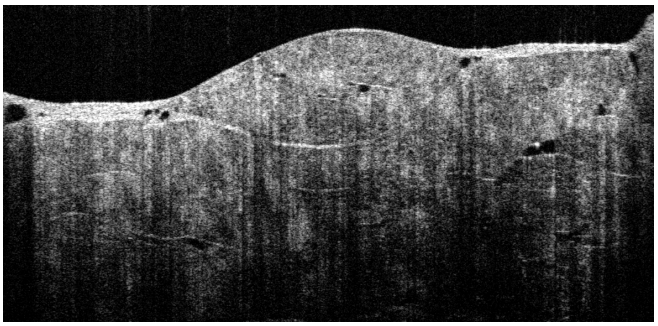
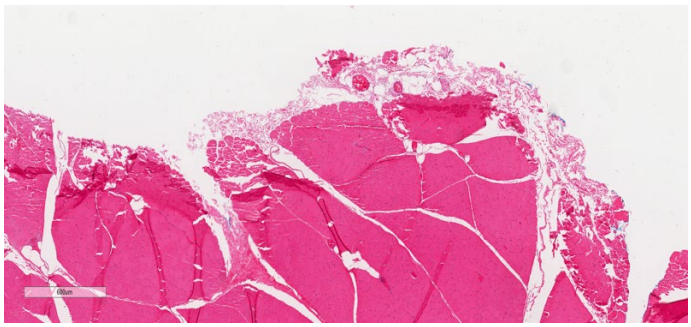


Figure 4: *Appearance of normal skeletal muscle with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the muscle are heterogenous high scattering tissue with linear white lines representing fascia surrounding muscle bundles.*





The results to date, demonstrate that OCT has the potential to delineate the margin between tumor and other connective tissues, as well as identify the diversity of tissue structures such as adipose, muscle, tumor, blood vessels, and connective tissue. We have completed case accrual of the 70 cases for aim 2 and 3, we created the training and datasets needed for the observer assessment phase of the study. We performed the observer assessment which revealed the diagnostic accuracy was very good (overall sensitivity 86.7%, specificity 84.6%) in the hands of clinicians with minimal prior experience interpreting OCT images.

Relevance & Impact to Canine Health:

Cancer is a common problem affecting an estimated 1 in 3 dogs in their lifetime and represents the leading cause of death in older dogs. The skin and subcutaneous tissues are common sites for development of tumors in older canines but incidence estimates have been hard to determine. In the veterinary literature, tumors of skin origin may represent 25.5-43% of all biopsy submissions, with 20-40% of these resulting from malignant skin lesions. In dogs these superficial tumors are often recognized leading people seek treatment for themselves or their dog. Initial diagnostics are performed and if a lesion is determined to be benign and causing symptoms, or malignant the recommended treatment will often be a surgery to remove the tumor. Complete surgical removal is important in dogs to decrease the chance of recurrence. Commonly histopathology is used to assess completeness of resection in both species representing an assessment of selected and small proportion of the surgical margins with results several days after surgery. There is a critical need for validation of improved imaging methods for microscopic tumor sample assessment real-time to improve accuracy of assessment, reduce patient morbidity and improve outcomes.

Conclusions:

Given the promising results of this study, our team will continue to work hard to perform further evaluation of this cutting-edge and promising technology for detection of residual cancer cells following surgery. Advancement of our knowledge of residual cancer cells at the time of surgery will help to improve options and outcome for dogs.

Publications/Presentations/Grant Submissions:

Cheng E, Jennings RN, Chen CL, Biggo MR, Erickson AK, Dornbusch JA, Linn SC, Lapsley J, Alva BM, Lorbach JN, Premanandan C, **Selmic LE**. Optical Coherence Tomography for Surgical Margin Evaluation of Excised Canine Cutaneous and Subcutaneous Tumors. Manuscript submitted to Veterinary and Comparative Oncology on 1/3/22.

Cheng E, Selmic LE, Jennings R, Chen C, Biggo M, Erickson A, Dornbusch J, Linn S, Lapsley J, Alva B, Lorbach J, Premanandan C. OCT & Canine Tumor Surgical Margin Evaluation. Presented in the resident forum at ACVS surgical summit October 2021.

Selmic, L.E. (PI), Dornbusch, J (Co-I), Jennings, R. (Co-I), Wavreille, V.W. (Co-I). **Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms.** Grant submission funded by American Kennel Club Canine Health Foundation, $43,443.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Use of radiation therapy and conforming intramedullary implant to treat canine appendicular OSA
Principal Investigator (PI)	Janis Lapsley
Co-PIs/Co-Is	Vincent Wavreille, Laura Selmic, Eric Green, Stephen Jones
Interim or Final	Final

Introduction:

Appendicular osteosarcoma (OSA) is locally aggressive and has a very high tendency to spread. Limb amputation followed by chemotherapy has been considered the standard of care treatment. Numerous limb salvage techniques have been described in dogs but the use of these techniques, including radiation therapy (RT), has been limited due to high complication rates. OSA lesions compromise the structural integrity of the affected bone, explaining the high incidence of pathological fractures encountered in dogs following RT. To prevent this complication, open surgical stabilization has been performed with RT. Unfortunately, this approach has also been associated with a high complication rate.

Application of a stabilizing implant via a minimally invasive approach to reduce soft tissue damage and associated complications is available and has been used in people with success. This study will evaluate the clinical outcome of a limb salvage technique using the combination of RT and this novel implant.

Approach:

The overall objective of this study is to describe the short- and long-term clinical outcome of dogs with primary OSA treated with the combination of stereotactic radiation therapy (SRT) and a conforming intramedullary implant (CII). We hypothesize that the combination of SRT and the minimally invasive application of a CII for the treatment of primary canine appendicular OSA will be feasible and safe, providing local tumor control and resulting in good to excellent limb function.

As such, the aims of the proposal are:
Aim 1: Determine the safety, tolerability and function of dogs with primary appendicular OSA treated with SRT-CII.
Aim 2: Determine the acute and long-term effectiveness of local tumor control of SRT-CII.

Results:

A total of 6 cases were enrolled in this study. Since enrollment, 5 cases have died or were euthanized due to progression of their disease. Three patients experienced catastrophic fracture around the implant. Two of these patients had an amputation performed and the other was humanely euthanized. One patient had local progression of disease in the face of SRT. Four patients had distant progression of disease (pulmonary metastasis) following SRT and systemic chemotherapy which ultimately resulted in owner election for humane euthanasia. One patient remains alive at this time and recently underwent limb amputation due to fracture of the limb and implant. Her restaging remains free of evidence of metastatic disease.

Relevance & Impact to Canine Health:

OSA is the most common primary bone tumor in dogs, usually affecting middle-aged, large breed dogs. More then 10,000 dogs per year are diagnosed with OSA in the USA accounting for 98% of canine primary skeletal malignancies. Traditionally, limb amputation with adjuvant chemotherapy is considered the standard of care for management of canine OSA. Though limb sparing options exist, and limb preservations attempts are generally performed in human patients, these techniques are not in widespread use in veterinary medicine. This is likely due to the lack of versatility of the current techniques and the high complication rate, and thus cost, associated with these therapies. Radiation therapy is an alternative limb sparing treatment modality which has recently become more accessible in veterinary medicine. Unfortunately, this therapy has been associated with major complications with reported post-treatment pathologic fracture rate of 62% at 9 months. Addition of an intramedullary stabilizing implant may be able to provide necessary stability to diseased and SRT damaged bone in order to prevent or treat pathologic fracture and allow OSA

patients to maintain a functional limb. This technique should offer dogs a safe and well tolerated treatment for preserving limb function and maintaining quality of life.

Conclusions:

This approach appears to be feasible in dogs with appendicular OSA following RT. However, the complication rate remains high with the approach and thus additional research is being conducted in in this area to provide additional stabilization of the implants to help reduce the incidence of catastrophic failure.

Publications/Presentations/Grant Submissions:

No publications have been submitted; however this base research has sparked multiple other related projects. One of these projects was recently awarded a $150,000 grant for further work in this area.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Utility of cardiac MRI to detect myocardial ischemia and fibrosis in dogs with mitral valve disease
Principal Investigator (PI)	Randolph Winter
Co-PIs/Co-Is	Bill Clark, Daniel Addison, Eric Green, Turi Aarnes, Jaylyn Rhinehart, Karsten Schober
Interim or Final	Final

Introduction:

Myxomatous mitral valve disease (MMVD) is a common disease in middle-aged to older dogs. Similar cardiac diseases in humans sometimes have myocardial ischemia and fibrosis, and in some cases, this can only be accurately diagnosed with advanced imaging such as cardiac MRI (CMR). These findings in humans have been shown to have prognostic importance. Cardiac fibrosis has been documented with cardiac biomarkers in dogs with MMVD, but the full extent of ischemia and fibrosis has not yet been identified in dogs with MMVD using cardiac MRI. This pilot study aims to better characterize the myocardial health of dogs with MMVD using cardiac MRI, serum cardiac troponin I (cTnI), and serum galectin-3 (Gal-3).

Approach:

Dogs with MMVD stage B2 (i.e. those with left heart enlargement) and normal healthy controls of a similar age will have diagnostic tests performed to assess systemic health, cardiac health with cardiac biomarkers, echocardiography, and thoracic radiographs, and also a cardiac MRI examination performed under general anesthesia. Analysis of myocardial ischemia and myocardial fibrosis using information obtained from the cardiac MRI will be performed.

Results:

Enrollment is completed, with 6 dogs with MMVD stage B2 and 6 healthy control dogs. No significant difference in age between groups exists (p=0.062). No significant differences were observed between the control and MMVD groups regarding Native T1 values (p=0.719), Post-contrast T1 values (p=0.575), T2 values (p=0.611), and serum Gal-3 concentrations (p=0.085). Dogs with MMVD had significantly greater cTnI (p=0.013). Two dogs in each group had identifiable gadolinium enhancement consistent with myocardial fibrosis.

Relevance & Impact to Canine Health:

In human with cardiac diseases, information obtained from cardiac MRI is used for prognostication but also for individualized therapy. Previous reports suggest that dogs with MMVD have increased amounts of myocardial fibrosis, and that this myocardial fibrosis may contribute to ongoing disease progression. We have demonstrated that CMR in anesthetized dogs with cardiomegaly secondary to MMVD can be safely performed, and that myocardial fibrosis is not a prominent factor in this disease process as has been previously suggested.

Conclusions:

No significant differences in detection of myocardial fibrosis and ischemia were observed between healthy control dogs and dogs with MMVD that were age-matched. This pilot study demonstrates that CMR can safely be performed in dogs with MMVD stage B2, and that myocardial fibrosis identifiable by CMR exists in both older control dogs and dogs with MMVD stage B2. This pilot study suggests that myocardial fibrosis is not a prominent aspect of the myocardial remodeling pathophysiology that occurs in dogs with MMVD stage B2 disease.

Publications/Presentations/Grant Submissions:

2022 CVM Research Day PowerPoint Travel Award Recipient:

Utility of cardiac MRI to diagnose myocardial ischemia and fibrosis in dogs with cardiomegaly secondary to myxomatous mitral valve disease. W. Clark, R. Winter, T. Aarnes, E. Green, P. Ruz, D. Addison, J. Rhinehart, K. Schober, and H. Friel. Dept of Veterinary Clinical Sciences, Dept of Medicine (Division of Cardiovascular Medicine), Philips Medical Systems.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Molecular and serologic surveys of shelter dogs and their ticks as sentinels for tick-borne disease risk in Ohio
Principal Investigator (PI)	Risa Pesapane
Co-PIs/Co-Is	Colleen Shockling Dent
Interim or Final	Final

Introduction:

Ohio is situated between two actively converging fronts of Lyme Disease in the northeast and upper Midwest regions of the United States due to the geographic distribution of the blacklegged tick (*Ixodes scapularis).* Since the blacklegged tick was introduced, Ohio has experienced an epidemic of Lyme Disease in both humans and dogs. Ohio has also seen an upward trend in tick-borne anaplasmosis and ehrlichiosis. Other neglected tick-borne diseases like spotted fever group rickettsiosis and babesiosis lack any surveillance at all. Because domestic dogs are susceptible to many of the same tick-borne pathogens, are more likely to become infected, and increase human exposure to ticks and tick-borne disease, dog surveillance has been proposed as an effective method for assessing human risk. The goal of our research is to assess whether dogs are effective sentinel animals for tick-borne disease risk and the geographic distribution of ticks in Ohio.

Approach:

Blood and ticks were obtained from shelter dogs from five counties in southern Ohio. DNA was extracted from blood for parallel screening for spotted fever group rickettsiae, *Anaplasma* spp., *Ehrlichia* spp., and *Borrelia burgdorferi* using serology and PCR. Ticks were examined under light microscopy to determine species before DNA extraction and PCR testing for pathogens. Sampling was conducted over a period of 12 months to capture peak activity of all tick life stages. Data on tick species, tick abundance, and pathogen prevalence in ticks and dogs from this study will be compiled by county. Risk factors for tick-borne disease in shelter dogs will be assessed by univariate and multivariate logistic regression analyses. This data will be compared to publicly available county-level data on human tick-borne disease from the Ohio Department of Public Health, local vector control agencies, and the Centers for Disease Control and Prevention to determine the efficacy of shelter dogs as sentinels for human health risk.

