THE OHIO STATE UNIVERSITY

College of Veterinary Medicine

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26 April 2024

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 7 final and 12 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
2023

Submitted to:
The Ohio General Assembly

May 2024

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

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 aimed to determine how oral administration of Ursodiol (21-day course) alters the canine intestinal ecosystem, specifically the gut microbiota (collection of microorganisms that live in the intestines) and bile acids (important metabolites best known for their role in digestion and absorption of fat). The goal is to improve our knowledge of Ursodiol-mediated effects to the canine intestinal ecosystem to facilitate rational incorporation of Ursodiol into a personalized medicine approach for dogs suffering from liver and gastrointestinal diseases in order to improve quality of life.

Approach:
Our central hypothesis is that oral administration of Ursodiol will alter the canine intestinal ecosystem, specifically the fecal microbial community structure and bile acid metabolome. To test this, we conducted a clinical trial in client-owned healthy dogs administered Ursodiol for 21 days at a clinically relevant dose (10-15 mg/kg/day). Freshly voided feces was collected from dogs at baseline (3 separate samples), weekly during Ursodiol administration, and at 1 week, 1 month, and 3 months post Ursodiol administration. Alterations in the gut microbiota and fecal bile acid metabolome were serially and simultaneously assessed with shot-gun sequencing (microbiota community structure) and targeted bile acid metabolomics, allowing for an integrated multi-omics approach. This study is the first comprehensive, multi-omics characterization of how Ursodiol impacts the healthy canine intestinal ecosystem.

Results:
In 15 healthy client-owned dogs at baseline 61 fecal bile acids were detected, including: 3 unconjugated primary bile acids, 4 conjugated primary bile acids, 21 unconjugated secondary bile acids, 7 conjugated secondary bile acids, and 26 amino acid microbially conjugated bile acids (MCBAs) (Figure 1). These newly described MCBAs included bile acid conjugates with alanine, serine, leucine, threonine, lysine, methionine, histidine, phenylalanine, tyrosine, arginine, and tryptophan. Using shot-gun sequencing, metagenome-assembled genomes (MAGs) were constructed and a comprehensive metagenomic analysis of bile acid biotransformation gene abundances was performed. In screening MAGs for bile acid transformation genes, we identified 14 genes (BSH, BaiA1, BaiA2, BaiB, BaiCD, BaiE, BaiF, BaiG, BaiH, BaiI, BaiJ, BaiK, BaiL, HDHA) across various species including from the family Oscillospiraceae and *Peptacetobacter spp*.

Alterations in the fecal microbial community structures was seen in dogs during Ursodiol treatment. As expected, oral Ursodiol administration significantly increased fecal ursodeoxycholic acid (UDCA) concentrations by day 7 in dogs (Freidman test with Dunn's multiple comparisons; all timepoints $P < 0.05$; Figure 2). Aside from UDCA, Ursodiol treatment also altered the canine fecal bile acid metabolome, including increases in the secondary bile acid murocholic acid (Figure 3). Ursodiol treatment also significantly altered the newly described amino acid MCBAs in dogs, specifically histidine-UDCA, phenylalanine-UDCA, tyrosine-UDCA, tryptophan-UDCA, and methionine-UDCA (Wilcoxon matched-pairs signed rank test; all $P < 0.0001$; Figure 4).

Figure 1: Comprehensive fecal bile acid panel in 15 healthy client owned dogs.

