THE OHIO STATE UNIVERSITY

College of Veterinary Medicine

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30 April 2021

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 4 final and 23 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
2020

Submitted to:
The Ohio General Assembly

May 2021

## 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.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Analgesic effects and tolerability of tapentadol in combination with NSAIDs in dogs with appendicular osteosarcoma |
| **Principal Investigator (PI)** | Dr. Megan Brown, DVM, MS, DACVIM (Oncology) |
| **Co-PIs/Co-Is** | Dr. Shannon Kenny, DVM<br>Dr. Turi Aarnes, DVM, MS, DACVAA<br>Dr. Nina Kieves, DVM, DACVS, DACVSMR, CCRT |
| **Interim or Final** | Final |

**Introduction**:
Osteosarcoma is a common bone tumor in dogs that causes pain and diminished quality of life. Oral pain medication options for affected patients are limited, with non-steroidal anti-inflammatory drugs (NSAIDs) being the mainstay of treatment. Tramadol, an opioid-like pain medication, is also used; however, growing evidence suggests that tramadol may not produce reliable analgesia in dogs due to minimal production of the active metabolite. In contrast, analgesic effects of tapentadol, a novel μ opoid receptor agonist, are mediated by the parent compound and a recent canine study demonstrated rapid oral absorption and good tolerance in healthy dogs. In addition, tapentadol demonstrated pain relief properties in healthy dogs in an experimental pain model but has not been evaluated in a spontaneous canine model of pain. The aim of this study is to assess the analgesic effects and tolerability of tapentadol in conjunction with NSAIDs in dogs with appendicular osteosarcoma.

**Approach:**
Dogs (n=25) with suspected osteosarcoma affecting a limb are eligible to enroll. At the time of screening, owners will complete a baseline pain assessment survey. On day 1, dogs will undergo a standardized pain assessment by a veterinarian and use of the affected limb (peak vertical force) will be objectively assessed via a pressure sensitive walkway evaluation. Patients will be sent home on tapentadol and an NSAID. Owners will also be given a daily drug log to document medication administration and adverse events associated with tapentadol treatment. Patients will have a repeat veterinarian and owner pain assessments and pressure sensitive walkway evaluation and on Day 5, at which point the study is completed.

We hypothesized that tapentadol, in conjunction with NSAIDs, will provide pain relief and be well tolerated in dogs with osteosarcoma of the limb. To compare the pain relief benefits of tapentadol therapy, owner and veterinarian pain scores and peak vertical force will be compared before and after treatment.

**Results**:
The trial opened in November of 2019. Since that time, five patients have been enrolled and completed the study. An additional five patients were screened, but they were deemed ineligible for enrollment (failed enrollment criteria). Unfortunately, as the result of COVID 19 pandemic and the pause on clinical trials at OSU CVM, we were not able to recruit statistically significant number of patients for this study.

**Relevance & Impact to Canine Health**:
Cancer is now the leading cause of death in older dogs, with approximately half of dogs that live to 10 years of age or older dying of cancer. Cancer associated pain can significantly impact the quality of life in cancer patients, either due to pain caused by the tumor itself, pain caused by treatment (surgery, chemotherapy and/or radiation therapy) or pain associated with non-cancerous comorbidities (e.g. osteoarthritis). Unfortunately, suboptimal treatment of cancer pain in small animals likely occurs due to a lack of published, scientifically rigorous studies on the subject, leading to continued challenges in assessing pain and determining optimal treatment plans for veterinary patients.

**Conclusions**:
Unfortunately, due to COVID 19 and the pause on clinical trial recruitment for many months and the departure of the PI, Dr. Brown (April 23,2021), this project will not be completed. The majority of the project funds will be returned to the general canine fund and be available for new project competition.

**Publications/Presentations/Grant Submissions**:
None to be submitted.

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Characterizing the microbiome in dogs with and without bladder cancer |
| **Principal Investigator (PI)** | Vanessa Hale, MAT, DVM, PhD |
| **Co-PIs/Co-Is** | William Kisseberth, DVM, Ohio State University<br>Deborah Knapp, MS, DVM Purdue University<br>Morgan Evans, PhD, Ohio State University |

**Introduction** :

Urothelial carcinoma, also known as transitional cell carcinoma (TCC) is the most common bladder cancer in dogs and is associated with environmental exposures such as tobacco smoke and pesticides. However, the mechanism underlying this association is unknown. Hereditary genes have been linked to TCC, but most bladder cancers are sporadic. One area that has not been examined in dogs with TCC is the gut and urinary microbiome. The microbiome, a collection of bacteria, viruses, and fungi that live within and on us, plays a critical role in host health and in metabolizing compounds from the environment – including tobacco smoke and pesticides. The microbiome can also promote the development of cancers including stomach, cervical, and colorectal cancer. This study aims to characterize the stool and urinary microbial communities in healthy dogs and dogs with TCC in order to understand if microbial communities may be interacting with chemicals found in tobacco smoke and pesticides in dogs with TCC.

**Approach**:

Our approach is to use 16S rRNA amplicon sequencing to characterize the urine and stool microbiota of dogs with and without bladder cancer. Urine microbial communities represent microbes that have direct proximity to the bladder and may be interacting with host cells or producing metabolites that interact with host cells including promoting tumor growth in bladder cancer. Our first goal is to determine what types of microbes are present in the urine of healthy dogs and those with bladder cancer. We are also sequencing stool samples because the gut microbiota represents a reservoir for urinary tract microbes. Finally, we will deep sequence (using shotgun metagenomic sequencing) a subset of urine and stool samples in order to compare microbial strains between urine and stool and to compare microbial gene abundances in individuals with and without bladder cancer. Specifically, we will be looking for genes that degrade the chemicals (polycyclic aromatic hydrocarbons) found in tobacco smoke and herbicides to determine if these microbes may be interacting with chemical associate with bladder cancer risk.

**Results**:

We have completed the sample collection and benchtop portions of our two-year study. All urine and stool samples have undergone 16S rRNA sequencing and a subset were selected and sequenced via whole shotgun metagenomics. Analysis is ongoing. We exceeded our projected sample accrual (n=35 samples from dogs with bladder cancer and 35 samples from dogs without bladder cancer) and were able to sequence (16S rRNA – short read sequencing) samples from a total of 59 dogs with and 56 without bladder cancer. We will analyze these results to characterize the urine and stool microbial communities in dogs with and without bladder cancer. We will analyze the shotgun metagenomic sequencing (deeper sequencing) deeper sequencing to compare all of the microbial genes present in dogs with and without bladder cancer to determine if the microbial communities in dogs with bladder cancer have an increased potential to metabolize chemicals like tobacco smoke or pesticides. Our shotgun metagenomic sequencing was performed in collaboration with Nationwide Children's Hospital (NCH)Institute for Genomic Medicine. These samples provided the basis for protocol development on low biomass DNA extraction (Hale lab) and library preparation (NCH). Urine has few cells, and thus a low concentration of DNA. Standard protocols for extracting DNA from urine may cause excess DNA loss. Specialized protocols were developed to prepare samples with low DNA concentrations for metagenomic sequencing. These protocols can potentially be applied to other types of low biomass samples (e.g. bile, cerebrospinal fluid) in animals or in humans.

**Relevance & Impact to Canine Health**:
Over 40,000 cases of canine bladder cancer are diagnosed each year, and there are some breeds, including Scottish terriers, Shetland sheepdogs, West Highland white terriers, beagles, and wire hair fox terriers that exhibit a much higher risk of developing bladder cancer. Elucidating factors that contribute to the development, progression, mitigation, or prevention of bladder cancer is especially important for these breeds. Additionally, canine and human bladder cancers are quite similar in histological appearance and behavior; both maintain a sex predilection (humans: males; canines: females); and the BRAF (V600E) mutation present in 85% of canine bladder cancers and is also common in many human cancers. Dogs are considered a relevant and valuable model for human bladder cancer. As such, our study is well aligned with the purpose of the OSU CVM canine funding in that, it is "for the research and study of diseases of dogs" and for research that will "provide information applicable to the prevention and treatment of both human and canine illnesses."