Results:

From January 2020 to January 2021 a total of 276 shelter dogs were sampled for this study. Among 241 dogs tested using serology, 95 (39.4%) were seropositive for at least one tick-borne pathogen and 45 (18.7%) were seropositive for multiple tick-borne pathogens. Most dogs had been exposed to *Borrelia burgdorferi* (33.2%) followed by *Ehrlichia* spp. (24.5%) and *Anaplasma* spp. (1.7%). Among 266 dogs tested using PCR, only three (1.1%) were positive for spotted fever group rickettsiae, but negative for *Rickettsia rickettsii*, the agent of Rocky Mountain Spotted Fever. Four species of ticks were observed, including blacklegged ticks (*Ixodes scapularis*), American dog ticks *(Dermacentor variabilis),* Lone star ticks *(Amblyomma americanum),* and the exotic Asian Longhorned tick *(Haemaphysalis longicornis).* This was the first detection of the Asian Longhorned tick in Ohio demonstrating continued expansion across the United States since its introduction in 2017. Analysis of canine risk factors for tick-borne disease and comparison with human health data is ongoing.

Relevance & Impact to Canine Health:

Our study has revealed that roughly two out of every five shelter dogs in southern Ohio have been exposed to tick-borne pathogens which is higher than the seroprevalence reported by the Companion Animal Parasite Council for the same counties. These pathogens correspond to the range expansion of blacklegged ticks and Lone star ticks in Ohio. Dogs were seropositive for tick-borne pathogens whether or not ticks were observed suggesting all dogs should be screened for exposure as part of routine health checks. The presence of Asian Longhorned ticks in southern Ohio represents a new threat to canine health. At this time, there are no reports of disease among dogs parasitized by Asian Longhorned ticks in the United States, but these ticks are associated with a wide range of pathogens in other countries suggesting they may become important vectors here in the future.

Conclusions:

Dogs in Ohio are experiencing an epidemic of tick-borne disease in association with the expanding range of medically important ticks in the United States. Surveillance data currently available from the Companion Animal Parasite Council may underestimate the risk of tick-borne disease in some areas or for some populations such as shelter dogs. Shelter medicine in Ohio should include assessment of tick-borne disease and participation in tick surveillance to track the spread of the Asian Longhorned tick. Shelter dogs are a sensitive tool for the surveillance of novel ticks and tick-borne pathogens that may be detrimental to the health of humans, livestock, or companion animals.

Publications/Presentations/Grant Submissions:

One professional student presented interim results of this study at the 2020 National Veterinary Summer Scholar Symposium hosted by the American Association of Veterinary Medical Colleges and the 2021 College of Veterinary Medicine Annual Research Day at The Ohio State University. One publication is in preparation. Results of this study served as preliminary data in the submission of a National Animal Disease Preparedness and Response grant under the USDA Animal and Plant Health Inspection Service and in the submission of an Animal Health and Disease Research grant under the USDA National Institute of Food and Agriculture. Both of these grant submissions were awarded funding.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Identifying behavior changes in dogs during the six months following adoption from a shelter
Principal Investigator (PI)	Jeanette M. O'Quin
Co-PIs/Co-Is	Kyle Bohland, M. Leanne Lilly, Meghan E. Herron, Andréia G. Arruda
Interim or Final	Final

Introduction:

Millions of dogs are adopted from shelters in the United States every year, but very little is known about their long-term behavior post-adoption. Shelter-conducted behavior evaluations unfortunately have mixed ability to predict behavior in homes. Our study aimed to fill this knowledge gap by identifying the prevalence of behavior problems in dogs after adoption and how those behaviors changed throughout the first six months post-adoption.

Approach:

Ninety-nine dogs adopted from five Ohio shelters between October 1, 2020 and June 1, 2021 were tracked for six months after adoption. Owners were sent online questionnaires about their dog's behavior at 10, 30, 90, and 180 days after adoption. The Canine Behavioral Assessment and Research Questionnaire (C-BARQ) was used to track behavior over time, a questionnaire that has been validated to match dog behavior in real life situations. A statistical model was created for each C-BARQ trait, controlling for multiple canine and household factors: use of behavior medications, acquiring other pets, household moves, work schedule changes, shelter origin, age, sex, length of stay, shelter intake reason, and health status in the shelter. At each timepoint owners were also asked about their overall satisfaction with their dog.

Results:

99 owners completed the initial questionnaire at 10 days, and 83 owners completed the fourth and final survey at 180 days. Sixty-two owners (62.6%) answered all four surveys. Based on the statistical modeling, the following C-BARQ traits changed during the study period compared to baseline (10 days after adoption):

1. Stranger-directed aggression – *Increased* at all time points
2. Excitability – *Increased* at 3 months and 6 months
3. Touch sensitivity – *Increased* at 3 months and 6 months
4. Chasing behavior – *Increased* at all time points
5. Trainability – *Increased* at all time points
6. Separation-related behaviors – D*ecreased* at 6 months
7. Attachment and attention-seeking – *Decreased* at 6 months
8. There were no significant differences found during the study period for familiar dog aggression, owner-directed aggression, dog-directed aggression, stranger-directed fear, nonsocial fear, dog-directed fear, or energy level.

A majority of the findings represented a worsening of behavior (1-4) versus an improvement (5-7). Despite these increases in several undesirable traits during the study period, owner satisfaction of their adopted dog's behavior was overall very high. Across all timepoints, 98% of owners indicated their dog adjusted to the new home extremely or moderately well. 93% rated their dog's overall behavior as excellent or good. Additionally, 72% of owners described their dog's behavior as improved throughout the study.

Relevance & Impact to Canine Health:

These findings will provide veterinarians, canine behavior professionals, and shelter staff information to better council owners on the potential behavior changes in dogs after adoption from a shelter. Owners can be counseled about what behavior changes to expect and when. For example, stranger directed aggression scores increased at each timepoint throughout the study, but separation-related behaviors tended to only after 3 months. The latter finding suggests more support may be beneficial early in the adoption period to help owners better manage the most difficult adjustments. By providing owners with accurate information on what behavior changes to expect owners may have more realistic expectations on their dog's future behavior. This may mean fewer dogs rehomed, returned, or euthanized, advancing canine health and welfare.

Conclusions:

This is the first study to use the C-BARQ to track behavior changes in individual dogs after adoption from a shelter over multiple timepoints. This research finds that certain behaviors do change, some negative and some positive, over the first six months after adopting a dog from a shelter. This information will allow new adopters to have realistic expectations of their dog's future behavior and encourage timely and targeted behavioral interventions post-adoption by shelters, veterinarians, and behavior professionals.

Publications/Presentations/Grant Submissions:

1. Publication in a peer-reviewed journal is expected during the second half of 2022 or first half of 2023.
2. The research will be presented at the American College of Veterinary Behaviorist Veterinary Behavior Symposium in June 2022.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Germ line and somatic genetics of canine soft tissue sarcoma
Principal Investigator (PI)	William C. Kisseberth
Co-PIs (if applicable)	Carlos Alvarez
Interim or Final	Interim

Introduction:

Soft tissue sarcoma (STS) is a common cancer in dogs, especially Labrador and Golden Retrievers. In the proposed study we are analyzing STS tumors from to identify copy number alterations, i.e. alterations in chromosome/gene number, to identify the most common STS alterations in these breeds. Based on what is known about STS in humans, tumor samples that have a relatively normal genome structure likely will carry a translocation-mediated gene-fusion (i.e. rearranged chromosomes). We will then perform RNA sequencing (RNAseq) of 12 of the tumors normal ploidy (relatively normal genomes) to determine which genes have fused and validate the candidate translocations using PCR or Southern blotting. If successful, this study will have high impact, establishing whether translationally relevant translocations/driver-gene fusions exist in canine STS and will provide important data for identifying and developing new therapies.

Approach:

In this study we propose to integrate genetic determinants of germ line STS-risk with analysis of additional dimensions of germ line risk and somatic alterations. Specifically, we will define the somatic copy number alterations (i.e. changes in the number of chromosomes) in STS tumors from Labrador and Golden Retrievers. By using an advanced canine genomic platform (test), we will identify the most common STS gene alterations and "hotspots" for structural gene mutation in these breeds. This analysis will establish which samples have a normal genome structure, presumably carrying a translocation-mediated gene-fusion (the type more common in pediatric STS in people) that drives that STS. We will then conduct RNAseq of 12 of the tumors with normal ploidy and thus presumed to carry a translocation-mediated gene fusion that drives that STS. Candidate translocations will be validated by PCR or Southern blotting. This analysis will reveal the identity of the most common fusions in Labrador/Golden Retriever STS.

Results:

The tumor samples required for this study have been identified in the OSU CVM Biospecimen Repository and Colorado State University veterinary tumor bank. Additional samples have been identified and requested from the NCI Division of Cancer Treatment and Diagnosis who are distributing remaining canine tumor samples that were collected by the Canine Comparative Oncology Genomics Consortium (CCOGC). We will be isolating DNA from blood and tumor samples over the next 2-3 months, followed immediately by genotyping and low-coverage whole genome sequencing (LC-WGS). Selected samples will then be submitted for further genomic analysis.

Relevance & Impact to Canine Health:

Soft tissue sarcomas (STSs) are among the most common of canine cancers, exceeding in incidence both lymphoma and osteosarcoma - two intensively studied cancers in dogs. STSs are a heterogeneous group of tumors including hemangiopericytoma, peripheral nerve sheath tumor, myxosarcoma, liposarcoma, and other connective tissue (mesenchymal) tumors of soft (non-bone) tissues. While low-grade tumors are potentially cured by complete surgical resection +/- radiation, incompletely excised, unresectable, or metastatic tumors require additional therapy. In humans, genomic studies have provided detailed insights into STS biology and have provided convincing evidence that molecular classification of STS more accurately describes the biology and clinical course of STS to guide therapeutic decisions and development of new therapies. Thus, in order to identify new targets for treatment of STS and develop new therapies for STS for dogs, a molecular understanding of canine STS is needed. Findings from this study, will reveal information on Golden/Labrador Retriever STS germ line risk and the resulting patterns of somatic mutations.

Conclusions:

At the conclusion of this project we will have an improved understanding of the underlying genomics of STS in the dog. This improved understanding will help guide the identification of new targets for treatment of STS and the development of new therapies for STS in dogs.

Publications/Presentations/Grant Submissions:

A grant was submitted and funded to the Steps for Sarcoma foundation to expand the molecular characterization of canine STS being done in this study. Further, data from this study will be used in a new sarcoma SPORE grant submission being planned by investigators at The Ohio State University Comprehensive Cancer Center and Nationwide Children's Hospital to further justify the use of dogs with soft tissue sarcoma to investigate new therapeutic strategies for people with this cancer.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Morphologic, morphometric and functional characterization of degenerative lumbosacral disease in Labrador Retrievers
Principal Investigator (PI)	Ronaldo C. da Costa
Co-PIs/Co-Is	
Interim or Final	Interim

Introduction:

Degenerative lumbosacral stenosis (DLSS, cauda equina syndrome) is a common degenerative disease affecting the lumbosacral spine of older, large breed dogs. It is seen frequently in both working dogs and companion animals. DLSS causes compression of the cauda equina, nerve roots and the vessels that innervate these nerves leading to caudal spinal pain and neurologic deficits involving the pelvic limbs, tail, and urinary and fecal control. Despite numerous studies, strict objective evaluation of lumbosacral disease is lacking. Previous studies looked at imaging characteristics on radiographs, CT and MRI, however severity of lesions found did not correlate with clinical signs. Because diagnostic criteria remain variable, there are few reliable studies on prevalence, treatment and outcome of DLSS.

Canine DLSS bears similarities to human degenerative lumbar spinal disease, as both affect the cauda equina resulting in a similar clinical presentation. As in canine medicine diagnosis in human medicine is difficult, with an estimated 85% of humans with lower back pain unable to be given a precise diagnosis.

The purpose of this project is to prospectively study the lumbosacral spine of Labrador Retrievers and other large breeds in both clinically affected and clinically normal dogs using conventional and a novel kinematic magnetic resonance imaging technique. We aim to identify the morphologic and morphometric features that cause clinical disease in Labrador retrievers, and to expand this knowledge to all canine breeds affected.

Approach:

Thirty large breed dogs will be studied, 15 clinically affected and 15 with no signs of DLSS. The inclusion criteria for DLSS-affected dogs will be the presence of clinical signs compatible with DLSS, no concurrent orthopedic abnormalities and radiographs of the lumbar spine with no evidence of orthopedic disease or neoplasia. Inclusion criteria for DLSS-unaffected dogs will be the absence of clinical signs compatible with DLSS, orthopedic disease and radiographs of the lumbar spine with no apparent abnormalities.

Physical exam, neurologic exam, orthopedic exam and blood work will be performed on all dogs.

All 30 dogs will undergo kinematic MR and CT imaging of the lumbosacral vertebral column under general anesthesia.

The morphologic and morphometric analysis will be performed by use of a computer software program for image analysis (ClearCanvas).

Results:

At this point 10 affected and eight normal dogs have participated in the study. No initial morphologic or morphometric assessments have been made, though clear differences between neutral and kinematic positioning have been found.
Hypothesis: On MRI we expect to see a more severe reduction in the width, height and area of the lumbosacral vertebral column as well as intervertebral neurovascular foramina on extension in dogs clinically affected with DLSS compared with those not affected. We also expect to see a reduction in area of the neurovascular foramina on parasagittal views in addition to transverse views.