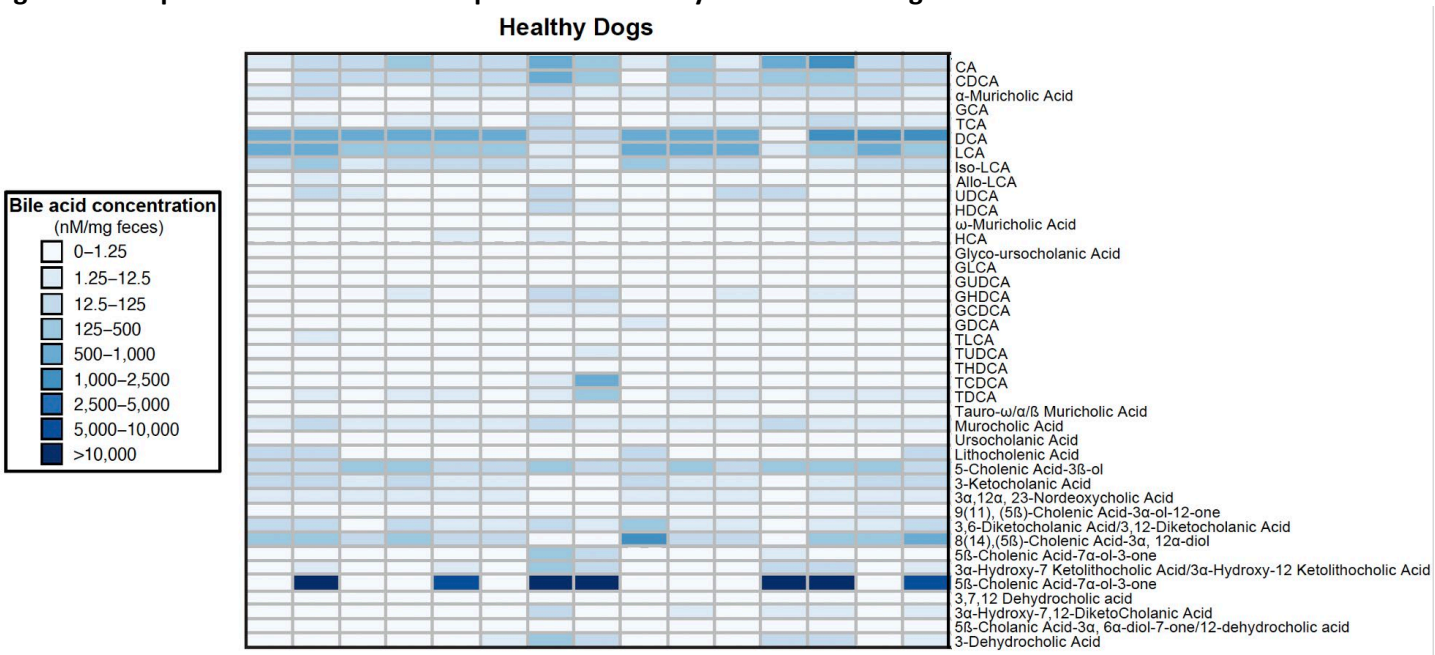

Figure 2: Oral Ursodiol administration in 15 healthy client-owned dogs significantly increased fecal UDCA concentrations.

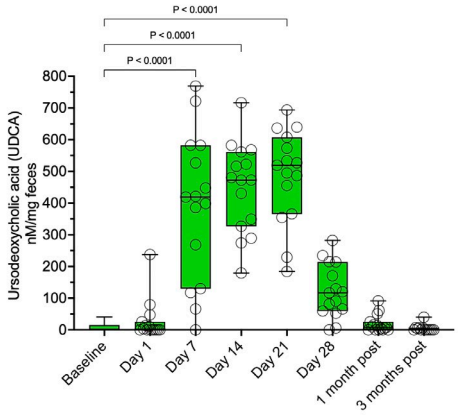

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Figure 3: Canine fecal bile acid pools are altered by oral Ursodiol administration in 15 healthy client-owned dogs.