**Conclusions**:
**Healthy dog urine data**: We compared DNA concentrations and microbial community sequencing data from the urine of 10 healthy dogs extracted using 5 different DNA isolation methods. Each method employed various mechanical, chemical, and thermal lysing techniques. Sex and dog, but not extraction method, significantly affected DNA concentrations and microbial diversity and composition. The Qiagen Bacteremia kit yielded the highest total and bacterial DNA concentrations, the greatest number of sequencing reads, and it extracted bacterial DNA from the greatest number of samples. Moreover, microbial diversity and composition did not significantly differ by kit indicating that no method dramatically biased the sequencing results. As such, Bacteremia was determined to be the most effective method for studies of the urine microbiota. For all subsequent analyses, we employed the Bacteremia kit for urine DNA extractions.

**Preliminary analyses of bladder cancer urine data**: First, we evaluated the urine microbiota of dogs with and without bladder cancer. Then, in dogs with bladder cancer, we compared the microbial communities of dogs that did or did not have a history of chemotherapy, and we compared urine collected via free-catch or non-free-catch methods. Dogs with bladder cancer had significantly higher urine DNA concentrations and altered urine microbial composition compared to healthy dogs. Differences in microbial composition but not diversity were observed in dogs with a history of chemotherapy and by collection method. Future work is warranted to assess the role of microbes in cancer development, progression, and treatment. Shotgun analyses are in process.

**Publications/Presentations/Grant Submissions**:
Our work this far has resulted in the following:

- **Publications**
  - R. Mrofchak[#], C. Madden[#], M.V. Evans[#], **V.L. Hale**. Evaluating extraction methods to study canine urine microbiota. *PLOS One.* January 2021. *In revision.*
- **Grant submissions**
  - AKC Canine Health Foundation Oncology Grant. *Examining chemical-microbe-host interactions in canine bladder cancer.* Submitted August 2020. PI: Vanessa Hale (Not funded)
  - **FUNDED**: CCTS Voucher Grant for Metabolomics. *Examining polycyclic aromatic hydrocarbon associated metabolites in urine and stool. Submitted May 2020.*
  - Morris Animal Foundation Established Investigator Canine Proposal. *Investigating the canine bladder cancer microbiome and metabolome in relation to polycyclic aromatic hydocarbons.* Submitted March 2020. PI: Vanessa Hale (Not funded)
  - National Institutes of Health R21. *Examining microbial-derived polycyclic aromatic hydrocarbon metabolism in bladder cancer.* Submitted April 2020. PI: Vanessa Hale (Not funded)
- **Presentations**
  - Accepted: R. Mrofchak, C. Madden, M.V. Evans, V.L. Hale. June 2021. E*valuating extraction methods to study canine urine microbiota.* World Microbe Forum.
  - Ryan Mrofchak's Master's Defense: *An Analysis of Canine Urine: Microbiota, Methods, and Changes in Health and Disease.* April 2021.
  - Poster: R. Mrofchak, C. Madden, M.V. Evans, V.L. Hale. April 2021. Evaluating extraction methods to study canine urine microbiota. OSU College of Veterinary Medicine Research Day.

- Poster – **2nd Place Poster Presentation Award**: R. Mrofchak, C. Madden, V.L. Hale. October 2020. *Evaluating Extraction Methods to Study the Urine Microbiome.* Global One Health Day Virtual Conference.
- V.L. Hale**.** *Canine bladder cancer urine and stool microbiome.* August 2020. Bladder Cancer Research Working Group Monthly Seminar. Ohio State University.
- Poster: V.L. Hale, R. Mrofchak, M.V. Evans, C. Madden, D. Dhawan, N. Chia, D.W. Knapp. June 2020. *Urine and Stool Microbiota in Dogs with Bladder Cancer*. ASM Microbe, Chicago, IL. *Cancelled due to COVID-19*.

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# PROGESS REPORT (lay report) to the Ohio General Assembly

| | |
|---|---|
| **Title** | Effects of gabapentin and trazodone on electroretinograms recorded in normal dogs |
| **Principal Investigator PI)** | Georgina Newbold, DVM, DACVO |
| **Co-PIs/Co-Is** | Nathaniel P. Violette, DVM; Chiaming Chen; Eric J. Miller, DVM, MS, DACVO; Anne J. Gemensky-Metzler, DVM, MS, DACVO |
| **Interim or Final** | Final |

**Introduction**:
Electroretinography (ERG) is a non-invasive eye test that records the electrical response, called "a-waves" and "b-waves", generated by retinal cells (e.g. rods, cones) and other supporting cells when stimulated by light. This clinical test is used to evaluate for the presence of retinal function prior to cataract surgery and also distinguishes between different types of vision loss. In anxious canine patients, mild sedation may be helpful in facilitating collection of ERG readings. The objective of this study is to compare ERG responses obtained in normal dogs both before and after administration of different doses of the oral sedative agents gabapentin and trazodone, and also following a combination of both medications.

**Approach**:
Twelve normal dogs recruited from the community were examined by a veterinary ophthalmologist to determine eligibility for study inclusion. A complete eye exam was performed for each dog. On week one of the study, a baseline short protocol mixed rod-cone response ERG with 20 minutes of dark adaption was performed, followed by oral study medication administration. A repeat ERG protocol was recorded 2 hours later. Following a 1-week washout period, the same procedure was repeated with the next oral study medication or combination of medications as outlined below. Statistical analyses were performed to determine significant variance from baseline ERG following oral sedative medications.

Week 1: 30 mg/kg of gabapentin PO
Week 2: 20 mg/kg of trazodone PO
Week 3: 5 mg/kg of trazodone PO
Week 4: 20 mg/kg of gabapentin and 5 mg/kg of trazodone PO

**Results**:
ERG tests following higher doses of trazadone (20mg/kg) and a combination of gabapentin and trazadone together, showed a significant reduction in "a-waves" and "b-waves" as compared to baseline in normal dogs. Low dose trazadone and gabapentin alone did not show a significant change in ERG readings.

**Relevance & Impact to Canine Health**:
If oral sedation can help to reduce canine anxiety to facilitate ERG recordings without causing a clinically significant change in the test results, these medications may be helpful to veterinary ophthalmologists that routinely perform this test.

**Conclusions**:
Higher doses of trazodone and the combination of gabapentin and trazodone statistically decreased the amplitudes of both "a-waves" and "b-waves" in normal dogs; however, although statistical significance was found, these observed effects have little clinical significance. No normal dog was found to have clinically abnormal ERG readings following oral sedation. The oral medications gabapentin and trazodone can be useful in the clinical setting to treat hospital anxiety in dogs before performing ERG testing.

**Publications/Presentations/Grant Submissions**:
Study abstract presentation at the OSU College of Veterinary Medicine Research Day (Clinical Research Category) April 2021. Manuscript in preparation for publication submission late 2021.

# PROGESS REPORT (lay report) to the Ohio General Assembly

| | |
|---|---|
| **Title** | Efficacy of a commercially available LH surge detection strip in the bitch |
| **Principal Investigator (PI)** | Erin Runcan |
| **Co-PIs/Co-Is** | Marco Coutinho da Silva |
| **Interim or Final** | Final |

**Introduction**:
Determining the ideal time for breeding is important to dog breeders when a male is unavailable, or in cases of infertility or limited semen. Many methods exist to determine the best day for breeding to maximize pregnancy, but these tests are not always accurate. Blood hormone testing can be performed to estimate ovulation, but requires multiple veterinary visits. Because of these limitations, various at-home tests are available to help predict the day of ovulation, however very few are actually validated for use. One such test, an "LH surge test strip," claims to change color when exposed to vaginal fluid at the time of the hormonal surge of luteinizing hormone (LH) which triggers ovulation. The time of the LH surge has been proven to be excellent in predicting ovulation and the optimum time for breeding. Determining the date of the LH surge using commercial testing is currently the gold standard used by veterinarians and breeders to choose the best day for breeding for maximal pregnancy rates in the bitch. Our research will determine if these strip tests are accurate by comparing them to validated hormonal blood tests.