Relevance & Impact to Canine Health:

Degenerative lumbosacral stenosis (DLSS) is a common condition resulting in back (caudal lumbar) pain and neurologic deficits. It commonly affects older dogs, with the Labrador Retriever being among one of the most commonly affected breeds (Egenvall et al, 2000). DLSS significantly affects the quality of life of the dogs and their families and can result in disability and early retirement in otherwise healthy working dogs (Steffen et al., 2007).

Degenerative lumbosacral stenosis is a frustrating disease because it lacks objective diagnostic criteria, and treatment is difficult, expensive and yields variable results. The key reason for this is a poor understanding of the mechanisms causing the disease to develop. Particularly, no large-scale prospective study has been performed comparing normal dogs to affected dogs using high-field conventional and kinematic MRI and CT. To date, there are no gold-standard diagnostic or treatment for dogs affected with DLSS. A superior understanding of the pathogenesis behind the disease will aid in the development of new and/or optimized diagnostic criteria and treatment options. The high prevalence of DLSS in certain breed suggests that the disease may have an inherited basis. Ultimately, after we thoroughly characterize the phenotype of DLSS, our goal would be to identify the genetic basis of DLSS to eventually minimize its incidence. However, successful genetic testing can only be developed with strict phenotypic characterization.

Conclusions:

No conclusions have yet been made as this project is still actively recruiting cases.

Publications/Presentations/Grant Submissions:

No publications or presentations have yet been made. Additional funding was provided by a grant through the Gray Lady Foundation.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Pilot study on the effects of intra-articular allogeneic stem cell therapy for the treatment of osteoarthritis
Principal Investigator (PI)	Nina Kieves
Co-PIs/Co-Is	Jennifer Barret, Eric Hostnik
Interim or Final	Interim

Introduction:

Osteoarthritis is estimated to affect approximately 20% of dogs in the US. Once the process begins in a joint, it is painful, irreversible and progressive. To date, no treatment has been shown to significantly decrease its development. If such a treatment option were available, it could have a significant impact on millions of dogs and people. Elbow dysplasia is a common cause of lameness, and cause for the development of osteoarthritis in dogs. This osteoarthritis development is predictable, and provides an excellent model for studying treatments of osteoarthritis. If a therapy is proven effective to treat osteoarthritis secondary to elbow dysplasia, it would likely be effective for other causes of osteoarthritis.

Mesenchymal stem cell therapy has been investigated for its ability to heal injured tissue such as tendons and ligaments, and its ability to treat inflammatory conditions. While studies thus far have shown promise, there is a need to optimize stem cell therapy. A practical approach to this would be to use optimized donor stem cells that would be available "off-the-shelf". The use of allogeneic stem cells has been shown to be safe in numerous animal models. Our laboratory has previously validated, screened and optimized three-dimensional cultured (3D) canine adipose-derived stem cells for their anti-inflammatory properties as an allogeneic treatment of osteoarthritis.

The aim of our study is to assess the effect of intra-articular allogeneic 3D stem cell treatment in dogs with naturally occurring elbow dysplasia. We hypothesize that such treatment will significantly improve patients' pain, joint inflammation, and reduce the progression of osteoarthritis.

Approach:

Dogs with naturally occurring elbow dysplasia undergoing surgical treatment will be prospectively enrolled in this study with written informed owner consent and IACUC approval. Dogs will act as their own control group with one elbow being randomly assigned to treatment and one to a placebo injection. The treatment elbow will receive an intra-articular injection of allogeneic stem cells suspended in autologous serum at the two-week post-operative exam, while the other elbow will serve as a control and receive placebo injection of autologous serum alone at the two-week post-operative exam. Dogs will be re-evaluated with objective data being gathered at 6 weeks, 3, 6, and 9 months post-operatively, including objective gait analysis, joint fluid analysis, and re-imaging via CT scan.

Results:

Enrollment is on-going, so no results are available at this time. To date, no adverse events have been recorded and 4 dogs have fully completed the study.

Relevance & Impact to Canine Health:

Osteoarthritis affects approximately 20% of dogs in the US. As the disease progresses, it can become debilitating to patients and have a significant impact on their quality of life, even leading to euthanasia. Currently, there is no treatment to significantly slow the progression of arthritis; only symptomatic treatment exists. Elbow dysplasia is a common cause of lameness in dogs, and causes the development of arthritis. Our study aims to evaluate the effectiveness of an "off-the-shelf" stem cell injection created from optimized donor cells for the treatment of arthritis. This stem cell treatment has already been proven safe in other animal models. If a therapy could be found that is effective at significantly treating arthritis, millions of dogs could be impacted. Additionally, the technology may be translatable for human treatment.

Conclusions:

Project is still ongoing.

Publications/Presentations/Grant Submissions:

Project is still ongoing.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Canine glioma as a model for testing Mklp2 inhibition in human glioblastoma
Principal Investigator (PI)	Sarah Moore
Co-PIs/Co-Is	Morgan Schrock
Interim or Final	Interim

Introduction:

Glioblastoma (GBM) is one of the most fatal human brain cancers with an average survival of 14 months after diagnosis despite aggressive surgery and chemotherapy. A major barrier to developing new treatments is the lack of a robust preclinical animal model for testing drug efficacy. Laboratory mice are the standard animal model, however, mice are not accurate predictors of success: approximately 97% of cancer drugs that are effective and safe in mice fail in human clinical trials, underscoring the need for an improved animal model. Veterinary clinical trials, which provide novel cancer treatments to pet dogs with naturally-occurring cancer, could fill this void. Not only are dogs more similar to humans in terms of drug metabolism and anatomy, a small percentage of dogs also develop GBM naturally in their old age. The enrollment of pet dogs diagnosed with brain cancer in veterinary clinical trials provides a mutual benefit to dogs and humans: pets receive a novel treatment at a fraction of the cost to the owner, while also providing invaluable data to inform research scientists on drug dosing and side effects for use in humans.

Approach:

The purpose of this study was to generate novel canine glioma cell lines needed for testing treatments before they can be used on pet animals as part of a veterinary clinical trial. We planned to establish these lines from tumor tissue that would be leftover following brain surgery in pet dogs to definitively diagnose their tumor type and therapeutically remove as much cancer as possible. Instead of growing the cancer cells on plastic culture dishes in an incubator, the method for establishing traditional cancer cell lines, we will inject our glioma samples into immunocompromised lab mice where they will grow in an *in vivo* environment. This latter method is standard in the human GBM field because it avoids genetic changes the cells accumulate when grown on plastic dishes. In order to obtain canine glioma samples we are enrolling eligible patients for tissue collection at OSU and have established two collaborative veterinary neurosurgeon teams (University of Purdue and University of Minnesota) to provide glioma samples. We plan to use our newly established canine glioma patient-derived lines to test our experimental GBM treatment, a small molecular inhibitor of the protein MKLP2. If the MKLP2 inhibitor is effective in our canine glioma patient-derived lines we can move forward with testing our drug in pet dogs diagnosed with glioma as part of a veterinary clinical trial.

Results:

Thus far, we have received two fresh glioma tissue samples from Dr. Tim Bentley (University of Purdue) and ten frozen glioma samples from Drs. Pluhar and Olin (University of Minnesota). Only one of the ten frozen samples revived from its frozen state successfully, but that one sample has been injected into mice and we are monitoring it for growth monthly. In regard to the fresh samples that Dr. Bentley sent, the first was injected into mice but after monitoring for a year, those cells failed to grow. However, the second sample was injected into mice and it did successfully grow in mouse brains. We have harvested those cells from four different mice and have frozen various aliquots. Therefore we are happy to report that we have successfully established at least one patient-derived line of canine glioma and that it is sensitive to our novel antimitotic drug (MKLP2 inhibitor), suggesting it may be a useful treatment for canine glioma. We have also established a new collaboration with Dr. Jey Koehler at Auburn University who will send us five additional frozen canine glioma samples for injecting into mice this month (April 2022). Finally, we are currently in the process of submitting our established canine glioma line for sequencing so that we can report on its establishment and genetic sequence when we publish our results later this year.

Relevance & Impact to Canine Health:

While we propose to study canine glioma to enhance drug testing for human GBM, this work will provide potential benefit to pet dogs as well. Currently, there is no standard of care for canine brain tumors. Treatment ranges from symptom management to surgery, radiation, and chemotherapy[27]. Most cases are diagnosed presumptively based on advanced imaging without histological confirmation and treated with symptomatic therapy (~2 month survival) or

radiation therapy (9-14 month median survival). Therefore obtaining unique canine glioma samples and testing the efficacy of a novel antimitotic will greatly benefit comparative oncology and inform veterinary treatment as well.

Conclusions:

We report the first successful establishment of a canine glioma patient-derived line. We have one more sample pending (we are monitoring its growth in mice) and plan to inject five more samples this month. We also plan to submit a manuscript within the year for publication that would describe our workflow, methods, engraftment rate, and the genetic characterization of the established patient-derived canine glioma line. Lastly, we find that our novel antimitotic drug (MKLP2 inhibitor) causes significant death in our newly established canine glioma patient-derived line.

Publications/Presentations/Grant Submissions:

We presented our findings at The Ohio State University College of Veterinary Medicine Research Day this year (April 2022) and plan to present them again this fall at the annual Veterinary Cancer Society Conference in Norfolk, Virginia (October 2022). We also plan to publish our findings in the journal Veterinary and Comparative Oncology to report on our techniques and engraftment rates.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs
Principal Investigator (PI)	Audrey Wanstrath
Co-PIs/Co-Is	Morgan Biggo, Turi Aarnes, Nina Kieves, Phillip Lerche, Carolina Ricco Pereira
Interim or Final	Interim

Introduction:

Gabapentin is a medication that is labeled to treat epilepsy and herpes neuralgia in humans. Gabapentin is safe for use in dogs and is commonly used to treat acute surgical pain or chronic osteoarthritic pain, with or without the use of a Non-Steroidal Anti-Inflammatory Drug (NSAID, such as carprofen). Despite being commonly used, there are few studies on the efficacy of gabapentin for pain control in dogs. We aim to determine if gabapentin administration reduces pain after elective orthopedic surgery in dogs. We hypothesize that gabapentin will not provide equivalent pain control compared to carprofen, and the addition of gabapentin to carprofen will not provide added pain control in dogs experiencing acute post-operative pain.

Approach:

We aim to enroll 45 dogs with unilateral cranial cruciate ligament rupture (similar to an ACL tear in humans). Dogs will be treated by tibial plateau leveling osteotomy (TPLO), which is the current gold standard surgical therapy. Dogs will be randomly assigned to 1 of 3 groups: gabapentin only, carprofen only, or gabapentin + carprofen treatment. All investigators will be blinded to each dog's treatment group. While in hospital, dogs will be examined regularly for pain using the Glasgow Composite Pain Scale, and treated with injectable rescue medication if perceived to be painful. Dogs will be discharged from the hospital 2 days after surgery and will receive their assigned pain medication(s) regularly for 2 weeks post-operatively, during which time owners will have access to an oral rescue medication if they feel their dog is painful. In addition to pain scoring, we will also walk the dogs on a pressure sensing mat - an objective measure of lameness - pre-operatively, 2-days post-operatively, and at 2-weeks post-operatively. Additionally, we will be testing drug levels in blood samples to ensure that the orally provided medications are reaching therapeutic levels. Based on pain scale and lameness evaluations, we will determine if there is a difference in post-operative pain between the 3 medication treatment groups and will be able to determine if gabapentin provides measurable pain relief.

Results:

We continue to enroll cases for this study. The enrollment has been slower than anticipated due to continued restrictions with our hospital caseload as a result of the COVID-19 pandemic. Additionally our elective surgical caseload from our Dublin satellite clinic is temporarily closed due to staff shortages. We are working with hospital administration and hope to be able to increase our elective surgical caseload in the upcoming months. We currently have approximately 40% of cases completed or enrolled. As we work to continue to recruit cases we hope to have the data collected within the next 15 months. We do not have any preliminary results to report at this point as the study is blinded.

Relevance & Impact to Canine Health:

This study will provide veterinarians with information regarding the efficacy of gabapentin for treatment of acute orthopedic surgical pain in dogs. If gabapentin does provide measurable pain relief in dogs, we will be able to recommend that it is prescribed regularly after orthopedic surgery given that it has a strong safety profile. If gabapentin does not provide measurable pain relief, we will recommend against prescribing it for the purpose of pain control after orthopedic surgery to avoid the cost and hassle of administering an unnecessary medication.

Conclusions:

This project is ongoing.

Publications/Presentations/Grant Submissions:

This project is ongoing.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the canine gut microbiota and bile acid metabolome
Principal Investigator (PI)	Jenessa A. Winston
Co-PIs/Co-Is	Valerie Parker; Adam Rudinsky; James Howard
Interim or Final	Interim

Introduction:

Ursodiol is an FDA approved naturally occurring bile acid that is used to treat a variety of liver and gastrointestinal diseases. Ursodiol is routinely administered in veterinary medicine; however, it is unknown how this drug impacts the canine intestinal ecosystem. Evidence is mounting that bile acids, such as Ursodiol, can alter the gut microbial composition and host physiologic response during health and disease. Our study aims to determine how oral administration of Ursodiol (21 day course) alters the canine intestinal ecosystem, specifically the gut microbiota (collection of microorganisms that live in the intestines) and bile acids (important metabolites best known for their role in digestion and absorption of fat). The goal is to improve our knowledge of Ursodiol-mediated effects to the canine intestinal ecosystem to facilitate rational incorporation of Ursodiol into a personalized medicine approach for dogs suffering from liver and gastrointestinal diseases in order to improve quality of life.