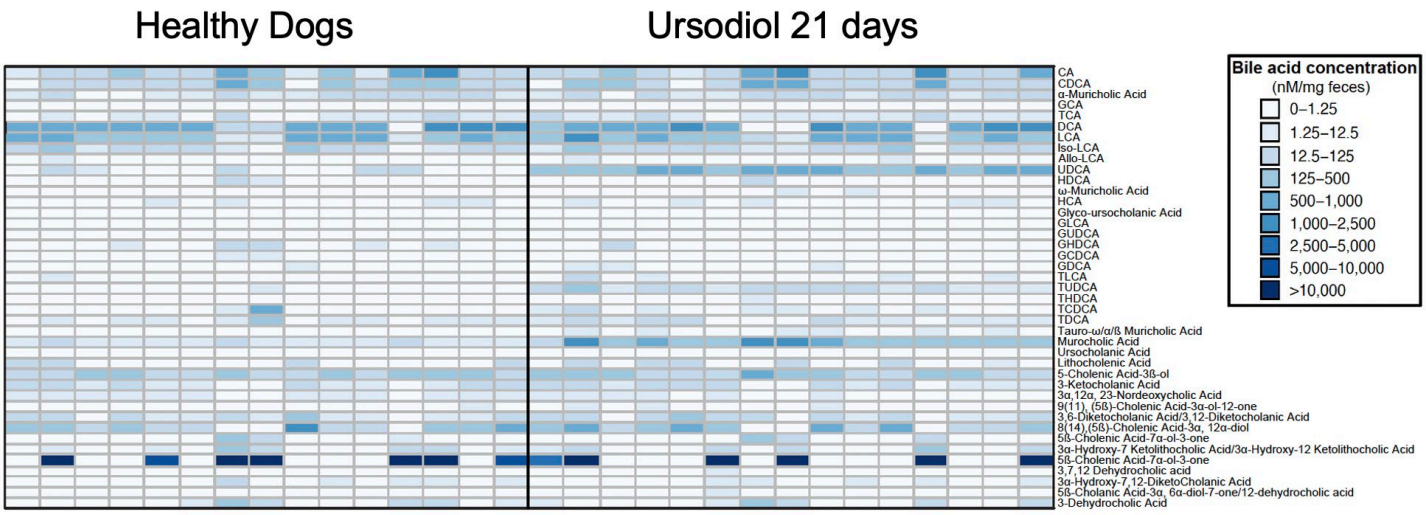
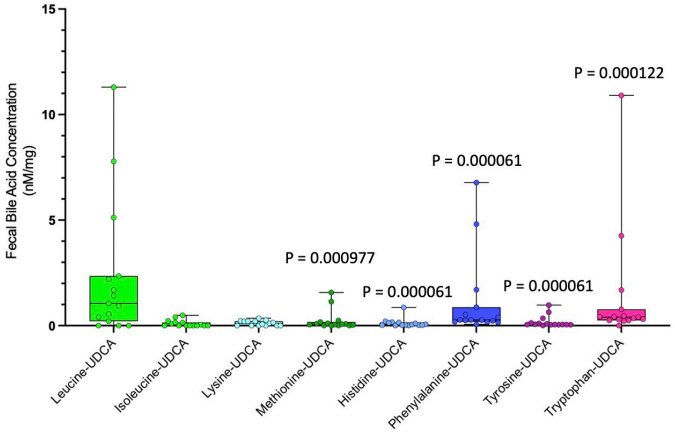


Figure 4: Canine UDCA-MCBAs are significantly altered by oral Ursodiol administration in 15 healthy client-owned dogs.



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 provides valuable data on the impacts of Ursodiol, administered at a clinically relevant dose, on the canine intestinal ecosystem. Specifically, this clinical trial is the first to provide a comprehensive characterization of Ursodiol mediated effects on the gut microbiota and bile acid metabolome in healthy dogs. Results of this study will be the catalyst that will ultimately allow us to make evidence-based recommendations on how to utilize Ursodiol to rationally manipulate the canine intestinal ecosystem. The ultimate goal is to understand how the canine bile acid metabolome contributes to health and disease in relation to chronic enteropathy, enteric pathogens, diabetes mellitus, chronic kidney disease (CKD), and obesity. To this effect, this study is the first to integrate multi-omics approaches to evaluate the canine gut microbiota-bile acid-host axis, which provides 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:

In conclusion, this study is the first to utilize a multi-omics approach to provide a comprehensive profile of how exogenously administered Ursodiol shapes the intestinal ecosystem in dogs. Additionally, this study provides a comprehensive overview of the diversity of bai-harboring bacteria in the canine gut highlighting the application of multi-omics approaches. These results expand the scope of bile acid pool diversity described in dogs, including the first documentation of α-muricholic acids in companion animals. Additionally, these results are the first to document microbial bile acid transformation genes and the gut microbiota's capability to produce MCBAs in dogs. This study opens a new perspective to investigate collaborative metabolism of bile acid between dogs and gut microbes in health and disease. Further studies to investigate how these changes in turn modify the host physiologic response are required. Lastly, with the widespread use of Ursodiol in veterinary medicine, ramifications of how this drug impacts the gut microbiota-bile acid-host axis in dogs during health and disease states is warranted.

Publications/Presentations/Grant Submissions:

Publications:

- Rowe, J.C. and Winston, J.A., 2024. Collaborative Metabolism: Gut Microbes Play a Key Role in Canine and Feline Bile Acid Metabolism. Veterinary Sciences, 11(2), p.94.
- Two additional manuscripts (*anticipated submission Fall 2024*; after ECVIM Congress)

Presentations:

The PI (J. Winston) had been invited to presented on this topic internationally:

- Australian and New Zealand College of Veterinary Scientists Science Week 2023, invited oral presentation titled: *"Gut Microbiota Derived Bile Acids: An Emerging Field in Veterinary Medicine"*

Research Abstracts:

- Comparative Gastroenterology Society GutSki 2024. Oral presentation titled: "*Expanding the scope of microbial derived bile acids in companion animals.*"
- European Society of Comparative Gastroenterology EuroGut 2024. Oral presentation titled: "*Expanding the scope of microbial derived bile acids in companion animals.*"
- European Congress of Veterinary Internal Medicine for Companion Animals (ECVIM-CA) Congress 2024 (*submissions pending acceptance*)
    - *Multi-omic Approach Identifies Expanded Bile Acid Pools, New Microbially Conjugated Bile Acids, and Bile Acid Biotransformation Genes in the Canine and Feline Gut Microbiota.* J. A. Winston, H. Klein, A. Wood, V. J. Parker, and A. J. Rudinsky.
    - *Ursodiol administration modulates the intestinal ecosystem of dogs and cats.* J. A. Winston, H. Klein, A. Wood, V. J. Parker, and A. J. Rudinsky.

Extramural grants:

This research project led to feline study:

- EveryCat Health Foundation grant titled: *Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the feline gut microbiota and bile acid metabolome.* Jenessa A. Winston (PI), Valerie Parker (co-I), and Adam Rudinsky (co-I). Total awarded: $26,437.

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

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

Results:
Nine dogs completed the study. This included 4 Labrador Retrievers, 2 German Shepherd Dogs, and 1 each giant Schnauzer, mixed breed, and Rottweiler. There were 3 neutered males, 1 intact males, 3 spayed females, and 2 intact females. The mean age at the time of enrollment was 13.1 months (SD +/- 4.9 mo), with a mean weight of 31.1 kg (SD +/- 9 kg). Mean body condition score was 6/9 (range 5-8). Flexion and extension of the elbow joints did not change significantly during the course of the study. At the time of enrollment, the mean Canine Brief Pain Inventory Score (CBPI) was 8.72 (SD +/- 5.08), which significantly decreased over the duration of the study to 1.61 (SD +/- 2.26). This trend was also seen in the Liverpool Osteoarthritis in Dogs (LOAD) scores over time, indicating an improvement in pain and quality of life over the duration of the study. Additional statistical analysis is undergoing to evaluate changes in objective gait analysis and computed tomography scoring over time. Most importantly, no adverse events were reported with treatment given during the duration of this study, indicating that it is safe for use in dogs.