**Approach**:
Client-owned bitches (n=10) were brought in five days after the onset of heat. Blood was be drawn daily and serum saved for hormonal testing. At each daily visit, a "LH surge test strip" was exposed to the vaginal fluid and evaluated for color change. Per the manufacturer's recommendation, any degree of color change (purple spots, bands, or complete color change) should be regarded as "positive" with no color change indicating a "negative" sample. A glucose reading of vaginal fluid will also be made. Once ovulation was detected via serum hormone testing, luteinizing hormone (LH) testing was also performed on stored serum. LH levels were determined using a commercial cage-side assay as well as Colorado State University's Veterinary Endocrinology Laboratory to determine quantitative values. The sensitivity, specificity and accuracy of the LH test strips will be determined against the true serum levels of LH.

**Results**:
The acquisition of study data is complete with final results pending statistical analysis. There appeared to be no correlation between LH strip color change (a "positive" sample) and the actual LH surge as determined by known testing methods. Interestingly, most vaginal fluid samples were unable to be read by a traditional glucometer designed to assess glucose levels in the blood. Progesterone levels correlated well with the LH surge, and the positive LH surge as detected by the cage-side assay had excellent correlation with true serum levels of LH.

**Relevance & Impact to Canine Health**:
We were hopeful that usage of this method of testing would provide an inexpensive way for breeders to determine the optimum time for breeding. This could have greatly decreased the need for multiple costly veterinary visits and the stress of handling and daily blood draws for the bitch. However, since the LH surge test strip had very poor correlation with true onset of the LH surge, we cannot recommend its use to replace currently available testing methods.

**Conclusions**:
Unfortunately, due to the inaccuracy of the LH surge test trip, it is not recommended that breeders use this approach in lieu of traditional testing methods available for accurate timing of breeding the bitch. While inexpensive, the unreliability of this testing method cannot replace serial blood draws for hormonal testing by a veterinarian. Final statistical analysis is pending, but will be supportive of the subjective findings of this study.

**Publications/Presentations/Grant Submissions**:
**Morelli, N. "**Efficacy of a commercially available LH surge detection strip in the bitch." Undergraduate Student Honors Research Thesis. The Ohio State University College of Food, Agricultural, and Environmental Science. 4/24/2020.

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| 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** | Interim |

**Introduction**:
Perfusion Index (PI) monitoring is a cutting edge technology used to determine vascular tone. In humans, PI increases after the vasodilation that occurs following epidural injection of local anesthetics. The objective of this study is to evaluate PI as a non-invasive method to determine epidural anesthesia onset and effectiveness in dogs. PI will be compared to the clinical gold standard used to evaluate epidural anesthesia in dogs under general anesthesia (hemodynamic responses after painful stimulation).

**Approach**:
Twenty-one dogs were used in a prospective, blinded, complete randomized design. Dogs were be anesthetized once using a standardized protocol. An epidural injection was performed using sterile technique. After baseline data collection, dogs were randomly assigned to two groups: morphine 0.05% at 0.2 mL/kg (0.1 mg/kg) [control group, n=6], and lidocaine 2% at 4 mg/kg (0.2 mL/kg) plus morphine 1% at 0.01 mL/kg (0.1 mg/kg) [test group, n=15) to be given epidurally. Data were collected before epidural injection and every 5 minutes thereafter for 30 minutes and will included PI, heart rate, and arterial blood pressure. Data were also recorded during surgery, and if heart rate and blood pressure increased after skin or bone incision, fentanyl was administered for additional analgesia.

**Results**:
There was no significant difference in PI between the control and treatment group at any time point following epidural injection prior to surgical stimulation. There was also no significant difference in PI between the two groups following skin incision or osteotomy.

**Relevance & Impact to Canine Health**:
Unfortunately, PI did not prove to be a reliable tool in detecting effective epidural anesthesia in dogs under general anesthesia. Several factors could have impacted the results, most notably, the fact that the patients were under isoflurane general anesthesia and it could have precluded further detection of regional vasodilation by the perfusion index monitor.

**Conclusions**:
Perfusion index did not provide an objective means for determining the onset or effectiveness of epidural anesthesia in anesthetized dogs and alternate methods of non-invasive assessment should be investigated.

**Publications/Presentations/Grant Submissions**:

- Manuscript submitted to the Veterinary Anaesthesia and Analgesia journal for review on March 3, 2021, it is still under review.
- A research abstract was submitted to the 2021 American College of Veterinary Anesthesia and Analgesia Annual Meeting for oral presentation and is under review.
- This research project was the Master's Thesis for Dr. Crystal Doyle. She defended the thesis on March 5, 2021 and is on track to graduate on Spring 2021.

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| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Pulse oximetry pleth variability index as a predictor of fluid responsiveness in dogs |
| **Principal Investigator (PI)** | Carolina H Ricco Pereira |
| **Co-PIs/Co-Is** | Natalia Henao-Guerrero, Turi Aarnes, Phillip Lerche, Richard Bednarski |
| **Interim or Final** | Interim |

**Introduction**:
Low blood pressure is very frequent during general anesthesia. One of the strategies to treat low blood pressure is to administer intravenous fluids. However, this treatment is not always effective. Pleth variability index (PVI) is new parameter that can be used to predict a patient's responsiveness to fluid administration in mechanically ventilated animals and guide fluid therapy in these patients. The objective is to determine the PVI value that will discriminate the patients who could benefit from intravenous fluids from the ones who need other therapies.

**Approach**:
With the dog under anesthesia a bolus of intravenous fluids will be administered. Before and after the fluids are administered several cardiovascular parameters will be measured, including cardiac output and PVI. This study will help veterinarians identify the patients who should be treated with intravenous fluids and the ones who should not.

**Results**:
Data collection will be concluded in 2021

**Relevance & Impact to Canine Health**:
We expect this study to determine the PVI value that discriminate responders from non-responders to a fluid challenge in mechanically ventilated healthy dogs.

This study will provide veterinarians a tool to quickly identify patients who need and the ones who don't need fluid replacement and volume expansion under anesthesia.

**Conclusions**:
Data collection not completed yet.

**Publications/Presentations/Grant Submissions**:
Data collection not completed yet.

| 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 96 dogs 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 96 STS tumors from Labrador and Golden Retrievers and from a mixture of other breeds. 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. Although additional samples were identified and requested from the Canine Comparative Oncology Genomics Consortium (CCOGC), they are not releasing specimens to investigators for an indeterminate period of time, thus we are proceeding without these samples. However, we have identified a sample set of DNA samples from retriever dogs at Cornell University that may be suitable control for a genome-wide association study (GWAS). 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).

**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**:
This study is still ongoing.

| 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 in both clinically affected and clinically normal dogs using conventional and a novel kinematic magnetic resonance imaging technique, as well as a functional assessment using electromyography and magnetic motor evoked potentials. We aim to identify the anatomic and functional features that cause clinical disease in Labrador retrievers, and to expand this knowledge to all canine breeds affected.

**Approach**:
Thirty Labrador Retrievers will be studied, 15 clinically affected and 15 with no signs of DLSS. The inclusion criteria for DLSS-affected Labrador Retrievers 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 Labrador Retrievers 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 Labrador Retrievers will undergo electrodiagnostics, 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 four affected and four 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. When performing electrodiagnosics, we expect the evoked MEP in the semimembranosis/semitendinosis muscles to not be significantly different between groups. We expect a significant delay in MEP latency in the cranial tibial and coccygeus muscles in the affected group compared to non-affected group.

**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, CT and electrodiagnostics. 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 (lay 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. Tencate, Dr. Yaxley, Dr. Mcloughlin, Dr Guillaumin |
| **Interim or Final** | Interim |

**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 enrollment has been completed. Data and statistical analysis are underway.

**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 proposes 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. If shown to be reliable, this technique could provide objective, real-time information about local perfusion and tissue viability. Thermal images are 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.