Approach:

Our central hypothesis is that oral administration of Ursodiol will alter the canine intestinal ecosystem, specifically the fecal microbial community structure and bile acid metabolome. To test this, we conducted a clinical trial in client-owned healthy dogs administered Ursodiol for 21 days at a clinically relevant dose. Freshly voided feces was collected from dogs at baseline (3 separate samples), weekly during Ursodiol administration, and at 1 week, 1 month, and 3 months post Ursodiol administration. Alterations in the gut microbiota and fecal bile acid metabolome will be serially and simultaneously assessed with 16S rRNA gene sequencing (microbiota community structure) and targeted bile acid metabolomics, allowing for an integrated multi-omics approach. Due to dietary and individual variations between subjects, each dog will serve as its own control for these analyses. This study is the first comprehensive, multi-omics characterization of how Ursodiol impacts the healthy canine intestinal ecosystem.

Results:

Due to the unforeseen circumstances with COVID research restrictions the original timeline for this study was delayed. As our university slowly eases restrictions, we were able to start recruiting patients. To date, all canine participates (n=15) have finished the clinical trial and all fecal samples have been collected. 16S rRNA gene sequencing (microbial community structure) and targeted bile acid metabolomics analysis is underway. Integration of the multi-omics datasets will follow.

Relevance & Impact to Canine Health:

Ursodiol is routinely and liberally administered to canines, however the ramifications of how this drug impacts the intestinal ecosystem remains unknown. This study will provide valuable data on the impacts of Ursodiol, administered at a clinically relevant dose, on the canine intestinal ecosystem. Specifically, this clinical trial is the first to provide a comprehensive characterization of Ursodiol mediated effects on the gut microbiota and bile acid metabolome in healthy dogs. Results of this study will be the catalyst that will ultimately allow us to make evidence-based recommendations on how to utilize Ursodiol to rationally manipulate the canine intestinal ecosystem. The ultimate goal is to understand how the canine bile acid metabolome contributes to health and disease in relation to chronic enteropathy, enteric pathogens, diabetes mellitus, chronic kidney disease (CKD), and obesity. To this effect, this study will be the first to integrate multi-omics approaches to evaluate the canine gut microbiota-bile acid-host axis, which will provide a foundation for unraveling the complex intricacies of bile acid metabolism within the canine intestinal ecosystem with the ultimate goal of improving canine health and quality of life.

Conclusions:

Analysis of the gut microbiota and bile acid metabolome is ongoing; therefore conclusions cannot be provided at this time.

Publications/Presentations/Grant Submissions:

Analysis is ongoing and resulting publications, presentations, and additional grant submissions will follow upon completion of this project.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Alveolar type II (ATII) cell function in dogs with severe acute respiratory distress syndrome (ARDS)
Principal Investigator (PI)	Ian C. Davis
Co-PIs/Co-Is	Ed Cooper, Chris Premanandan, Valerie Bergdall
Interim or Final	Interim

Introduction:

Acute respiratory distress syndrome (ARDS) is characterized by rapid onset of severe hypoxemia accompanied by evidence of non-hydrostatic pulmonary edema and reduced lung compliance. There are >300,000 cases of ARDS in humans in the USA alone each year. It is estimated that 5-10% of ill dogs also develop ARDS. Treatment options are currently limited to protective mechanical ventilation, conservative fluid administration, and supportive care. Development of more effective targeted ARDS therapies will require better understanding of the impact of injurious insults on host lung function. Alveolar type II (ATII) cells are central to normal lung function and important players in ARDS pathogenesis. Unfortunately, however, our understanding of how ATII cells behave in ARDS is almost entirely based on experimental models – it is virtually impossible to obtain viable ATII cells from human patients with ARDS because these patients are clinically extremely fragile, making biopsy very risky. We hypothesize that studying ATII cells from dogs euthanized for spontaneous severe ARDS will provide us with unique insights into ARDS pathogenesis. We will exploit this invaluable but untapped resource, using lung tissue from healthy adult dogs as a control. Our goals are: 1) To show that ARDS results in altered canine ATII cell mitochondrial energetics and metabolism *ex vivo* (in ATII cells isolated from canine lung by digestion and negative selection); and 2) To show that ARDS results in alterations in the lung secretome *in vivo* and *in vitro* (by analyzing bronchoalveolar lavage fluid and using the precision cut canine lung slice model, respectively).

Approach:

We will identify dogs that are to be euthanized for severe VetARDS that is unlikely to be responsive to treatment. Client consent for use of tissues in this study will be obtained, with the understanding that euthanasia is not contingent upon willingness to donate tissues. Tissues from a cohort of normal dogs will be used as controls - these will be from purpose-bred research dogs euthanized at end of other research studies not involving infectious diseases. After collecting arterial and venous blood, dogs will be euthanized. Lungs will be removed and assessed for gross pathology. Bronchoalveolar lavage will then be performed. Cellular pathology will be assessed in H & E-stained sections from FFPE tissue and by transmission electron microscopy. We will isolate ATII cells from fresh lung tissue by a digestion and negative selection method to determine effects of ARDS on the ATII cell phenotype, mitochondrial energetics, transcriptomics, lipidomics, and targeted metabolomics. For *in vitro* studies of the ARDS lung secretome, we will utilize the precision cut lung slice (PCLS) culture model.

Results:

Unfortunately, the ongoing SARS CoV-2 pandemic continued to impede our ability to perform the proposed studies over Year 2. Research in the lab was partly refocused towards COVID-19, which proved to be very labor intensive. Moreover, 2 research technicians departed from the group at the end of 2021. Consequently, we lacked the manpower to begin these studies. No additional expenditures have been made.

Relevance & Impact to Canine Health:

The proposed studies will help us to define mechanisms underlying the pathogenesis of ARDS and VetARDS. Since they could lead to new FDA-approved ARDS drugs, our findings will have the potential to transform critical care resulting in both improved survival and reduced health care costs for human patients and animal owners. Hence, our proposal is closely aligned with one goal of this program, which is to "research diseases of dogs that, by their nature, will provide information applicable to the prevention and treatment of both human and canine illnesses."

Conclusions:

Although the pandemic continues to have a significant detrimental impact on the proposed studies, we anticipate that we will be able to achieve our goals within the next project period.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	A pilot study on the role of *Staphylococcus pseudintermedius* toxins and virulence regulators in canine pyoderma
Principal Investigator (PI)	Lorch, Gwendolen
Co-PIs/Co-Is	Cole, Lynette Diaz-Campos, Dubraska Diaz Vergara, Sandra Montgomery, Christopher Lorch, Gwendolen Yang, Ching Matusicky, Missy
Interim or Final	Interim

Introduction:

Staphylococcus pseudintermedius is a bacterium that is a leading cause of skin infections in dogs and can be transmitted to humans. Currently, no effective vaccine is available for preventing *S. pseudintermedius*-induced infections in dogs. This bacterium produces several toxins, namely pore-forming toxins, which cause injury to cells in a laboratory setting. *S. pseudintermedius* is similar to a bacterium named *S. aureus*, which is the major cause of human skin infections. The immune response, specifically the antibody response, induced by *S. aureus* pore-forming toxins has been demonstrated to protect human patients against recurrent infections, and therefore, these toxins are considered potential vaccine candidates for staphylococcal infections in humans. However, the relationship of *S. pseudintermedius* pore-forming toxins during infection to the disease severity and protective immunity in dogs is unknown. The goal of this study is to investigate whether *S. pseudintermedius* pore-forming toxins play a role in worsening skin infections in dogs, and whether antibody-mediated immunity induced by these toxins will prevent dogs from recurrent infections. Successful completion of this study will provide valuable information for developing vaccines to prevent *S. pseudintermedius* infection in dogs.

Approach:

To define the role of toxin expression in the severity of skin infections (pyoderma) and the relationship between the immune response to the presence of infection and recurrent infection, a clinical trial recruiting both healthy dogs and dogs with pyoderma will be conducted, in which dogs with pyoderma will be evaluated. Bacterial and sera samples at the determined time will be collected. A thorough medical examination and scorings for clinical skin lesions and itch will be performed by board-certified veterinary dermatologists. We will characterize the gene expression of *S. pseudintermedius* pore-forming toxins and those regulating the toxin secretion (virulence regulators) in *S. pseudintermedius* clinical bacterial isolates from dogs using molecular techniques. The gene expressions will be correlated with the clinical lesion scores for pyoderma. To evaluate if the bacterial toxins will induce an antibody response in dogs during infections, we will compare the antibody levels between healthy dogs and dogs presented with a first-time pyoderma. To define the relationship of the immune response induced by bacterial toxins and the presence of recurrent infections, we will compare the antibody levels between 13 dogs that are healthy and have never had pyoderma or otitis externa to 18 dogs that have had either first time or recurrent pyoderma.

Results:

The results for this project are pending. Patient recruitment is ongoing. Currently, the healthy dog with colonization cohort (G1) has been completed with 13 dogs. Ten dogs have enrolled and completed the Staph pyoderma cohort (G2). This cohort needs to enroll an additional 8 dogs for study completion.

Relevance & Impact to Canine Health:

S. pseudintermedius is a major bacterial pathogen causing various infections in dogs and can infect humans. Resistance to multiple classes of antibiotics has become more frequently detected in *S. pseudintermedius* clinical isolates due to the indiscriminate use of antibiotics; therefore, an alternative preventive is needed for improving canine health. This study will advance the development of vaccines for preventing *S. pseudintermedius* infection in dogs by determining the role of *S. pseudintermedius* pore-forming toxins and the protective immune response induced by these toxins in canine patients during infection. This will be the first study to evaluate the changes in serum antibody levels to the bacterial toxins and to define the protective immunity against recurrent pyoderma in dogs, which is critical for future vaccine development.

Conclusions:

The conclusions for this study are pending.

Publications/Presentations/Grant Submissions:

A $3,000.00 CCTS voucher was awarded for a REDCap build and data management

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Scientific and clinical assessment of fecal microbiota transplantation to enhance weight loss in obese dogs (SLIM pilot study)
Principal Investigator (PI)	Jenessa A. Winston
Co-PIs/Co-Is	Valerie Parker Adam Rudinsky James Howard
Interim or Final	Interim

Introduction:

The obesity epidemic is rampant in canines and is ultimately resulting in physical impairment, comorbidities, and reduced quality of life and healthspan. Evidence is mounting that the intestinal microbiota (microbes living in the intestinal tract) contributes to obesity, and rational manipulation of this ecosystem may confer a health benefit. The overall objective for this clinical trial is to provide a comprehensive scientific and clinical assessment of the efficacy of fecal microbiota transplantation (FMT) as an adjunctive therapy for canine obesity management. FMT is the transfer of feces from a healthy donor to a recipient in order to confer a health benefit.

Approach:

Hypothesis: We hypothesize that capsular FMT, added to a standard dietary obesity management, will amplify weight loss compared to the use of dietary obesity management alone or with placebo. We also hypothesize that dogs receiving FMT treatment will experience rapid shifts away from the "*obesogenic*" intestinal ecosystem compared to receiving only dietary obesity management alone or with placebo.

Specific Aims: We plan to accomplish our objective for this project by pursing the following:

Specific Aim 1: Determine the clinical efficacy of FMT as an adjunctive therapy to enhance standard canine obesity management compared to standard dietary management alone or with placebo. Client-owned obese, but otherwise healthy dogs are being prospectively enrolled into a randomized, double-blinded, placebo controlled, cross-over clinical trial. Throughout the 24-week clinical trial, serial monitoring of body weight, body condition score (BCS), activity and quality of life questionnaires are being conducted to assess clinical and owner perceived improvement.

Specific Aim 2: Assess longitudinal alterations within the canine "*obesogenic*" intestinal ecosystem throughout a structured obesity management program with FMT compared to standard dietary management alone or with placebo. Using an integrated multi-omics approach, the intestinal ecosystem will be comprehensively evaluated every 6 weeks throughout the trial via 16S rRNA gene sequencing (for microbial community composition) and global untargeted metabolomics (for microbial community function).

Results:

Due to the unforeseen circumstances with COVID restrictions, the SLIM study has been delayed. As our university slowly eased restrictions, the Ohio State University Companion Animal Fecal Bank recruited four lean and healthy canine fecal donors for the SLIM study. Over 8 pounds of feces was collected from each donor in order to make all the fecal capsules for fecal microbiota transplant (FMT) that will be administered to obese dogs during the SLIM study. We officially launch the SLIM study January 2021. To date, we have 22 dogs that have successfully completed the 24-week clinical trial. Currently, we have 3 dogs actively enrolled and are actively recruiting one remaining dog. Once all patients (n=26) have completed the 24-week study, 16S rRNA gene sequencing (microbial community structure) and global metabolomics will be submitted and analyzed.

Relevance & Impact to Canine Health:

This clinical trial is the ***first*** to assess the efficacy of FMT for obesity management in dogs. Additionally, this study is the ***first*** to provide comprehensive, integrated multi-omics data on obese dogs throughout a structured obesity management program. This study will shed light on the role(s) that the canine intestinal ecosystem plays during treatment and recovery from an "*obesogenic*" disease state and could change standard of care practices for our canine patients. Success of this clinical trial will be of immediate benefit to obese dogs by providing an adjunctive option for canine obesity. Understanding the obesity-specific key microbial community members and their metabolic function will help to facilitate development of precision canine microbiome-targeted therapies aimed at facilitating accelerated metabolic improvements to promote healthspan and improve quality of life in dogs suffering from obesity.