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 :
While full statistical analysis is on-going we were able to show that the use of allogenic stem cell injection for the treatment of osteoarthritis in a dog model was safe, with no reported adverse events in any study participants for the entire duration of the study. While the study duration was 9 months long, longer term follow-up has been maintained by the principal investigator and to date, no adverse events have been reported. The significant change in validated pain scores (the Canine Brief Pain Inventory and Liverpool Osteoarthritis in Dogs) provides objective data that patient comfort and quality of life improved over the duration of the study with treatment. Additional statistical evaluation will enable us to fully evaluate if these changes are also seen with objective gait analysis in terms of weight distribution between forelimbs and hindlimbs and symmetry between left and right forelimbs. Additionally, computed tomography osteoarthritis scores will be evaluated to determine if there is a difference in progression of osteoarthritis between the treatment joints and placebo injected joints.

Publications/Presentations/Grant Submissions:
Submission to Journal of Orthopedic Research expected by summer 2024, abstract submission to Veterinary Orthopedic Society Meeting, and Orthopedic Research Meeting are planned for presentation winter 2025.

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

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

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

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

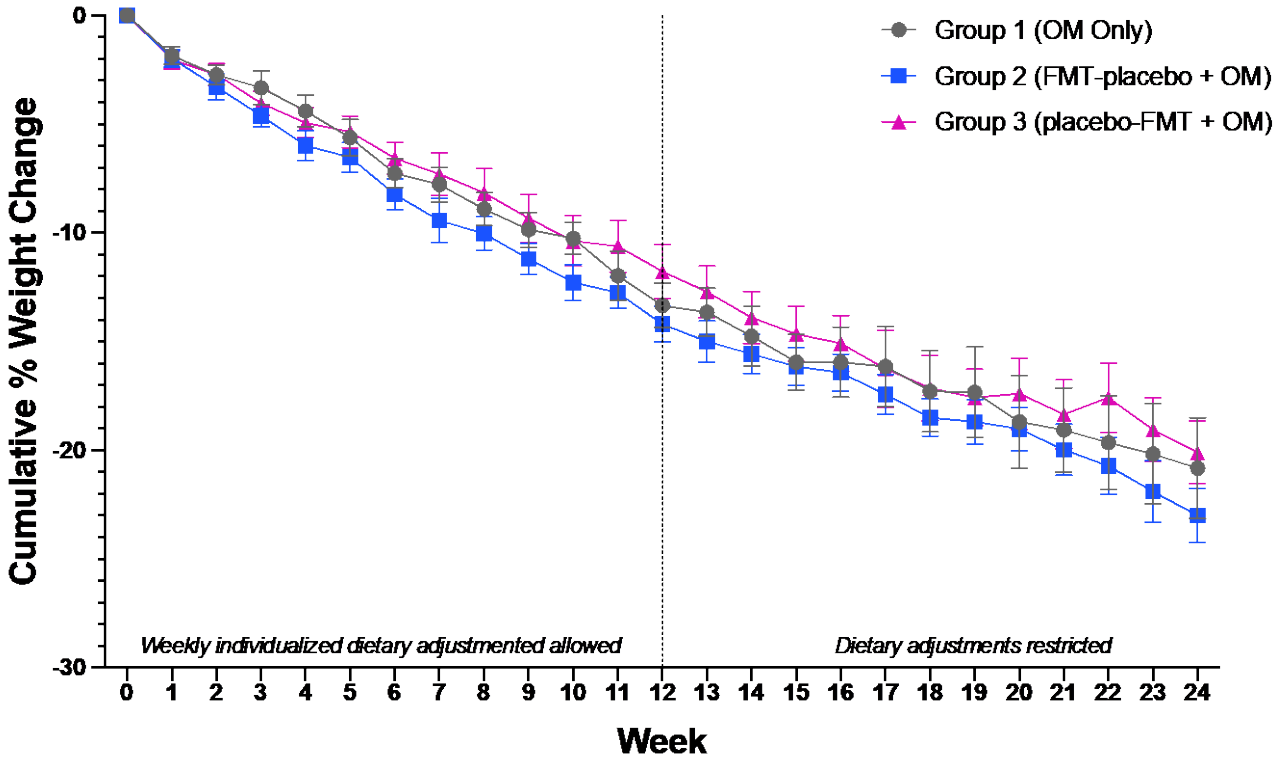
Specific Aim 1: Determine the clinical efficacy of FMT as an adjunctive therapy to enhance standard canine obesity management compared to standard dietary management alone or with placebo. Client-owned obese, but otherwise healthy dogs are being prospectively enrolled into a randomized, double-blinded,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