**Conclusions**:
Conclusions are not available at this time as data analysis is underway.

**Publications/Presentations/Grant Submissions**:
Manuscript preparation has been initiated and will be completed once the data has been finalized. Expected submission Fall 2021.

| PROGESS REPORT (lay 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. At the time of surgery, dogs will be randomly assigned to one of two groups using a computer-generated randomization program. **Group 1** will receive an intra-articular injection of allogeneic stem cells suspended in autologous serum at the two-week post-operative exam, while **Group 2** 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 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, no results are available at this time.

**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**:
This project is ongoing.

**Publications/Presentations/Grant Submissions**:
This project is ongoing.

| 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** | Interim |

**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 (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 collaborator Dr. Tim Bentley (University of Purdue). The first sample was received and injected into two mice mid-February 2020. Unfortunately, these cancer cells never grew in the mice and the mice were humanely euthanized after one year. However, we very recently (April 7th, 2021) received a second sample from Dr. Bentley that contained enough tissue to inject 5 different mice at two different locations (brain and flank) using varying cell numbers. We are hopeful these cancer cells will engraft and we will monitor the growth of the cancer cells injected into the brain monthly with MRI imaging. In addition, we have secured ten high grade canine glioma samples from additional collaborators, Dr. Liz Pluhar and Dr. Mike Olin (The University of Minnesota) which we plan to engraft by the beginning of June. According to published reports, approximately 30-50% of human glioma cells engraft into mice and we expect a similar take rate with the canine cells.

**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**:
Due to the COVID-19 pandemic, we were unable to receive glioma samples from our collaborators until early 2021 which set our work back nearly one year. However, since the beginning of this year, we have received ten frozen glioma samples from University of Minnesota, which we plan to inject in June 2021. We have also received one glioma sample from University of Purdue that we are currently monitoring for engraftment. We monitor growth of the cancer cells injected into the brain with MRI imaging every month, while cancer cells placed in the flank of the mice can be monitored by visually inspecting the area for growth. Because approximately 30-50% of human glioma cells engraft into mice, we expect a similar take rate for our canine cells and we will monitor growth for up to 9 months.

**Publications/Presentations/Grant Submissions**:
Due to our COVID-19 related delays in creating the cancer lines, we have not yet presented this work at a conference or published any results. However, we do plan to present our findings at OSU's College of Veterinary Medicine Research Day (2022) and the Veterinary Cancer Society Annual Conference (2021) once we have been able to inject all of our glioma samples. We plan to publish our findings in the journal Veterinary and Comparative Oncology to report on our techniques and engraftment rates.

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Optical Coherence Tomography for Margin Evaluation of Canine Skin and Subcutaneous Neoplasms |
| **Principal Investigator (PI)** | Laura E. Selmic |
| **Co-PIs/Co-Is** | Ryan Jennings |
| **Interim or Final** | Interim |

**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 will focus 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**:
The *purpose of this study* is to evaluate the diagnostic accuracy and clinical utility of optical coherence tomography (OCT) imaging for assessment of surgical margins for resected canine cutaneous and subcutaneous neoplasia. The *central hypotheses* of this study are that OCT imaging **will have a high sensitivity for detection of incomplete margins** for canine cutaneous and subcutaneous tumors, and that OCT will be superior to standard pathology: detecting a greater number of dogs with incomplete margins. We further hypothesized that dogs with incomplete resection on OCT imaging will have a higher rate of tumor recurrence on follow-up. Our hypotheses are based on the high reported sensitivity of OCT for detection of residual human breast cancer, preliminary data using OCT for surgical margin assessment in canine and feline tumors and assessment of comparative pathology in these studies. We plan to test these hypotheses with the following specific aims:
Aim 1: Compare normal and abnormal histological features with OCT images for surgical margins from resected cutaneous and subcutaneous tumor specimens in dog. These OCT images and corresponding correlations with histopathology will form a training set for observers.

Aim 2a: Determine the diagnostic accuracy of OCT for assessment of surgical margins for resected canine cutaneous and subcutaneous tumors.
Aim 2b: Determine the frequency that OCT and standard surgical pathology assessment detect incomplete margins for the same canine cutaneous and subcutaneous tumors.

**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 have been 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.*

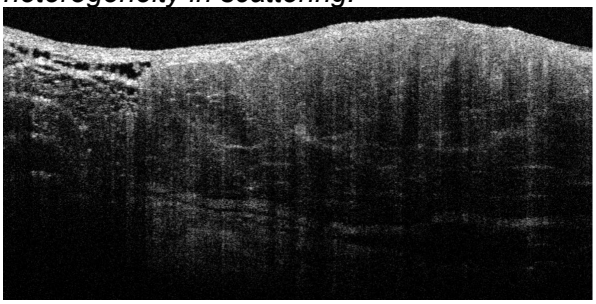
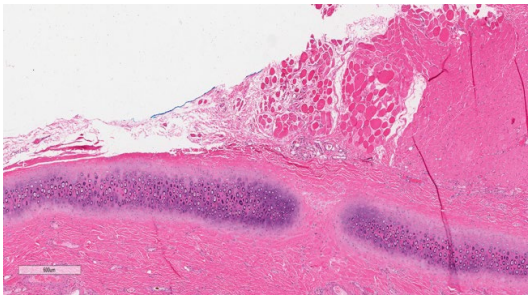
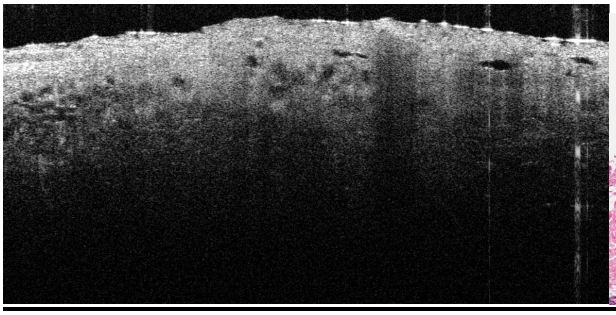
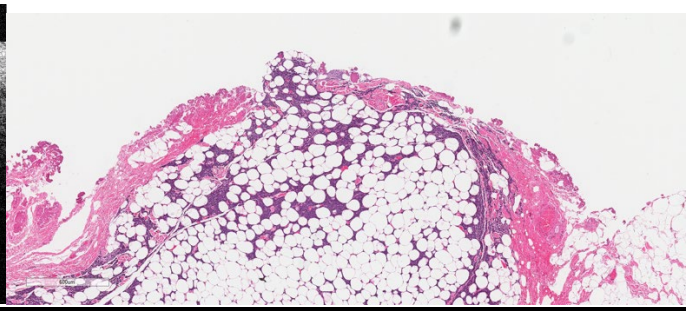





***Figure 2:*** *Appearance of a fibroma with narrow excision with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the fibroma are heterogenous with generally high scattering.*

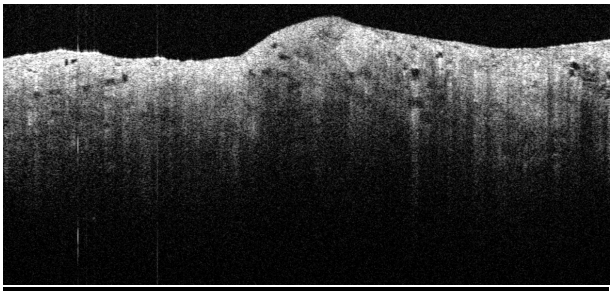
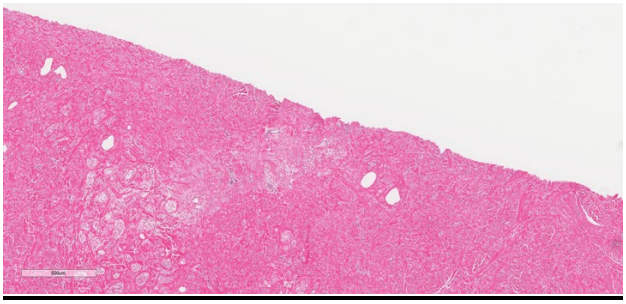