Conclusions:

This study is still ongoing; therefore conclusions cannot be provided at this time.

Publications/Presentations/Grant Submissions:

This study is still ongoing and resulting publications, presentations, and additional grant submissions will follow upon completion of this project.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Examining urine microbiota, urinalysis, and urine protein over time in healthy dogs
Principal Investigator (PI)	Vanessa Hale
Co-PIs/Co-Is	Jessica Hokamp, Sheryl Justice, Adam Rudinsky
Interim or Final	Interim

Introduction:

Urinary tract diseases – such as urinary tract infections – are amongst the most commonly diagnosed diseases in veterinary medicine. Urine samples are often collected to aid in disease diagnosis, prognosis, or response to treatment. However, little is known about if or how microbes and proteins present in urine vary in healthy dogs over time. The microbiota play a critical role in host health including immune development and defense against pathogens. While studies on the gut microbiome have increased exponentially over the last decade, knowledge of the urinary microbiome remains limited. Similarly, while there have been some studies to evaluate urine pH and urine specific gravity in dogs over time, less is known about how protein type and content can vary temporally in healthy dogs, or if and how protein levels may vary with other urine attributes. Proteins identified in urine are important to diagnosing renal disease and also guiding treatment and prognosis.

Approach:

Urine is a highly variable matrix that undergoes hourly changes based on host health, hydration status, body mass index, concentrating ability, and diet. In a clinical setting and in cross-sectional research studies, it is common to evaluate a single urine sample to characterize the urinary microbiome, urogenital pathogens, or to look for the presence of disease. However, little is known as to how much urine microbial communities or urine protein may vary over time in healthy dogs. In this study, we compared urine properties, proteins, and urine microbial communities in 14 dogs at 12 time points.

- Aim 1 - Compare urine microbial communities over time in healthy dogs.
- Aim 2 - Characterize urine, urine cells, and urine protein over time in healthy dogs.

All dogs (n=14, 7 males, 7 females) underwent physical exam, bloodwork, urinalysis, and urine culture and sensitivity upon enrollment. We collected mid-stream free-catch urine samples from each dog over 12 time points (Morning and Afternoon of Days 1, 2, 3, end of Weeks 1, 2, 3, 4, and end of months 1, 2,3). At each time point, urine was aliquoted for immediate urinalysis, protein analysis (SDS-PAGE), and DNA extraction for 16S rRNA microbial community sequencing.

Results:

We have completed recruitment and sample collection for this project, and all urine pH, dipstick, protein, cytology, and culture analyses are also complete. Microbiome extraction and sequencing has also been completed and sequencing results were just returned to us on April 14, 2022. We will be analyzing the sequencing data this summer (2022). Three students have been involved in this work, and their accomplishments are described under Publications/Presentations/Grant submissions below.

- Ryan Mrofchak, Masters student, focused on urine pH and specific gravity analyses over time
- Rushil Madan, undergraduate, involved in culture and bacterial identification in urine over time
- Andrew McGlynn, veterinary summer scholar student (2021 and 2022)
    - 2021: Focused on protein analysis in urine over time
    - 2022: Will focus on longitudinal urine microbiome analyses

Relevance & Impact to Canine Health:

Urinary tract disease is one of the most common diagnoses in veterinary medicine, and the incidence of urinary tract infection (UTI) in a dog over its lifetime is reported to be 14%. Despite this, there are still many aspects of canine urine that not well characterized. Previous studies have demonstrated that urine specific gravity and proteinuria varies over time in healthy dogs; however, little is known about the microbial communities and proteins found in healthy dog urine over time. Deepening our understanding of variation within dog urine over time has relevance for dog urogenital health, for dogs as a translational model for human urogenital health, and for specific breeds of dogs that are disproportionately

affected by urogenital diseases, such as Scottish Terriers and bladder cancer, or miniature schnauzers and urolithiasis / bladder stones.

Conclusions:

Key findings from our current work:

- Urine pH, urine specific gravity (USG), number of protein bands, and protein concentrations varied significantly between dogs.
- pH was highly variable within dogs over time and should be measured via pH meter (not dipstick) at multiple time points before making clinical recommendations.
- USG was consistent over time within dogs, and measurement of USG at a single timepoint is likely to be an accurate representation of a dog's urine concentrating ability.
- Varying but low concentrations of Tamm Horsfall / albumin proteins were present in most healthy dogs.
- A few additional protein bands of unknown identity were consistently identified in urinesamples suggesting that these bands can be considered normal findings.
- Cultured bacterial profiles varied significantly between dogs; but was less variable within dogs over time, indicating some stability in the urinary microbiota.
- We found no differences in presence or number of cultured bacterial taxa by sex.
- The most commonly cultured organisms, *Streptococcus canis* and *Staphylococcus pseudintermedius* are skin contaminants frequently found in voided urine. Cystocentesis or catheterized urine collection is recommended when feasible to avoid these contaminants.
- Multiple healthy, asymptomatic dogs cultured high abundances of bacteria (>10^5 CFU/mL); although, these bacteria were likely skin contaminants.
- Uropathogens associated with urinary tract infections like *E.coli* and *P. auruginosa* were also cultured at low levels in healthy dogs, and the presence of these taxa in cultures from asymptomatic dogs does not necessarily warrant treatment.

Publications/Presentations/Grant Submissions:

- Ryan Mrofchak, incorporated preliminary data from this study into his Masters and he successfully defended in Spring 2021.
  - Thesis: An Analysis of Canine Urine: Microbiota, Methods, and Changes in Health and Disease
- Rushil Madan presented his work OSU Spring Undergraduate Research Festival (April 2022): R. Madan, et al., V.L. Hale. April 2022. *Longitudinal Examination of Urine Cultures from Healthy Dogs*
- Rushil was also awarded:
  - Undergraduate Research Apprenticeship Program (URAP) Funding from May 2021-December 2022 to work on this project.
  - CREATES-Undergrad Fellowship to continue work on the urine microbiome during summer 2022.
- Andrew McGlynn presented his work in the following venues:
  - A. McGlynn, et al., V.L. Hale. August 2021. Urine Trouble: Examining urine pH, specific gravity, and urine protein over time in dogs.
    - OSU College of Veterinary Medicine Research Day. April 2022.
    - National Veterinary Scholars Symposium. August 2021.
  - Andrew is leading a manuscript in preparation on his findings. A separate manuscript will be prepared for urine microbiome analyses during the summer of 2022.
- Both Andrew and Rushil will also be submitting their work for presentation at the Purdue Microbiome Symposium in May 2022.
- V. Hale submitted and NIH K08 in February 2022 that is relevant to the urine microbiome: Getting into the weeds: Herbicidal compounds as modifiers of microbiomes, metabolomes, and urothelium.
- V. Hale, S. Justice, A. Rudinsky, B. Husbands were awarded funds for a clinical trial involving the canine urine microbiome: Can probiotics improve clinical outcomes in bladder cancer?: Addition of probiotic *Escherichia coli Nissle 1917* to a vinblastine / piroxicam protocol for the treatment for urothelial carcinoma in dogs

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Clinical utility of corticosteroids and point of care monitoring in canine acute pancreatitis
Principal Investigator (PI)	Rudinsky, Adam
Co-PIs/Co-Is	Winston J, Chen M, Parker V, Howard J
Interim or Final	Interim

Introduction:

Pancreatitis is a common inflammatory disease of the pancreas with studies reporting up to 58% of affected dogs dying. This results from a lack of knowledge on how to treat and monitor patients. Currently, there are no specific treatments or monitoring tools available to veterinarians, and most dogs are treated only with supportive care. Alternatively, in human medicine, corticosteroids (a low-cost anti-inflammatory medication) and intensive monitoring improve outcomes. This study aims to determine the efficacy of corticosteroids and a bedside test (VetScan cPL Rapid Test) in the treatment, prognosis and monitoring of canine pancreatitis. Dogs will be entered into 14-day clinical study where they receive standard of care supportive treatment in addition to either corticosteroids or a placebo. Dogs will then be monitored with the expectation that corticosteroids and cPL monitoring will result in faster improvement, decreased hospitalization, improved survival, and a reliable marker of prognosis and disease severity.

Approach:

We are conducting a blinded, placebo-controlled, randomized clinical trial to evaluate the efficacy of anti-inflammatory corticosteroids and serial POC laboratory monitoring using the cPL in CAP. This study will accomplish both of our proposed specific aims in the previous grant proposal by establishing the clinical utility of intravenous corticosteroids (Dexamethasone SP) compared to placebo (saline) in CAP and demonstrating the clinical utility of the VetScan cPL Rapid Test through serial assessment of CAP and correlation to disease severity and prognosis. Enrolled dogs will be hospitalized on standardized therapies, with one group receiving corticosteroids and another group receiving placebo. Clinical severity index, cPL activity, inflammatory markers, and clinical scoring will be assessed throughout the study. Dogs will be hospitalized until clinical score improve. Dogs will return for a recheck with the OSU-VMC Internal Medicine service 14 days after initial presentation.

Results:

Pending completion of study enrollment. No results are available at this time. Enrollment has been limited thus far due to study inclusion criteria. The study team is evaluating options for altering the inclusion criteria to boost enrollment and maintain the high level of scientific rigor currently in this project.

Relevance & Impact to Canine Health:

Although CAP is considered a completely reversible condition, the financial burden of treatment can be tremendous. The impact of a novel treatment strategy like glucocorticoids or a technique for disease monitoring could save the lives of countless dogs and guide their owners in the decisions they make for the best welfare of their pets. In summary, the absence of evidence-based medical literature on CAP, accentuates the prolific need for investigation into treatment and serial monitoring of CAP. In dogs, we currently lack this information and further studies are desperately needed to turn the tides on the enormous financial and emotional impact this deadly disease inflicts annually.

Conclusions:

This project is ongoing. Conclusions from this study will be determined upon completion of enrollment and data analysis.

Publications/Presentations/Grant Submissions:

This study is ongoing. At study completion we plan to present this work at college, national, and international venues as well as pursue publication to disseminate the information for the betterment of canine health.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Understanding and stopping persistent *Ancylostoma caninum* egg shedding in chronic shedders
Principal Investigator (PI)	Marsh, AE
Co-PIs/Co-Is	O'Quinn, Winston (substitution in for Horvath)
Interim or Final	Interim

Introduction:

Ancylostoma caninum (hookworm) is a nematode of the canine gastrointestinal tract. This parasite is zoonotic through cutaneous contact with infectious larvae, resulting from eggs shed in the feces. The OSU CVM is seeing an increase in persistently infected greyhounds, requiring a combination drug therapy developed at OSU. The combination therapy includes three different classes of dewormers: pyrantel, febantel (pro-fenbendazole), and moxidectin. However, unverified reports suggest that this combination therapy is beginning to fail due to multi-drug resistant hookworms. The study aims are to evaluate the combination therapy, including a drug substitution, along with pre- and post-treatment worm genetics.

Approach:

For the treatment substitution, we plan to use fenbendazole to determine if a lower cost drug could be used in lieu of febantel. This study involves privately-owned adult Greyhounds (including recent racetrack dogs) or other breeds presenting with persistent *A. caninum* egg shedding despite prior deworming. Eggs will be collected at the start for genetic analysis. The dogs will receive a combination treatment protocol comprised of the original combination therapy (topical moxidectin, followed by oral pyrantel and febantel within 24 hours) or a modification combination therapy (topical moxidectin, followed by oral pyrantel within 24 hours and three days of oral fenbendazole). At 14 days post-treatment, a fecal examination will monitor for parasite eggs and for egg count reduction. Dogs will remain on the monthly combination treatment protocols and fecal egg monitoring until they ceased shedding detectable eggs or for six months whichever is sooner.

Results:

During the study, one kennel of 20 dogs cleared hookworm shedding following a single treatment of moxidectin, 3 dogs failed to clear and continued to shed hookworm eggs in their feces despite receiving the triple dewormer therapy, ranging from 3-6 months. These dogs were then treated with a single oral dose emodepside. Fecal evaluation 2 weeks post-treatment demonstrated no detectable fecal hookworm eggs. One dog failed to clear after 4 months of combination treatment with fenbendazole and moxidectin, then cleared after switching from fenbendazole to febantel, pyrantel, and praziquantel. Limitations of the current study include small sample size, and client compliance with fecal collection and animal care. Genetic analysis to date demonstrates drug resistant alleles in this dog population.

Relevance & Impact to Canine Health:

Follow-up fecal examinations were important for verifying the presence or absence of egg shedding despite the use of anthelmintic treatment. It is also important that greyhounds are more frequently checked for hookworm shedding as they occasionally revert to positive status despite being on gastrointestinal worm prevention.

Conclusions:
Comparison to a prior study using monthly doses of combination pyrantel, febantel and moxidectin, it appears this treatment maybe insufficient to completely clear some hookworm positive dogs from shedding eggs in their feces, suggesting a change to the hookworm population. This study is ongoing and data collection is ongoing.