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). *NOTE: Portion of project funded by American Kennel Club Canine Health Foundation (AKC CHF).*

Results:
We screened (comprehensive physical examinations, bloodwork, and fecal analysis) 51 dogs for the SLIM study. Of these, 31 met the inclusion criteria and were enrolled. Of these enrolled dogs, 25 dogs completed the 24-week clinical trial. Unfortunately, 6 dogs were removed from the study early with the most common reason related to administration of antimicrobials needed for underlying health conditions including dog bite wounds, urinary tract infections, and surgical procedures. Based on recalculating our power analysis for sample size estimation, our current samples size (n=25) is sufficient to find a significant difference in weight loss between the treatment groups. Our last SLIM patient finished the clinical trial at the end of October 2022. Since this time, we have entered the analysis phase and sample processing for microbiome sequencing and metabolomics (Specific Aim 2). Specific Aim 2 was expanded by AKC and CHF to include metagenomics, which will take additional time to analyze.

Since completion of the SLIM study, we have unblinded the dogs' treatment groups and are providing a ***confidential*** interim weight loss analysis for Specific Aim 1. Over the 24-week clinical trial, regardless of treatment group all dogs

reached their individualized weight loss goals. There was no significant difference in cumulative percentage weight loss between the treatment groups (p=0.4580; total n=25; Group 1 (Purina OM diet alone), n= 6, 20.8% weight loss +/- 5.7; Group 2 (Purina OM diet + FMT-placebo), n=10, 23% weight loss +/- 3.9; Group 3 (Purina OM diet + placebo-FMT), n=9, 20.1% weight loss +/- 4.3).

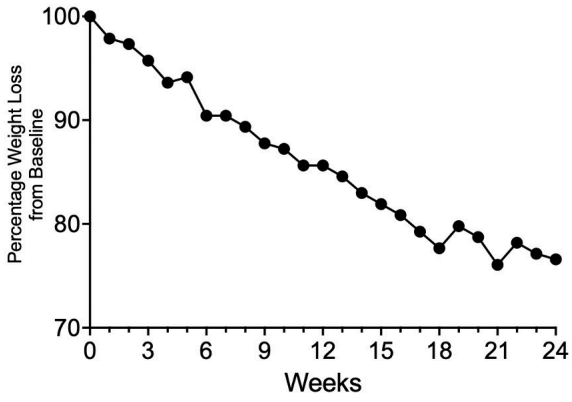


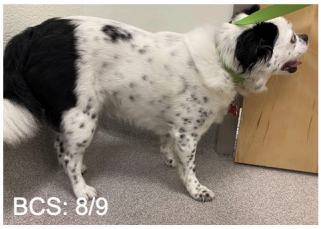
Relevance & Impact to Canine Health:

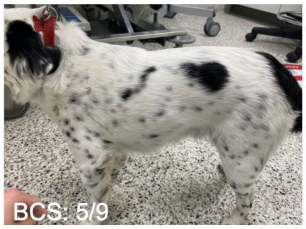
This clinical trial is the *first* to assess the efficacy of FMT for obesity management in dogs. Additionally, this study is the *first* to provide comprehensive, integrated multi-omics data on obese dogs throughout a structured obesity management program. This study will shed light on the role(s) that 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. Understanding the obesity-specific key microbial community members and their metabolic function will help to facilitate development of precision canine microbiome-targeted therapies aimed at facilitating accelerated metabolic improvements to promote healthspan and improve quality of life in dogs suffering from obesity.