***Figure 3:*** *Appearance of normal tissues of fascia and fat with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the fascia are heterogenous high scattering thin layer of tissue with underlying honey comb structure showing mature adipocyte cells.*

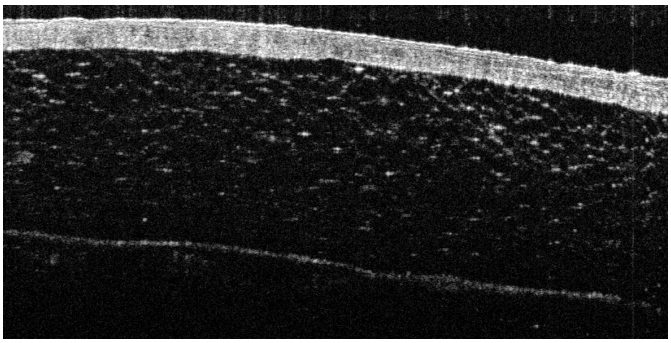
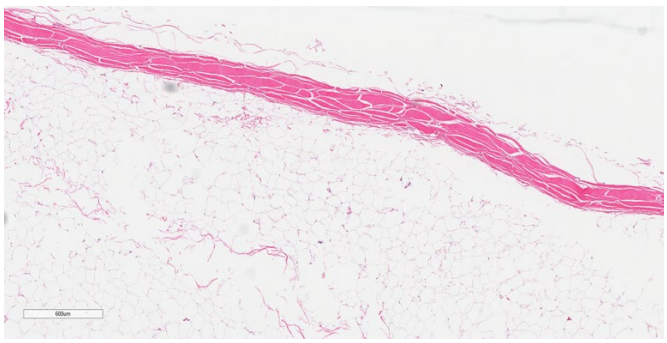
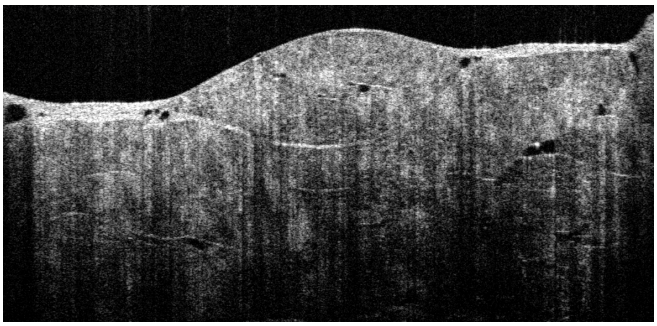
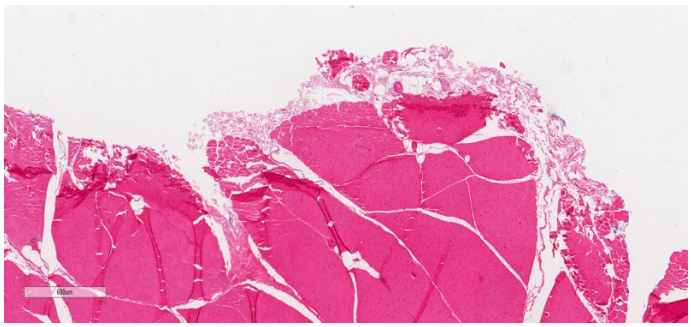



***Figure 4:*** *Appearance of normal skeletal muscle with OCT (on the left-hand side) and histopathology (on the right-hand side). The optical characteristics of the muscle are heterogenous high scattering tissue with linear white lines representing fascia surrounding muscle bundles.*

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 obtained additional funding and have completed case accrual of the 70 cases for aim 2, we have created the training and datasets needed for the observer assessment phase of the study. The observers will receive the training within the next two weeks.

**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,[11-16] with 20-40% of these resulting from malignant skin lesions.[12,13] Skin cancer is also one of the most common forms of cancer in the US in humans.[17] In dogs and humans 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 and people 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**:

This project is investigating an emerging diagnostic imaging tool, optical coherence tomography that uses light waves to generate real time high-resolution images of tissues for detection of residual cancer cells immediately following surgical removal. Our team involves collaboration between veterinary medicine and pathology at the Ohio State University. We have had excellent progress in our patient enrollment, we completed enrollment of the 80 cases planned already! We completed the first phase of this project where we are looking at imaging skin tumors after surgical removal in dogs. In this first phase we performed initial comparisons between the images from optical coherence tomography with biopsy slides of these areas. This phase allowed us to identify features of the tissues and helped to train our imaging operators for the second phase of the project. To date, our assessments have been encouraging and we are seeing correlation between optical coherence tomography imaging features seen and biopsy results in the second phase of our study. We will provide training and test sets of images to observers in the next two weeks. In this next phase of the project observers will look at these images for evidence of residual cancer and allow us to assess the accuracy of the technique.

**Publications/Presentations/Grant Submissions**:

No publications have been submitted. An abstract submission for the American College of Veterinary Surgery Meeting in October 2021 is planned.

A grant was submitted to AKC and funding was obtained for $43,443 to increase the scope of this work and allow accrual of more cases and obtain long term follow up on cases.

# PROGESS REPORT (lay report) to the Ohio General Assembly

| | |
|---|---|
| **Title** | Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs |
| **Principal Investigator (PI)** | Selena Tinga |
| **Co-PIs/Co-Is** | Morgan Biggo, Turi Aarnes, Stephen Jones, Nina Kieves, Phillip Lerche, Carolina Ricco Pereira, Audrey Wanstrath |
| **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**:
The study's IACUC was approved >1 year ago, but case enrollment has been challenged by COVID19 restrictions. The appointment caseload at the OSU campus location remains restricted, meaning that we are seeing fewer chronic/elective conditions such as cranial cruciate ligament degeneration. We are still seeing a high caseload of this condition at the Dublin location but this facility does not have the equipment necessary to perform the research. We have, however, been able to enroll cases from Dublin that were unable to schedule the procedure at Dublin for a variety of reasons and have been successful at capturing 1 or 2 cases some weeks, in this way. We have approximately 10% of cases either completed or enrolled, and if we are able to continue in this manner we hope to have data collection completed within approximately 1 year from now. As the study is blinded, we do not have any preliminary results to report.

**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** | Use of radiation therapy and conforming intrameduliary 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** | Interim |

**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. Two patients experienced catastrophic fracture around the implant. One 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 will continue to be routinely restaged with thoracic imaging.

**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**:
No conclusions have yet been drawn. Final data analysis is pending the long term follow up of our one remaining case as well as further histopathologic evaluation of the bone and implant samples.

**Publications/Presentations/Grant Submissions**:
No publications have been submitted.

| 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<br>Adam Rudinsky<br>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 are conducting a clinical trial in client-owned healthy dogs administered Ursodiol for 21 days at a clinically relevant dose. Freshly voided feces will be 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 timeline for this study was delayed.
As our university slowly eases restrictions, we were able to start recruiting patients. To date, we have 10 patients that have fully completed the trial and 2 patients that will finish in the next 6 weeks. This study is still ongoing and actively recruiting normal healthy canine patients. We are aiming to enroll another 3-4 patients. Once all patients have completed the study 16S rRNA gene sequencing (microbial community structure) and targeted bile acid metabolomics will be submitted and analyzed.

**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, the proposed 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**:
This study is still ongoing and actively recruiting normal healthy canine patients, 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.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| 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** | Interim |

**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. 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.

**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 near completion for the project. The enrollment goal is 6 dogs with MMVD stage B2 and 6 healthy control dogs. Thus far, all 6 MMVD stage B2 dogs and 4 normal healthy control dogs have completed the study. Once all cardiac MRI examinations have been completed, the data will be analyzed in regards to myocardial ischemia and 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. It is possible that information obtained from the cardiac MRI examinations in dogs with MMVD will help advance the understanding about how best to medically manage this disease.

**Conclusions**:
As more information is obtained, a better understanding of myocardial health in dogs with MMVD will be gained.