Publications/Presentations/Grant Submissions:
College of Veterinary Medicine, All VMC (presentation)
Greyhound and Parasitology Student Clubs (presentation)

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Effect of isotonic versus hypotonic maintenance fluid therapy on urine output, fluid balance, and electrolyte homeostasis in healthy dogs
Principal Investigator (PI)	Jiwoong Her
Co-PIs/Co-Is	Dr. Daniel Gordon, Dr. Cathy Langston
Interim or Final	Interim

Introduction:

Maintenance and replacement fluids in hospitalized patients account for the majority of mean daily fluid volume, exceeding the volume provided by resuscitation fluids. The use of isotonic crystalloids as a maintenance fluid is an important source daily sodium and chloride, which leads to an increase in volume retention. Previous studies have shown that isotonic fluids administered rapidly are excreted slower than an equal amount of hypotonic fluids. The principle of fluid tonicity contributing to fluid retention in the maintenance phase has been shown in a both healthy and hospitalized patients. The detrimental effects of salt and fluid overload are well known in human medicine; thus, the goal of this study is to document fluid balance and related effects during maintenance fluid therapy. We hypothesize that dogs receiving the hypotonic fluid will have a higher urine output over a 48-hour period.

Approach:

Healthy dogs will receive two different types of fluids at a maintenance rate with different electrolyte like compositions during each hospital stay. Total fluid volume administered, and urine output will be measured in dogs to assess overall fluid balance between the two fluid types. To investigate physiological mechanisms and compare effects on electrolyte concentrations balance between both using two types of crystalloid fluids, aldosterone, anti-diuretic hormone levels will be measured.

Results:

A total of 9 dogs are included in the study. Blood and urine samples for anti-diuretic hormone, aldosterone, Complete blood count, Chemistry, Urinalysis, and Urine profile are collected. Sample will be analyzed.

Relevance & Impact to Canine Health:

This study will provide evidence to the general knowledge of maintenance fluid therapy in hospitalized patients. Much of the recommendations for maintenance fluid therapy is based on expert opinion and theory, with a lack of clinical data to support the recommendations. The theory is logical, but concrete evidence will set the stage for change in fluid therapy for all patients. The significance of this pilot study will allow us to determine if hypotonic fluids should be preferred in the maintenance phase in a normal population of dogs, leading to further investigation whether hypotonic fluids should be preferred in a patients with critical-illness and known renal dysfunction in acute kidney injury.

Conclusions:

This project is ongoing. Conclusion will be stated based on the analysis of final results.

Publications/Presentations/Grant Submissions:

This project is ongoing.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Evaluation of OCT for metastatic lymph node identification in dogs with oral malignant melanoma
Principal Investigator (PI)	Janis Lapsley
Co-PIs/Co-Is	Laura Selmic, Eric Hostnik, Ryan Jennings
Interim or Final	Interim

Introduction:

Tumors of the oral cavity represent 5-7% of all canine tumors with oral malignant melanoma (OMM) being most common malignancy. OMM is known to metastasize (spread) via lymphatic pathways and presence of nodal metastasis reduces reported median survival times from 818 to 131 days. Lymph node (LN) metastasis is reported in up to 37% of cases with distant metastasis present in 25-55%. Due to variable patterns of lymphatic drainage of the head and unreliability of preoperative diagnostic techniques as predictors of metastasis, nodal metastasis may be missed during preoperative patient evaluation. Currently, primary tumor removal and nonselective cervical lymph node removal is standard of care for OMM. More selective lymphadenectomy techniques may be advantageous by reducing patient morbidity while still providing full staging information. Selective lymphadenectomy approaches rely on successful identification and removal of first order draining LN(s), termed sentinel lymph nodes (SLN). Indirect computed tomographic lymphangiography (ICTL) is one SLN identification technique which has been used successfully in dogs. However, ICTL cannot be used as a sole diagnostic to differentiate normal vs metastatic nodes which is critically valuable information to determine if selective lymphadenectomy approaches are feasible in this population. Optical coherence tomography (OCT) is a rapid noninvasive imaging modality used for identification of nodal metastasis in humans. OCT has the potential for intraoperative use, thus reducing unnecessary LN dissection and associated patient morbidity. This novel technique has not been applied to LN analysis in veterinary patients and represents a new frontier.

Approach:

The purpose of this study is to evaluate the ability of optical coherence tomography (OCT) imaging to identify metastatic disease in lymph nodes from canine OMM patients. Use of indirect computed tomographic lymphangiography (ICTL) to identify sentinel lymph nodes (SLN) will facilitate the aim of OCT evaluation of SLNs as well as determining if SLN status is an accurate predictor of cervical lymphatic basin metastatic status and determining the frequency of metastasis beyond the SLN. The central hypothesis of this study is that OCT imaging will have a high sensitivity for detection of lymph node metastasis and high correlation with histopathologic findings of nodal metastasis in patients with OMM. This hypothesis is based on the reported high sensitivity and specificity of OCT for nodal metastasis detection in human breast carcinoma and translational research using rat models. This novel application of an innovative imaging technique has the potential to offer intraoperative nodal assessment and limit extent of surgery necessary for patients with OMM.

Aim 1: Correlate normal and metastatic LN OCT imaging features with corresponding histopathology to create an image training set for observers.
Aim 2: Evaluate diagnostic accuracy of OCT imaging for identifying metastatic LNs.
Aim 3: Preliminary evaluation of SLN metastatic status as predictor of cervical lymphatic basin metastatic status and determine frequency of metastasis beyond the SLN.

Results:

No results are currently available. This study is currently enrolling patients with 5 patients enrolled at this time.

Relevance & Impact to Canine Health:

Cancer is a common problem faced by veterinary patients and is the leading cause of death in older dogs. Oral tumors represent 5-7% of all canine tumors with oral malignant melanoma (OMM) being the most common. This tumor has a high metastatic rate and poor prognosis, similar to oral mucosal melanoma in humans. OMM is known to metastasize via the lymphatic system to regional lymph nodes and presence of nodal metastasis is a poor prognostic indicator. Complete surgical removal of the tumor and nonselective cervical lymphadenectomy is current standard of care. However, blanket application of nonselective cervical lymphadenectomy potentially subjects over 50% of patients to unnecessary surgical morbidity without actual benefit to the patient. Establishing new techniques to allow accurate selective lymphadenectomy will reduce patient morbidity and allow therapy to be tailored to individual patients. There is a critical need for accurate intraoperative diagnostics to help guide selective lymphadenectomy in veterinary and human cancer patients. This research may act as a steppingstone for additional translational research as canine OMM has been proposed as a model for human oral mucosal melanoma.

Conclusions:

This project aims to investigate an emerging diagnostic imaging tool, optical coherence tomography, which uses light waves to generate real time high-resolution images of tissues for detection of cancer cells. Currently this tool is being used for evaluation of presence of residual cancer cells in surgically resected tissue. This project aims to broaden the scope of application of this technology for use in evaluating canine lymph node tissue. Our team involves collaboration between veterinary medicine and pathology at the Ohio State University.

Publications/Presentations/Grant Submissions:

This project is ongoing.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Evaluation of SpO2 to FiO2 ratio with PaO2 ratio in Dogs Receiving High Flow Nasal Cannula Oxygen Therapy for Hypoxemic Respiratory Failure
Principal Investigator (PI)	Jiwoong Her
Co-PIs/Co-Is	Dr. Edward Cooper, Page Yaxley, Anda Young
Interim or Final	Interim

Introduction:

High-flow nasal cannula (HFNC) oxygen therapy is a safe and effective method of oxygen delivery for critically ill patients with respiratory disease. This respiratory support offers more comfortable, superior oxygen support than conventional oxygen supplementation. While patients are receiving supplemental oxygen, clinicians use the PF ratio of oxygen in arterial blood (PaO2) to the level of oxygen being inspired (FiO2) to assess disease severity, response to therapy and predict outcome. Determination of this ratio requires a sample of arterial blood, which can be challenging in some patients. For those patients, pulse oximetry provides non-invasive, continuous monitoring of blood oxygen levels without the requirement for repeated arterial puncture. Recent human studies have found that oxygen saturation measured via pulse oximetry (SpO2) to FiO2 (SF) ratio as a surrogate for the PaO2 to FiO2 (PF) ratio. We hypothesize that SF and PF ratios will track in a similar fashion in dogs receiving HFNC and demonstrate the utility of SF in patients with severe respiratory disease.

Approach:

This is a prospective observational study. Dogs with hypoxemic respiratory failure requiring HFNC at the Ohio State University Veterinary Medical Center will be eligible for inclusion in the study. Arterial blood samples) will be obtained utilizing commercial blood gas syringes and run immediately within 3 minutes using a blood gas analyzer. SpO2 values, measured simultaneously or within 3 minutes of blood gas analysis, will be recorded. Statistical analysis will be performed to calculate correlation coefficients, Pearson or Spearman correlation as appropriate as well as repeated measures correlation.

Results:

The study targeted to obtain 240 data collection. So far, 120 data have been collected. The correlation analysis will be performed after the study obtain target sample size.

Relevance & Impact to Canine Health:

This canine grant targets studies investigating respiratory diseases that will provide information that will affect treatment decision and outcome prediction of canine respiratory medicine, specifically acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Importantly, the results of this study will be relevant to emergency clinicians and diplomates of the American College of Emergency and Critical Care across the spectrum of care worldwide. If successful, our studies will provide compelling evidence that SF ratio can be incorporated into severity stratification and predictive modeling in human and canine patients with ALI and ARDS.

Conclusions:

This project is ongoing.

Publications/Presentations/Grant Submissions:

This project is ongoing.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Efficacy of the topical antihistamine olopatadine hydrochloride in dogs with experimentally induced allergic conjunctivitis
Principal Investigator (PI)	Georgina Newbold, DVM, DACVO
Co-PIs/Co-Is	Esther Mamo
Interim or Final	Interim

Introduction:

Allergies causing itchy, red eyes (allergic conjunctivitis) are common in dogs, but there is little published research assessing antihistamine eye drops in this species. Usually, allergic conjunctivitis is treated with topical steroids, such as prednisolone acetate drops, which can have unwanted side effects. If an over-the-counter topical antihistamine (0.7% olopatadine hydrochloride) proves helpful in dogs, this will provide veterinarians an alternative to steroid eye drops for ocular allergies. This study will compare signs (redness, swelling, discharge and itching of eyes) in dogs receiving antihistamine eye drops compared to those receiving artificial tears when allergy simulant eye drops (histamine) are given.

Specific aims: Goals of the study are to determine if the antihistamine eye drop 0.7% olopatadine hydrochloride is effective in preventing or improving ocular clinical signs of allergic conjunctivitis as induced experimentally by an ophthalmic histamine solution.

Significance: Allergic conjunctivitis is common in dogs, but there is little research assessing topical antihistamines in this species. Commonly allergic conjunctivitis is treated with topical corticosteroids, which can be contraindicated in some patients. If a once a day, over the counter topical antihistamine (0.7% olopatadine hydrochloride) proves effective in dogs, this will provide veterinarians an alternative to prescribing topical corticosteroids.

Approach:

The study will assess clinical signs of allergic conjunctivitis, such as conjunctival hyperemia, chemosis, ocular discharge, and ocular pruritis in 15 healthy dogs receiving a topical antihistamine eye drop (0.7% olopatadine hydrochloride) compared to those receiving a placebo (saline/artificial tears). In a 2-phase study, dogs will receive the antihistamine drops 1 hour after and 1 hour prior to application of ophthalmic histamine solution. These sessions will be separated by a 2- week washout period. A previously published grading rubric and photographs will be used to document clinical signs. Data analysis, including ANOVA, will be used to compare results, and values $P < 0.05$ will be considered statistically significant. Photographs of the eye changes will be taken to help compare the response to this therapy over time. It is expected that olopatadine hydrochloride 0.7% eye drops will reduce the length of time and severity of the clinical signs of allergic conjunctivitis in normal dogs as compared to artificial tears.

Result:

The study is expected to take place in the summer of 2022

Relevance & Impact to Canine Health:

Allergic conjunctivitis is common in dogs, but there is little research assessing topical antihistamines in this species. Commonly allergic conjunctivitis is treated with topical corticosteroids, which can be contraindicated in some patients. If a once a day, over the counter topical antihistamine (0.7% olopatadine hydrochloride) proves effective in dogs, this will provide veterinarians an alternative to prescribing topical corticosteroids.

Conclusions:

This project was recently funded and is in its beginning stage.

Publications/Presentations/Grant Submissions:

This project was recently funded and is in its beginning stage.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Retrospective histologic and immunohistochemical characterization of canine exocrine pancreatic adenocarcinoma and correlation to clinical features and outcome
Principal Investigator (PI)	Megan Schreeg
Co-PIs/Co-Is	Yea Ji Jeong, Laura Selmic
Interim or Final	Interim

Introduction:

Exocrine pancreatic adenocarcinoma (EPAC) is a tumor that has a poor outcome and short survival time in humans and animals. In humans, extensive research has been conducted in an effort to fight this disease, but similar studies have not been performed in dogs. In humans, microscopic characteristics and biomarkers are used to define different subtypes of EPAC, which in turn are associated with improved survival and treatment response. We predict that in dogs, similar markers are present that may aid pet owners and veterinarians in choosing treatment and care options. Currently in animals, up to 6 different subtypes of EPAC have been described: ductal, acinar, hyalinizing, clear cell, mixed exocrine-endocrine, and undifferentiated. Some of these EPAC subtypes have been described in low numbers of dogs. However, there is lack of consensus on defining microscopic features of each subtype in dogs, as well as lack of known association between subtypes and clinical outcomes in affected patients. Collectively, these shortcomings render the diagnostic utility of these subtypes questionable at best. Moreover, diagnostic biomarkers to reliably diagnose these subtypes have not yet been established in dogs. Therefore, the overarching goal of this study is to identify and establish standardized diagnostic criteria for canine EPAC subtypes in order to predict clinical outcome and aid in patient management.