Conclusions:

In conclusion, our patients' owners were thrilled with how their pets progressed in their weight loss goals and were greatly appreciative for the improvement in their pets' quality of life since completing the SLIM study. For example, over the 24-week SLIM study, Pax's achieved a total weight loss of 23% of his body weight (*see below*). His body condition score (BCS) improved from 8/9 to 5/9 during the SLIM study. His owners' report a marked improvement in his energy and actively levels since completing the SLIM study.







Although FMT did not accelerate weight loss in dogs in SLIM study, cumulative percentage weight loss may not be the best measure to evaluate efficacy of FMT as an adjunctive treatment for canine obesity. If the principle behind FMT is that microbes from lean donors are less efficient at energy extraction, with the expansion of the original proposal and support from AKC CHF, we are utilizing metagenomics to look at microbial enzymes and transporters that may be contributing to obesity phenotype.

Publications/Presentations/Grant Submissions:

Extramural grant submission:

- American Kennel Club Canine Health Foundation Oak grant titled: *Scientific and clinical assessment of fecal microbiota transplant to enhance weight loss in obese dogs (SLIM Study).* Total awarded: $113,129. Funds utilized to complete a portion of Aim #1 and all of Aim #2.

Presentations:

PI (Dr. Winston) was invited to record podcasts, related to FMT and the SLIM study:

- Morris Animal Foundation's Fresh Scoop podcast show titled "*The Amazing Science of Fecal Microbiota Transplantation*". Aired on January 13, 2022.
- Fully Vetted podcast show titled "*The Power of Poop: Fecal Transplants for Treating Disease*". Aired on January 3, 2022.

ACVIM Forum 2022 invited oral research abstract titled: "*Standardized preparation of canine fecal transplant material does not alter microbial community structure*". Randolph, N., Klein, H., Salerno, M., and J. Winston.

- This data presented by a fourth-year veterinary student (M. Salerno), who received a second-place award from the Comparative Gastroenterology Society for this oral presentation.

PI (J. Winston) has been invited to regional, national, and international conferences to oral presentations on the canine SLIM study including:

- Comparative Medicine Program at the University of Mizzou in November 2022
- American College of Veterinary Internal Medicine (ACVIM) Forum June 2023
- AKC Canine Health Foundation 2023 National Parent Club Canine Health Conference
- European Congress of Veterinary Internal Medicine for Companion Animals (ECVIM-CA) Congress September 2023

Manuscripts: Two manuscripts in preparation (*anticipated Summer 2024*) and at least two additional manuscripts from the work outlined above.

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

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. Recent studies have demonstrated that there are live microbes present in the urine of healthy individuals, and that there are links between the microbiota and urinary tract diseases. 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, specific gravity, or urine protein content? Are there body sites that share microbes with the urinary tract? Similarly, little is known about how urine protein type and content varies temporally in healthy dogs, or if and how protein levels vary with other urine attributes. Proteins identified in urine are important to diagnosing renal disease and also guiding treatment and prognosis. Evaluating urine properties and urine, fecal, perineal, and genital microbial communities over time in healthy dogs is key to furthering our understanding and use of urine for diagnostic or prognostic purposes in health and disease.

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

Aim 1: Characterize urine properties and urine protein over time in healthy dogs. We used standard urinalysis methods to characterize and compare urine properties in all dogs over time. Urinalysis included urine dipsticks that approximated values for urine pH and concentrations of glucose, ketones, and other compounds. We also used SDS gels to characterize urine proteins, a pH meter to measure urine pH, a refractometer to measure urine specific gravity, and selective culture and identification of urine isolates to assess antimicrobial resistance to antibiotics commonly used in veterinary medicine.

Aim 2: Examine urine, fecal, perineal, and genital (vulval/preputial) microbial communities over time in healthy dogs. We compared microbial community diversity and composition (16S rRNA gene sequencing) in 14 healthy dogs over 12 time points.