**Publications/Presentations/Grant Submissions**:
A grant required for completion of this study has been submitted to the American Kennel Club and is currently under review.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| 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 has significantly impeded our ability to perform the proposed studies. Work was scheduled to begin in March but was unable to proceed due to the subsequent 5-month shutdown of all non-COVID research at OSU. Moreover, the pandemic has resulted in a significant reduction in the numbers of canine patients presenting to the OSU Veterinary Medical Center with VetARDS. We have fully developed the protocol for generation of canine PCLS and are ready to proceed with the project as soon as a suitable dog becomes available.

**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 project has been delayed as the result of the COVID19 pandemic, we anticipate that we will be back on track and will be able to recruit enough patients and achieve our goals in 2021/2022.

**Publications/Presentations/Grant Submissions:**

Project is still in progress.

| 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** | Barrett, Susan; Cole, Lynette; Diaz-Campos, Dubraska; Diaz Vergara, Sandra; Horvath, Stephan; Matusicky, Michelle; Montgomery, Christopher; Van Balen Rubio, Joany; Yang, Ching |
| **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 predict prior 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, a clinical trial recruiting both healthy dogs and dogs with pyoderma will be conducted. Clinical follow-up one month after enrollment will be performed in dogs with pyoderma. 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 induce an antibody response in dogs during infections, antibody levels of healthy dogs and dogs with pyoderma will be compared. Antibody levels in dogs with pyoderma during the enrollment and one month after will also be compared.

**Results**:
The results for this project are pending.

**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 serum antibody levels to the bacterial toxins and to define the association of antibody level with disease severity, which is critical for future vaccine development.

**Conclusions** :
The conclusions for this study are pending.

**Publications/Presentations/Grant Submissions**:
The conclusions for this study are pending.

# PROGESS REPORT (lay 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 R. Pesapane |
| **Co-PIs/Co-Is** | Colleen Shockling Dent |
| **Interim or Final** | Interim |

**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, *Babesia* spp., *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 168 dogs tested thus far, 67 (39.8%) were seropositive for at least one tick-borne pathogen and 32 (19%) were seropositive for multiple tick-borne pathogens. The majority of dogs had been exposed to *Borrelia burgdorferi* (31.5%) followed by *Ehrlichia* spp. (26.8%) and *Anaplasma* spp. (1.8%). Direct pathogen detection via PCR and assays for *Rickettsia* spp. and *Babesia* spp. exposure are ongoing. 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 is the first detection of the Asian Longhorned tick in Ohio demonstrating continued expansion across the United States since its introduction in 2017. Analysis of risk factors 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 2.5-6.8X 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 has 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. Two publications are 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 (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<br>Adam Rudinsky<br>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 health span. 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 will be prospectively enrolled in 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 will be 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 16 patients actively enrolled in the study, with our first patient completing week 12 of 24. The study is actively recruiting obese canine patients. We are aiming to enroll the remaining dogs by the end of the summer. Once all patients 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 health span and improve quality of life in dogs suffering from obesity.

**Conclusions**:
This study is still ongoing and actively recruiting obese canine patients, 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 (lay 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. Despite this, there are still many aspects of canine urine that not well characterized. Urine is a highly variable matrix that undergoes hourly changes based on host health, hydration status, body mass index, concentrating ability, and diet. Little is known about the microbial communities and proteins found in healthy dog urine 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, studies on the urine microbiome have lagged behind. Recent studies have demonstrated that there are microbes present in the urine of healthy individuals, and that there may be links between the microbiota and urinary tract diseases including bladder stones, urinary incontinence, and urogenital tract cancers. However, knowledge of the urinary microbiome and its relationship with other urine properties remains limited. How stable is this microbial community? Does it shift with changes in urine pH, ion concentrations, specific gravity, or urine protein content? Evaluating urine properties like pH, urine specific gravity (USG), and proten presence, type, and variability over time in healthy dogs along with the microbial community will key to providing more context for urine samples analyzed in both healthy and sick dogs.

**Approach**:
**Examine urine microbial communities over time in healthy dogs.** We will identify and compare microbial communities (16S rRNA gene sequencing) in healthy dog urine samples from 12 dogs over 12 time points (Day 1 morning and afternoon, Day 2 morning and afternoon, Day 3 morning and afternoon, end of Week 1, end of Week 2, end of Week 3, end of Week 4, end of Month 1, end of Month 2, end of Month 3).

**Characterize urine, urine cells, and urine protein over time in healthy dogs.** We will use standard urinalysis methods to characterize and compare urine from 12 dogs over 12 time points (listed above). Urinalysis includes urine dipsticks that approximate values for urine pH and concentrations of glucose, ketones, and other compounds. We will also use cell counting chambers to enumerate cells, special stains to evaluate urine sediment, and gels to characterize urine protein (tubular and glomerular), a pH meter to measure urine pH, and a refractometer to measure urine specific gravity.

Microbial community results will be correlated with urine properties to determine if if or what urine properties are associated with changes in the urine microbiota.

**Results**:
To date, we have enrolled 9 dogs out of a total of 12 intended dogs. Five have completed samplings at all 12 timepoint of the study, and four are in their final rounds of sampling. We aim to recruit up to 6 more dogs for this study and will continue enrollement through summer 2021. Urine microbial community extraction, sequencing, and analysis will occur once all sampling is complete to prevent batch effects. Protein analyses will begin in summer 2021 on samples from dogs that have completed the study. One veterinary student, Andrew McGlynn, and one undergraduate, Rushil Madan, have received summer funding from the OSU Veterinary Scholar Summer Research Program and the OSU Undergraduate Research Apprentice Program respectively to work on this project.

**Preliminary analyses**: In this study, we evaluated urine pH and urine specific gravity (USG) in 5 dogs over 3 months. We also compared two methods for measuring urine pH: a pH meter and dipsticks. We found that both urine pH and USG varied widely within and between dogs over time; although, USG was more consistent by dog than pH. We also found both statistically and clinically significant differences between pH values recorded by meter and dipstick.

**Relevance & Impact to Canine Health**:
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. Dr. Rudinsky is involved in the clinical treatment of dogs with urinary tract infections (UTIs) and he along with Dr. Justice also investigate microbe-host interactions in UTIs to aide in evaluation of new therapeutics. As a Clinical Pathologist, Dr. Hokamp plays a key role in the diagnosis and follow up urogenital diseases that present in the Veterinary Medical Center, and her research is also focused on kidney diseases in domestic animals. Dr. Hale studies naturally-occurring bladder cancer in dogs. Her studies not only advance veterinary care, but dog bladder cancer closely mimics human muscle-invasive bladder cancer in terms of histopathology, biological behavior including distant metastases in 50% of cases, and response to treatment. This project has high relevance to both dog and human health and to the clinical and research endeavors of all of the individuals involved. Understanding temporality and variation in urine in healthy dogs provides context for interpreting urine in sick dogs and utilizing urine as an effective diagnostic tool.

**Conclusions**:
We have completed the first year of this study. Based on preliminary analyses on 5 dogs that have completed this study, we recommend the use of a pH meter for accurate pH measurement. We also note that due to high variability, urine pH but not necessarily urine specific gravity should be measured at multiple time points in healthy dogs prior to making clinical decisions. We will reanalyze all data once we have complete enrollment and reexamine these conclusions. We will also forumate additional analyses and conclusions around urine microbial communitites and protein content during the second year of this study.

**Publications/Presentations/Grant Submissions**:

**Submissions**

- FUNDED: Andrew McGlynn, OSU Veterainry Scholar Summer Research Program. *Examining urine protein over time in healthy dogs.*
- FUNDED: Rushil Madan, OSU Undergraduate Research Apprentice Program. *Evaluating urine culture and susceptibility in healthy dogs over time.*

**Presentations**

- Ryan Mrofchak's Master's Defense: *An Analysis of Canine Urine: Microbiota, Methods, and Changes in Health and Disease.* April 2021.