Approach:

We have tumor tissues from 25 dogs diagnosed with EPAC identified from The Ohio State University College of Veterinary Medicine Anatomic Pathology autopsy and surgical biopsy service archives. Additional cases are actively being recruited from collaborators at North Carolina State University College of Veterinary Medicine in order to increase numbers of dogs included. In this study, we will 1) characterize unique microscopic features from these tumors in order to develop guidelines for microscopic subtypes of EPAC; 2) develop novel biomarkers for each subtype in order to further aid in diagnosis and 3) correlate these microscopic subtypes and biomarkers to clinical information (example: breed, age, sex, survival rate) for each dog. We hypothesize that a combination of unique microscopic features and novel biomarkers will allow for identification of distinct subtypes of canine EPAC. In turn, by correlating microscopic and biomarker features with clinical data, we predict that we will identify links between these distinct subtypes and clinical outcomes for canine patients.

Results:

Twenty-five cases of suspected canine EPAC have been identified in the Ohio State University College of Veterinary Medicine Anatomic Pathology service tissue archives. The mean and median age of the cohort is 11 years and includes 13 males and 12 females. The cohort predominantly consists of mixed breed dogs (n=11) followed by Weimaraners (n=2) and boxers (n=2). Based on the original microscopic diagnoses, the tumors were diagnosed as following subtypes: undetermined (n=13), ductal (n=3), hyalinizing (n=3), acinar (n= 2), adenoma (n= 2), acinar to undifferentiated (n= 1) and islet cell carcinoma with amyloidosis (n=1). Out of 25 dogs, 16 had reported regional and/or distant metastasis, with the highest frequency in the liver (11/16). Additionally, 7/25 canine patients exhibited additional pancreatic pathology such as nodular hyperplasia and/or pancreatitis. Intriguingly, 9/25 dogs had at least 1 concurrent neoplasm in other organs.

Relevance & Impact to Canine Health:

Despite the poor clinical outcome, there is a huge knowledge gap on canine EPAC, including diagnosis, clinical signs, and best treatment approaches. Canine EPAC has historically been considered rare, which contributes to this knowledge gap, but anecdotal evidence suggests it may be more common than previously described. Therefore, it is critical to identify diagnostic biomarkers to aid in predicting clinical outcomes and guiding therapy for this devastating disease. While this study will establish these biomarkers by utilizing tissue from previous patients that have since passed away, we predict that information gained in this study will ultimately allow for new opportunities for development of real-time biomarkers that can be used for early detection of EPAC in live canine patients. Furthermore, development of these markers will improve accuracy of diagnosis of canine EPAC, which in turn will support clinical decision-making for both owners and veterinarians.

In addition to the impact this study will have on canine health, this study will also serve as a critical step in career development for Dr. Schreeg and Dr. Jeong, two veterinary pathologists who are passionate about understanding and, in turn, combatting pancreatic disease and cancers in dogs as well as other veterinary patients.

Conclusions:

This project was recently funded and started.

Publications/Presentations/Grant Submissions:

This project was recently funded and started.

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	How low can you go?: Low volume, low biomass urine microbiome sequencing optimization
Principal Investigator (PI)	Vanessa Hale
Co-PIs/Co-Is	Seth Faith, Sheryl Justice, Adam Rudinsky, Jessica Hokamp
Interim or Final	Interim

Introduction:

Studies on the urine microbiome in both dogs and humans have been stymied by technical challenges with capturing and sequencing rare microbes that are often in limited volumes of urine. Additionally, urine can contain host cells and host DNA that interferes with obtaining microbial sequencing results. These challenges limit our ability to study the urine microbiome and its potential role in disease detection, development, progression, prognosis and treatment. Urinary tract diseases are amongst the most commonly diagnosed diseases in veterinary medicine and characterizing the urine microbiome and its role in disease will be critical to advancing clinical care for both dogs and humans.

Approach:

Studies on the urine microbiome have lagged behind studies on the gut microbiome in large part due to technical challenges with extracting and sequencing low biomass substrates like urine. However, urine microbial community studies are critical to furthering our understanding of urinary tract health and disease. The aims below will allow us to optimize and expand urine microbiome studies and facilitate more mechanistic examinations of the urine microbiota in diseases like canine bladder cancer, urolithiasis / stone disease, kidney disease, and urinary tract infection. This work also has high translational potential as human medicine faces similar challenges with urine microbiome studies.

- **Aim 1**: **Determine the lowest volume of healthy canine urine that reliably generates valid shotgun metagenomic sequencing reads.** We will extract 5 different volumes of urine (5ml, 3ml, 1ml, 500ul, 200ul) across 7 different dogs using a single extraction method. We will measure total and bacterial DNA, total number of sequencing reads, proportions of host, microbial, and contaminant reads, and microbial community taxonomic and functional diversity and composition to assess the microbiome results by urine volume.
- **Aim 2**: **Identify an optimal host DNA removal method for shotgun metagenomic sequencing of canine urine by comparing results across 5 methods.** Based on Aim 1, we will select a standard urine volume for all extractions conducted in Aim 2. Urine samples from 7 different dogs will be aliquoted into 5 different host decontamination methods including: Molzym Molysis, NEBNext Microbiome DNA Enrichment, QIAamp DNA Microbiome Kit, Zymo HostZERO, and propidum monoazide.

Results:

All urine samples have been collected and extraction kits for aims 1 and 2 have been purchased. Extractions will begin in May 2022 followed by sequencing and analysis.

Relevance & Impact to Canine Health:

Urinary tract diseases are amongst the most common diagnoses in veterinary medicine and approximately 14% of dogs will present with a urinary tract infections (UTIs) at least once in their lifetime. Human medicine also carries a high burden of urinary tract disease as over 150 million UTIs are reported in humans each year, representing well over 6 billion dollars in health care expenditures. Despite this, there are still many aspects of the urine microbiota that are not well characterized in human or veterinary medicine. The microbiota play a critical role in host health including immune development, and colonization resistance. Moreover, a growing number of studies have identified a role for

microbes in disease processes like cancer, and microbes have been linked directly to tumor development, tumor progression, patient prognosis, and cancer treatment efficacy. Critically, to achieve these mechanistic findings in urine microbiota, we must be able to examine strain level differences and putative microbial functions in health and disease. This level of granularity is essential for future studies evaluating the functional role of microbes in canine bladder cancer, the contribution of urine / bladder microbes to xenobiotic metabolism *in vivo*, antimicrobial resistance in urinary tract infections, or the transmission and exchange of microbial strains within and between the gut and bladder or in relation to probiotic treatments and microbiota transplants. Enabling deeper examination of the dog urine microbiome and putative microbial community function has relevance for dog urogenital health, for dogs as a translational model for human urogenital health, and for specific breeds of dogs that are disproportionately affected by urogenital diseases, such as Scottish Terriers and bladder cancer or miniature schnauzers and urolithiasis / bladder stones.

Conclusions:

This project is ongoing.

Publications/Presentations/Grant Submissions:

- Rushil Madan was awarded:
    - CREATES-Undergrad Fellowship to work on this project from May 2022-August 2022.
- V. Hale submitted and NIH K08 in February 2022 that is relevant to the urine microbiome: Getting into the weeds: Herbicidal compounds as modifiers of microbiomes, metabolomes, and urothelium.
- V. Hale, S. Justice, A. Rudinsky, B. Husbands were awarded funds for a clinical trial involving the canine urine microbiome (January 2022): Can probiotics improve clinical outcomes in bladder cancer?: Addition of probiotic *Escherichia coli Nissle 1917* to a vinblastine / piroxicam protocol for the treatment for urothelial carcinoma in dogs

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Analysis of detection and genotyping of *Giardia*, an elusive zoonotic protozoa.
Principal Investigator (PI)	Marsh, AE
Co-PIs/Co-Is	
Interim or Final	Interim (project start date 2/1/2022)

Introduction:

Giardia is a gastrointestinal (GI) parasite that can cause diarrhea or may be asymptomatic in dogs. It is the second most common GI parasite diagnosed at the Veterinary Medical Center (VMC). There are genetic assemblages of the parasite associated with particularly hosts or found more commonly in humans. However, some *Giardia* do not maintain strict host boundaries, suggesting greater zoonotic potential. Some of these assemblages can be found in dogs, including asymptomatic shedders that can be a source of infection for the owner. In 2019, we found 115 positive *Giardia* shedding dogs. Our project plans to characterize the *Giardia* genotypes isolated from dogs seen at the VMC.

Approach:

Giardia will be identified by centrifugal flotation using fecal flotation solution. Cysts will be concentrated from positive samples. The resulting cyst will undergo DNA extraction followed by genotyping. Analysis will include reviewing the clinical history, time of year, and genotype of the Giardia isolated.

Results (250 word limit):

As this project started in the last few months, we have continued to bank samples, obtaining approximately 5 additional *Giardia* positive dog samples. We are now planning the genotyping analysis the nearly 40 samples.

Relevance & Impact to Canine Health:

We anticipate that genotypes associated with human infections will be detected in dogs. The results will assist in address risk factors associated with dogs shedding *Giardia* and the likelihood of having an assemblage capable of being transmitted to humans.

Conclusions:

As this study just started (2/1/2022), we do not have conclusions to report. We anticipate results will provide a prevalence of the different assemblages for the dog *Giardia* samples analyzed and potential risk to other dogs or humans.

Publications/Presentations/Grant Submissions:

This project is ongoing.

FUNDING OF PROJECTS	
TITLE	**BUDGET**
Perfusion index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs	$11,588
Assessment of regional intestinal perfusion by infrared thermography during foreign body surgery	$ 8,037
Effects of antimicrobial therapy on virulence and antimicrobial resistance of canine EPEC UTIs	$22,600
Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms	$15,255
Use of radiation therapy and conforming intramedullary implant to treat canine appendicular OSA	$22,421
Utility of cardiac MRI to diagnose cardiac fibrosis in dogs with mitral valve disease: a pilot study	$22,727
Molecular and serologic surveys of shelter dogs and their ticks as sentinels for tick-borne disease risk in Ohio	$21,020
Identifying behavior changes in dogs during the six months following adoption from a municipal shelter	$ 9,980
Germ line and somatic genetics of canine soft tissue sarcoma	$22,493
Morphologic, morphometric and functional characterization of degenerative lumboscacral stenosis in Labrador Retrievers	$23,422
Pilot study on the effects of intra-articular allogenic stem cell therapy for the treatment of osteoarthritis	$22,727
Canine glioma as a model for testing MKlp2 inhibition in human glioblastoma	$22,619
Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs	$22,727
Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the canine gut microbiota and bile acid metabolome	$22,633
Alveolar type II (ATII) cell function in dogs with severe acute respiratory distress syndrome (ARDS)	$27,000
A pilot study on the role of *Staphylococcus pseudintermedius* toxins and virulence regulators in canine pyoderma	$26,919
Scientific and clinical assessment of fecal microbiota transplantation to enhance weight loss in obese dogs (SLIM pilot study)	$27,233
Examining urine microbiota, urinalysis, and urine protein over time in healthy dogs	$25,781
Clinical utility of corticosteroids and point of care monitoring in canine acute pancreatitis	$27,273
Understanding and stopping persistent *Ancylostoma caninum* egg shedding in chronic shedders	$27,188
Effect of isotonic versus hypotonic maintenance fluid therapy on urine output, fluid balance, and electrolyte homeostasis in healthy dogs	$23,732
Evaluation of OCT for metastatic lymph node identification in dogs with oral malignant melanoma	$25,358

Evaluation of SpO2 to FiO2 ratio with PaO2 ratio in dogs receiving high flow nasal cannula oxygen therapy for hypoxemic respiratory failure	$23,732
Efficacy of the topical antihistamine olopatadine hydrochloride in dogs with experimentally induced allergic conjunctivitis	$14,410
Retrospective histologic and immunohistochemical characterization of canine exocrine pancreatic adenocarcinoma and correlation to clinical features and outcome	$22,520
How low can you go?: Low volume, low biomass urine microbiome sequencing optimization	$27,271
Analysis of detection and genotyping of Giardia, an elusive zoonotic protozoa	$12,474

APPENDICES

- **Intramural Grant Application Template**
- **County Canine Tag Payments**

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Application Deadline Date

Canine/Equine
Spring ☐
Fall ☐

This is a:
☐ New Proposal
☐ Resubmission

Intramural Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR CFR USE ONLY.

Grant Number		Meets Guidelines ☐
Grant Funded Yes ☐ No ☐		
Score	Range	Date Received

1. TITLE OF PROJECT *(Do not exceed space provided.)*

2a. INDICATE TYPE OF GRANT
Equine ☐ Canine ☐ Paladin ☐ Feline ☐

2b. IS THIS A RESIDENT PROJECT?
YES ☐ NO ☐

3. PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)

3b. DEGREE(S)/BOARD CERTIFICATION

3c. POSITION TITLE

3d. MAILING ADDRESS *(Street, city, state, zip code)*

3e. DEPARTMENT

3f. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: FAX:

3g. E-MAIL ADDRESS:

4. HUMAN SUBJECTS RESEARCH
☐ No ☐ Yes

4b. Human Subjects Assurance No.