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Identifying Behavior Changes in Dogs During the Six Months Following Adoption from a Municipal Shelter |
| **Principal Investigator (PI)** | Dr. Jeanette O'Quin, DVM, MPH, DABVP, DACVPM |
| **Co-PIs/Co-Is** | Dr. M. Leanne Lilly, DVM, DACVB, Dr. Kyle Bohland, DVM, MS, Dr. Meghan Herron, DVM, DACVB |
| **Interim or Final** | Interim |

**Introduction**:
Millions of dogs enter and are adopted from dog shelters in the United States every year, but very little is known about their behavior long-term after adoption and how the shelter experience and subsequent rehoming affects their behavior. Shelters typically conduct behavior evaluations in the shelter, but evidence is mixed as to the predictive value for future behavior in their adoptive home. Furthermore, there is a general sentiment among shelter staff and behavioral professionals that there is a "honeymoon" period after adopting a dog, where dogs do not show their full repertoire of behaviors, both positive and negative, until getting more comfortable in their new home. There is currently a gap in the knowledge on canine behavior problems after adoption and whether a "honeymoon" period actually exists. To assess post adoption behavior, we are surveying owners of newly adopted dogs from four Ohio shelters at 1 week, 1 month, 3 months, and 6 months after adoption. We will also collect information regarding where owners seek out behavioral advice and record outcomes of euthanasia, rehoming, or relinquishment. Lastly, we will compare dogs' behavior and health status in the shelter to their long-term behavioral outcomes.

**Approach**:
The research team is collaborating with four shelters (Franklin County Dog Shelter, CHA Animal Shelter, Columbus Humane, and Cleveland Animal Protective League) to survey owners at specific time points after they adopt a dog. The owners will be surveyed about their newly adopted dog's behavior at 1 week, 1 month, 3 months, and 6 months after adoption using the Canine Behavioral Assessment & Research Questionnaire (C-BARQ). This questionnaire is a standardized, validated method for measuring behavior and behavior problems in dogs. It is specifically designed to measure the frequency and severity of behavioral problems in privately-owned dogs. The questionnaire collects information on excitability, aggression, fear, separation behaviors, trainability, and other behaviors. To encourage recruitment, participants will receive a $10 gift card for completing the first survey and $20 after completing all four of the surveys.

**Results**:
Recruitment is ongoing through June 1, 2021. All data will be acquired by December 2021 and results will be analyzed with plans to publish in a peer reviewed journal in early 2022.

**Relevance & Impact to Canine Health**:
This research will provide veterinarians, behavior professionals, and shelter staff information on the post-adoption behavior of dogs adopted from a shelter setting. More specifically, this research will fill a gap in the knowledge of how behavior changes or does not change during the 6 months following adoption from a shelter, which behaviors are more likely to impact the human-animal bond (based on relinquishment, rehoming, or behavioral euthanasia data), what resources are utilized by owners to manage behavior problems, and if certain characteristics or behavioral observations inside the shelter can help predict future behavior. This information will allow shelter staff, behavioral professionals, and veterinarians to better council owners on the management of behavior problems of dogs after adoption.

Additionally, dog bites are a major public health issue in the United States, with approximately 4.7 million dog bites reported each year. This study will help dogs and their owners by improving the understanding of canine behavior after

adoption from a shelter, and identifying risk factors for aggression after adoption, which can be used to help prevent future dog bites.

**Conclusions**:
The research is ongoing; thus no conclusions have been made at this time.

**Publications/Presentations/Grant Submissions**:
The research is expected to result in a publication in a peer-reviewed journal in 2022. As part of Dr. Bohland's residency training program requirements, he will also present results to the Ohio State College of Veterinary Medicine and at the 2022 American College of Veterinary Behaviorist Veterinary Behavior Symposium.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Clinical utility of corticosteroids and point of care monitoring in canine acute pancreatitis |
| **Principal Investigator (PI)** | Rudinsky, AJ |
| **Co-PIs/Co-Is** | Winston, JA; Parker, VJ, Chen, M; Howard, JP; Yaxley, P. |
| **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.

**Approach**:
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.

**Results**:
There are no results yet for this proposal as the donated equipment required to complete this project has been significantly delayed at the contracting and supply chain level.

**Relevance & Impact to Canine Health**:
The impact of a novel treatment strategy like corticosteroids 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 this proposal, we hope to establish evidence-based recommendations to turn the tides on the enormous financial and emotional impact this deadly disease inflicts annually.

**Conclusions**:
There are no results yet for this proposal as the donated equipment required to complete this project has been significantly delayed at the contracting and supply chain level. We are hopeful this will be resolved soon and the project can move forward in the Spring/Summer of 2021.

**Publications/Presentations/Grant Submissions**:
This project was recently funded.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| PROGESS REPORT (lay report) to the Ohio General Assembly | |
|---|---|
| **Title** | Understanding and stopping persistent *Ancylostoma caninum* egg shedding in chronic shedders. |
| **Principal Investigator (PI)** | A.E. Marsh |
| **Co-PIs/Co-Is** | S. Horvath and J. O'Quin |
| **Interim or Final** | Interim (initial) |

**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. 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**.

This study is critical to determine the presence of multi-drug resistant *A. caninum* in the dog population.

**Approach**:
We to use fenbendazole in place of febantel to determine if a lower cost drug and downstream active metabolite 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. 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) ora 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:**
Study started March 2021, no results to date.

**Relevance & Impact to Canine Health**:
Will determine the extent of drug resistant hookworms in dogs seen at the OSU CVM and whether a lower cost combination therapy can be used to effectively treat the dogs.

**Conclusions**:
This study was recently started.

**Publications/Presentations/Grant Submissions:**
This study was recently started.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

| PROGESS REPORT (lay 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**:
This study was recently funded and is pending IACUC approval.

**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 study was recently funded and is pending IACUC approval.

**Publications/Presentations/Grant Submissions** (250 word limit):
This study was recently funded and is pending IACUC approval.

| 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 in process for IACUC approval and thus no patients have yet been enrolled.

**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%[1] 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[6,7].

**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. We hope to have IACUC approval in the very near future and then will begin active recruitment of patients.

**Publications/Presentations/Grant Submissions**:
No publications have been submitted.

| FUNDING OF PROJECTS | |
|---|---|
| **TITLE** | **BUDGET** |
| Analgesic effects and tolerability of tapentol in combination with NSAIDS in dogs with osteosarcoma | $22,575 |
| Characterizing the microbiome in dogs with and without bladder cancer | $22,682 |
| Effects of trazodone and gabapentin on electroretinograms recorded in normal dogs | $10,676 |
| Efficacy of a commercially available LH surge detection strip in the bitch | $ 8,046 |
| Perfusion index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs | $11,588 |
| Pulse oximetry pleth variability index as a predicter of fluid responsiveness in dogs | $22,728 |
| 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 |
| Assessment of regional intestinal perfusion by infrared thermography during foreign body surgery | $ 8,037 |
| Pilot study on the effects of intra-articular allogenic stem cell therapy for the treatment of osteoarthritis | $22,727 |
| Effects of antimicrobial therapy on virulence and antimicrobial resistance of canine EPEC UTIs | $22,600 |
| Canine glioma as a model for testing MKlp2 inhibition in human glioblastoma | $22,619 |
| Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms | $15,255 |
| Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs | $22,727 |
| Use of radiation therapy and conforming intramedullary implant to treat canine appendicular OSA | $22,421 |
| Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the canine gut microbiota and bile acid metabolome | $22,633 |
| Utility of cardiac MRI to diagnose cardiac fibrosis in dogs with mitral valve disease: a pilot study | $22,727 |
| 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 |
| Molecular and serologic surveys of shelter dogs and their ticks as sentinels for tick-borne disease risk in Ohio | $21,020 |
| 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 |
| Identifying behavior changes in dogs during the six months following adoption from a municipal shelter | $ 9,980 |

| | |
|---|---|
| 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 |

## 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 | | | |
|---|---|---|---|---|---|---|
| County | Invoice | Amt Paid $ | 1 - YR | 3 - YR | PERMNT | KENNEL REG |
| Adams County Auditor | 1 | $703.20 | 6,677 | 75 | 10 | 30 |
| Allen County Auditor | 1 | $1,637.60 | 15,609 | 148 | 31 | 13 |
| Ashland County Auditor | 1 | $875.70 | 7,999 | 145 | 25 | 73 |
| Ashtabula County Auditor | 1 | $1,115.00 | 9,775 | 377 | 19 | 54 |
| Athens County Auditor | 1 | $983.50 | 9,561 | 22 | 10 | 106 |
| Auglaize County Auditor | 1 | $904.40 | 8,107 | 219 | 28 | |
| Belmont County Auditor | 1 | $915.20 | 7,711 | 360 | 26 | 101 |
| Brown County Auditor | 1 | $1,026.60 | 9,651 | 104 | 29 | 13 |
| Butler County Auditor | | | | | | |
| Carroll County Auditor | 1 | $832.40 | | | | |
| Champaign County Auditor | 1 | $825.10 | 7,768 | 82 | 11 | 127 |
| Clark County Auditor | 1 | $2,173.60 | 2,030 | 405 | 20 | 21 |
| Clermont County Auditor | 1 | $1,728.40 | 15,566 | 389 | 53 | 21 |
| Clinton County Auditor | 1 | $809.20 | 7,315 | 156 | 20 | 109 |
| Columbiana County Auditor | 1 | $2,290.30 | 20,097 | 567 | 109 | 15 |
| Coshocton County Auditor | 1 | $1,023.30 | 9,580 | 16 | | 605 |
| Crawford County Auditor | 1 | $847.70 | 7,841 | 142 | 20 | 10 |
| Cuyahoga County Auditor | 1 | $7,370.00 | 55,328 | 4,630 | 447 | 12 |
| Darke County Auditor | 1 | $1,238.60 | 11,580 | 112 | 12 | 350 |
| Defiance County Auditor | 1 | $749.90 | 6,859 | 127 | 19 | 69 |
| Delaware County Auditor | 1 | $2,054.40 | | | | |
| Erie County Auditor | 1 | $1,309.70 | 13,068 | 4 | 1 | 7 |
| Fairfield County Auditor | 1 | $2,496.10 | 22,222 | 700 | 60 | 39 |
| Fayette County Auditor | 1 | $422.30 | 3,789 | 88 | 16 | 10 |
| Franklin County Auditor | | | | | | |
| Fulton County Auditor | 1 | $817.10 | 7,478 | 177 | 11 | 52 |
| Gallia County Auditor | 1 | $227.30 | 1,793 | 5 | 2 | 445 |
| Geauga County Auditor | 1 | $1,082.20 | 10,157 | 140 | 14 | 105 |
| Greene County Auditor | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Guernsey County Auditor | 1 | $667.70 | 6,031 | 91 | 29 | 83 |
| Hamilton County Auditor | 1 | $3,988.20 | | | | |
| Hancock County Auditor | 1 | $1,349.30 | 12,741 | 145 | 16 | 157 |
| Hardin County Auditor | 1 | $730.70 | 7,273 | 1 | 1 | 21 |
| Harrison County Auditor | 1 | $331.30 | 3,199 | 22 | 1 | 38 |
| Henry County Auditor | 1 | $623.50 | 6,057 | 47 | 2 | 37 |
| Highland County Auditor | 1 | $545.30 | 4,897 | 123 | 13 | 57 |
| Hocking County Auditor | 1 | $469.20 | 4,402 | 67 | 5 | 39 |
| Holmes County Auditor | 1 | $990.80 | 9,431 | 4 | | 465 |
| Huron County Auditor | 1 | $821.50 | 7,771 | 78 | 13 | 80 |
| Jackson County Auditor | 1 | $789.00 | 7,307 | 87 | 18 | 142 |
| Jefferson County Auditor | 1 | $512.50 | 4,235 | 199 | 29 | 3 |
| Knox County Auditor | 1 | $952.20 | 8,476 | 208 | 34 | 82 |
| Lake County Auditor | 1 | $2,956.60 | 26,185 | 775 | 103 | 26 |
| Lawrence County Auditor | | | | | | |
| Licking County Auditor | 1 | $2,925.20 | 28,787 | 55 | 18 | 120 |
| Logan County Auditor | 1 | $632.10 | 6,063 | 40 | 13 | 8 |
| Lorain County Auditor | | | | | | |
| Lucas County Auditor | 1 | $5,131.90 | | | | |
| Madison County Auditor | 1 | $629.20 | 5,239 | 251 | 30 | |
| Mahoning County Auditor | 1 | $2,832.60 | 26,028 | 534 | 47 | 226 |
| Marion County Auditor | 1 | $886.10 | 7,753 | 211 | 39 | 85 |
| Medina County Auditor | 1 | $2,399.10 | 19,449 | 1,068 | 126 | 78 |
| Meigs County Auditor | 1 | $211.40 | 1,828 | 29 | 7 | 129 |
| Mercer County Auditor | | $425.10 | 4,124 | 20 | | 67 |
| Miami County Auditor | 1 | $1,903.30 | 16,043 | 619 | 112 | 13 |
| Monroe County Auditor | 1 | $350.60 | 3,280 | 2 | | 220 |
| Montgomery County Auditor | 1 | $5,872.30 | | | | |
| Morgan County Auditor | 1 | $217.60 | 1,916 | 56 | 7 | 22 |
| Morrow County Auditor | 1 | $562.10 | 5,010 | 131 | 17 | 48 |
| Muskingum County Auditor | 1 | $1,145.20 | 10,936 | 47 | 20 | 175 |
| Noble County Auditor | 1 | $170.30 | 1,467 | 32 | 6 | 80 |
| Ottawa County Auditor | 1 | | | | | |
| Paulding County Auditor | 1 | $329.60 | 2,971 | 58 | 12 | 31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Perry County Auditor | 1 | $654.90 | | | | |
| Pickaway County Auditor | | | | | | |
| Pike County Auditor | | | | | | |
| Portage County Auditor | 1 | $2,911.90 | 25,574 | 734 | 127 | 73 |
| Preble County Auditor | 1 | $339.50 | | | | |
| Putnam County Auditor | 1 | $697.80 | 6,294 | 29 | 9 | 507 |
| Richland County Auditor | 1 | $1,785.70 | 17,273 | | | 584 |
| Ross County Auditor | 1 | $1,354.80 | | | | |
| Sandusky County Auditor | 1 | $1,208.70 | 11,400 | 150 | 20 | 37 |
| Scioto County Auditor | | | | | | |
| Seneca County Auditor | 1 | $1,014.20 | | | | |
| Shelby County Auditor | 1 | $854.40 | 7,770 | 178 | 15 | 90 |
| Stark County Auditor | | | | | | |
| Summit County Auditor | 1 | $3,890.60 | | | | |
| Trumbull County Auditor | | | | | | |
| Tuscarawas County Auditor | 1 | $1,623.00 | 15,074 | 191 | 52 | 63 |
| Union County Auditor | 1 | $978.30 | 7,480 | 536 | 69 | 1 |
| Van Wert County Auditor | 1 | $486.80 | 4,449 | 86 | 14 | 21 |
| Vinton County Auditor | 1 | $2,795.60 | 22,252 | 1,260 | 190 | 24 |
| Warren County Auditor | | | | | | |
| Washington County Auditor | 1 | $1,128.70 | 10,022 | 231 | 56 | 12 |
| Wayne County Auditor | | | | | | |
| Williams County Auditor | 1 | $400.00 | | | | |
| Wood County Auditor | 1 | $2,332.70 | 17,978 | 1,117 | 192 | 78 |
| Wyandot County Auditor | 1 | $444.10 | 3,951 | 120 | 7 | 60 |
| | | | | | | |
| | **Total:** | **$103,712.00** | **675,977** | **18,822** | **2,462** | **6,469** |
| ****NOTES:** | | | | | | |
| Invoice 1 sent out | | | | | | |
| | | | | | | |
| **Payment not yet received as of 4/27/2021** | | | | | | |
| | | | | | | |
| Breakdowns of type of tags sold not provided as requested | | | | | | |
| | | | | | | |