5. Is this a Clinical Trial or are client owned animals being utilized?
☐ No ☐ Yes
If yes, requirement for CTO Consultation for Trial Design and Budget Formulation; Signature sign off below

6. VERTEBRATE ANIMALS ☐ No ☐ Yes

4a. Research Exempt
☐ No ☐ Yes

If "Yes," Exemption No.

6a. IACUC Approval and Date

6b. ILACUC Number

7. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		8. COSTS REQUESTED FOR FIRST YEAR	9. COSTS REQUESTED FOR TOTAL PERIOD OF SUPPORT
From	Through	8a. Direct Costs ($)	9a. Direct Costs ($)

10. Checklist:

- ☐ Page 1 (*Form - Cover Page*)
- ☐ Page 2 *(Form – Technical & Lay Abstracts and Personnel)*
- ☐ Pages 3 & 4 (*Budget pages and justification*)
- ☐ Page 5 (*Form - Resources*)
- ☐ Resubmission? Response to Reviewer Criticism (Form Pages-2 page limit)
- ☐ Research Plan (*Sections A through F – 8 page limit)*
- ☐ Letter(s) of Cooperation
- ☐ Curriculum Vitae *(use 5 page NIH biosketch)*
- ☐ Packet contains Original and 3 copies turned into the College Research Office
- ☐ ILACUC approval and BBVCTO approval when applicable
- ☐ Submitted electronic version to Morscher.1@osu.edu

11. CLINICAL TRIALS OFFICE: I certify that the Principle Investigator has met with the Blue Buffalo Clinical Trials Office to discuss the clinical trial work outlined in this grant application and that the proposed trial is feasible and budget for trial work is accurate.	SIGNATURE OF CTO REPRESENTATIVE *(In ink. "per" signature not acceptable.)*	DATE
10. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that if a grant is awarded as a result of this application I will accept responsibility for the scientific and technical conduct of the research project; provide an annual and final report to the College Research Office; present the results of this project at the next College Research Day; submit a grant application based on this work to an extramural funding agency	SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*	DATE
11 DEPARTMENT CHAIR I certify that the Principal Investigator has approval to conduct the research described in this grant, and will be provided with adequate research space. I also agree to monitor expenditures charged against said grant and to cover any overage charged to the grant account.	SIGNATURE OF DEPARTMENT CHAIR. *(In ink. "Per" signature not acceptable)*	DATE

Principal Investigator (Last, First, Middle):

Abstract and Key Personnel

Intramural Grant Application

College of Veterinary Medicine

TECHNICAL ABSTRACT: See instructions. Provide a concise summary of the proposal, including, but not limited to specific aims, methods and procedures, expected outcomes and significance.

DO NOT EXCEED THE SPACE PROVIDED (300 words).

LAY ABSTRACT: See instructions. Provide a summary of the proposal in layman's terms. Do not exceed the space provided. **Limited to 150 words.**

KEY PERSONNEL. See instructions.
Start with Principal Investigator. List all other key personnel in alphabetical order, last name first. Do not include technician or other support personnel. In general, graduate student stipends are not supported without compelling justification (see Budget page and justification)

Name	Department	Time Commitment to Project	Signature

Principal Investigator (Last, First, Middle):

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
Year 1
INTRAMURAL GRANT APPLICATION
COLLEGE OF VETERINARY MEDICINE

FROM	THROUGH

PERSONNEL			% EFFORT ON PROJ.		DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
SUBTOTALS →							

ANIMALS AND PER DIEM (*Provide price justification below*)

EQUIPMENT *(Itemize and provide justification below)*

SUPPLIES *(Itemize by category and show estimated cost for individual items)*

VMC SUPPLIES & SERVICES *(Itemize costs to be charged to the Veterinary Medical Center)*

OTHER EXPENSES *(See instructions; Itemize by category; include services to be purchased)*

COST JUSTIFICATION (*See instructions: where partial support is requested for personnel, please provide source for the remainder of the salary; provide justification for the per cent effort of including graduate students if applicable; justify animal purchase price [conditioned vs unconditioned]; justify equipment purchase if applicable Use continuation pages as needed)*

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	**$**
FACILITIES AND ADMINISTRATIVE COSTS (10%)	
TOTAL COSTS FOR INITIAL BUDGET PERIOD	**$**

Principal Investigator (Last, First, Middle):

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

INTRAMURAL GRANT APPLICATION
COLLEGE OF VETERINARY MEDICINE

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 3)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
		2nd			
PERSONNEL: *Salary and fringe benefits. Applicant organization only*.					
ANIMAL COST and PER DIEM					
EQUIPMENT					
SUPPLIES					
OTHER EXPENSES					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
TOTAL DIRECT COSTS					
F&A (10%)					
TOTAL COST PER YEAR					
TOTAL COSTS FOR ENTIRE PROPOSED PROJECT PERIOD					**$**

JUSTIFICATION. (*justify any significant variation in cost within each budget category over the life of the grant; justify equipment cost that appear beyond the first year*).

Principal Investigator (Last, First, Middle):

RESOURCES

INTRAMURAL GRANT APPLICATION
COLLEGE OF VETERINARY MEDICINE

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Clinical:

Animal:

Computer:

Office:

Other:

MAJOR EQUIPMENT: (*List the most important equipment items already available for this project, noting the location and pertinent capabilities of each).*

I. RESPONSE TO REVIEWER CRITICISMS (*for resubmission only; limited to 2 pages*)

II. RESEARCH PLAN (*limited to 8 pages for sections A through F. Font to be used is Arial 11 point with margins in all directions of at least ½ inch.)*

A. Specific Aims: (*recommended length 0.5 to 1 page*)

B. Significance:(*see instructions; recommended length 2 pages*)

C. Species/Program Relevance: (*recommended length 0.5 page*)

D. Preliminary Data: (*recommended length 1 page)*

E. Experimental Plan: (*recommended length 3-4 pages)*

F. Time Line for Experimental Plan:

G. Literature Cited

III. INVESTIGATOR INFORMATION

A. Plan for Future Support: (*recommended length 0.5 page*)

B. Previous Intramural Funds Record: (*explain how previous intramural funding received in the past five years from any source, has been used to enhance the PI's research program and apply for extramural; include extramural grant application information [title, funding agency, submission date, direct cost], publications, and graduate student thesis arising from these funds)*

C. New Area of Investigation: (*If this grant application is a new area of investigation for the PI, describe how this integrates with other research programs in the College/University and availability of research collaborators with expertise in this area*)

D. Role of Investigators: (*Describe roles of PI and Co-investigators, including descriptions of graduate student roles, the relationship of this proposal to their achieving their degree and time schedules for the graduate student*)

E. Project Integration: (*Describe how this project integrates with and facilitates collaboration among other programs in the College and/or University*)

F. Letters of Cooperation: (*List name(s) of individual(s) providing letters of cooperation; attach letter(s) to the end of the document*)

G. Biosketch Forms: (*Attached biosketch forms for each key personnel; use the **CURRENT** NIH Biosketch format*) NIH Website: https://grants.nih.gov/grants/forms/biosketch.htm

IV. APPENDICES (*List Appendice items [not to exceed 10]; appendices shall be limited to manuscripts accepted for publication or published, data collection forms or statistical calculations in direct support of the grant proposal. Include here ILACUC or HEC approval letter and Owner Consent Form(s). Appendices should be attached to the end of the application after the Biosketch Forms.*

			NUMBER OF EACH TYPE OF TAG SOLD IN 2021				
County	**Invoice**	**Amt Paid $**	**1 - YR**	**3 - YR**	**PERMNT**	**KENNEL REG**	NOTES
Adams County Auditor	1	$720.30	6,570	156	13	35	
Allen County Auditor	1	$1,689.80	15,975	180	37	13	
Ashland County Auditor	1	$973.80	8,308	278	52	76	
Ashtabula County Auditor	1	$1,263.30	9,808	535	93	290	
Athens County Auditor	1	$921.60	9,011	15	7	90	
Auglaize County Auditor	1	$915.90	7,873	312	35	0	
Belmont County Auditor	1	$903.30	7,248	386	53	97	
Brown County Auditor	1	$1,012.90	9,057	253	30	13	
Butler County Auditor 2020 and 2021	1	$6,392.00	57,587	1,052	278	397	
Carroll County Auditor	1	$834.20	7,906	83	17	17	
Champaign County Auditor	1	$801.40	7,321	93	31	104	
Clark County Auditor	1	$2,202.50					
Clermont County Auditor	1	$1,762.20	13,171	832	194	15	
Clinton County Auditor	1	$786.90	6,668	260	36	61	
Columbiana County Auditor	1	$2,266.50	19,296	680	131	15	
Coshocton County Auditor	1	$1,006.40	9,289	16	5	677	
Crawford County Auditor	1	$882.90	7,689	257	36	9	
Cuyahoga County Auditor	1	$8,615.40	56,687	6,474	1,003	15	
Darke County Auditor	1	$1,240.90	11,543	217	17	45	
Defiance County Auditor	1	$760.90	6,463	233	37	77	
Delaware County Auditor	1	$2,049.20					
Erie County Auditor	1	$1,217.50	12,154	2	1	5	
Fairfield County Auditor	1	$2,415.72	21,173	683	86	15	
Fayette County Auditor	1	$413.70	3,600	112	19	11	
Franklin County Auditor	1	$10,181.00					

Fulton County Auditor	1	$808.60	7,047	208	37	45	
Gallia County Auditor	1	$168.50	1,335	10	8	240	
Geauga County Auditor	1	$1,164.50	9,126	545	86	24	
Greene County Auditor	1	$2,606.30	20,505	1,241	180	35	
Guernsey County Auditor	1	$715.90	6,187	226	22	74	
Hamilton County Auditor	1						
Hancock County Auditor	1	$1,293.80	12,125	163	21	114	
Hardin County Auditor	1	$724.00	7,216	1	0	21	
Harrison County Auditor	1	332.5	3105	46	6	22	
Henry County Auditor	1	$632.50	5,615	106	28	112	
Highland County Auditor	1	$552.20	4,648	149	41	17	
Hocking County Auditor	1	$469.00	4,193	89	20	30	
Holmes County Auditor	1	$1,019.60	9,105	15	6	986	
Huron County Auditor	1	$1,088.00	9,750	242	39	14	
Jackson County Auditor	1	$820.40	7,331	170	21	153	
Jefferson County Auditor	1	$411.10	3,375	153	22	57	
Knox County Auditor							
Lake County Auditor	1	$3,085.20	25,681	1,095	181	76	
Lawrence County Auditor	1	$775.20	7,346	91	13	3	
Licking County Auditor							
Logan County Auditor	1	$626.00	5,887	61	18	10	
Lorain County Auditor	1	$2,592.50	23,409	406	115	148	
Lucas County Auditor	1	$4,903.70	41,108	1,984	195	27	
Madison County Auditor	1	$620.10					
Mahoning County Auditor	1	$2,792.10	24,311	832	85	264	
Marion County Auditor	1	$930.50	7,690	299	66	58	
Medina County Auditor	1	$2,455.60	18,802	1,201	170	79	
Meigs County Auditor	1	$453.40	3,103	272	48	135	
Mercer County Auditor	1	$379.60	3,721	8	0	51	
Miami County Auditor	1	$1,895.70	15,064	768	153	59	
Monroe County Auditor	1	$354.90	3,177	76	14	4	

Montgomery County Auditor	1	$5,691.10					
Morgan County Auditor	1	$279.30	2,284	77	10	178	
Morrow County Auditor	1	$589.20	4,985	170	35	47	
Muskingum County Auditor	1	$1,138.60	10,936	19	21	183	
Noble County Auditor	1	$168.60	1,364	48	9	88	
Ottawa County Auditor	1	$788.00	7,058	187	26	1	
Paulding County Auditor	1	$374.40	3,121	128	21	29	
Perry County Auditor							
Pickaway County Auditor	1	$500.50	3,934	183	24	282	
Pike County Auditor							
Portage County Auditor	1	$3,191.80					
Preble County Auditor	1	$147.90					
Putnam County Auditor	1	$676.70	6,487	14	3	208	
Richland County Auditor	1	$1,845.70	17,997	0	0	460	
Ross County Auditor	1	$1,358.10					
Sandusky County Auditor	1	$1,216.90	10,957	243	35	133	
Scioto County Auditor	1	$549.70	4,198	175	29	484	
Seneca County Auditor	1	$992.20	8,896	179	47	19	
Shelby County Auditor	1	$869.90	7,432	212	54	91	
Stark County Auditor							
Summit County Auditor							
Trumbull County Auditor	1	$1,768.80	15,127	566	82	43	
Tuscarawas County Auditor	1	$1,615.80	14,741	268	55	63	
Union County Auditor	1	$778.20					
Van Wert County Auditor	1	$448.60	3,959	109	15	50	
Vinton County Auditor	1	$312.50	2,941	5	4	129	includes 2020 tags
Warren County Auditor	1	$2,963.40	21,755	1,849	231	22	
Washington County Auditor	1	$1,117.00	9,180	435	66	25	
Wayne County Auditor	1	$1,826.00					
Williams County Auditor	1	$503.50					
Wood County Auditor	1	$2,339.60	16,844	1,265	268	77	

Wyandot County Auditor	1	$444.50	3,575	233	12	51	
	Total:	**$121,398.12**	**782,130**	**29,931**	**4,853**	**7,598**	
****NOTES:**							
Invoice 1 sent out							
Payment still not received as of 4/25/2022							
Breakdowns of type of tags sold not provided as requested							