Results:
We have completed enrollment and analysis of urine properties on all 14 dogs. We have also completed analysis on urine, fecal, perineal, and genital microbiomes over time. We now plan to correlate microbial community results with urine property and protein results. Veterinary student Andrew McGlynn spent 2 summers working in my laboratory on this project as part of the Veterinary Summer Scholars program. Andrew led one manuscript on this work, now published at the Journal of Veterinary Internal Medicine. There is a second manuscript on the urine, fecal, perineal, and genital microbiomes over time that is in preparation by Andrew. Three other students have also been involved in this project:

- Ryan Mrofchak, Masters student whose Master's thesis focused on canine urine pH and specific gravity analyses over time

- Rushil Madan, undergraduate, involved in culture and bacterial identification in urine over time
- Mohammad Jahid, MPH/VPH student, performed his practicum in Hale laboratory testing antimicrobial resistance of urine isolates.

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

Conclusions:
Key findings from our current work:

- Urine pH, urine specific gravity (USG), number of protein bands, and protein concentrations varied significantly between dogs.
- pH was highly variable within dogs over time and should be measured via pH meter (not dipstick) at multiple time points before making clinical recommendations.
- USG was consistent over time within dogs, and measurement of USG at a single timepoint is likely to be an accurate representation of a dog's urine concentrating ability.
- Varying but low concentrations of Tamm Horsfall / albumin proteins were present in most healthy dogs.
- A few additional protein bands of unknown identity were consistently identified in urine samples suggesting that these bands can be considered normal findings.
- Cultured bacterial profiles varied significantly between dogs; but was less variable within dogs over time, indicating some stability in the urinary microbiota.
- We found no differences in presence or number of cultured bacterial taxa by sex.
- The most commonly cultured organisms, *Streptococcus canis* and *Staphylococcus pseudintermedius* are skin contaminants frequently found in voided urine. Cystocentesis or catheterized urine collection is recommended when feasible to avoid these contaminants.
- Multiple healthy, asymptomatic dogs cultured high abundances of bacteria (>10^5 CFU/mL); although, these bacteria were likely skin contaminants.
- Uropathogens associated with urinary tract infections like *E. coli* and *P. auruginosa* were also cultured at low levels in healthy dogs, and the presence of these taxa in cultures from asymptomatic dogs does not necessarily warrant treatment.
- We found phenotypic resistance to ampicillin and oxacillin in 75% of the bacterial isolates obtained from healthy canine urine underscoring the importance of antimicrobial stewardship in veterinary medicine.
- Urine microbial composition and diversity differed significantly between dogs but were relatively stable over time within dogs.

Publications/Presentations/Grant Submissions:
Publications

- McGlynn*, R. Mrofchak*, R. Madan, C. Madden, M.J. Jahid, D. Mollenkopf, T. Wittum, S.S. Justice, A. Rudinsky, J. Hokamp, **V.L. Hale.** 2023 Longitudinal examination of urine pH, specific gravity, proteins, culture, and resistance profiles in healthy dogs. 2023. JVIM. 37(6):2219-2229. doi: 10.1111/jvim.16860.

Submissions

- 2021, 2022: Andrew McGlynn, OSU Veterinary Scholar Summer Research Program. *Examining urine protein over time in healthy dogs.*
- 2021-2022: Rushil Madan, OSU Undergraduate Research Apprentice Program. *Evaluating urine culture and susceptibility in healthy dogs over time.*
- V. Hale, S. Justice, A. Rudinsky, B. Husbands were awarded funds for a clinical trial involving the canine urine microbiome: Can probiotics improve clinical outcomes in bladder cancer?: Addition of probiotic *Escherichia coli Nissle 1917* to a vinblastine / piroxicam protocol for the treatment for urothelial carcinoma in dogs
- V. Hale received an NIH K08 in September 2023. The grant is relevant to the urine microbiome and included preliminary data gathered in the course of this project. Title of K08: Bladders and biomes: Environmental compounds as modifiers of microbiomes, metabolomes, and urothelium.

Presentations

- Poster: A. McGlynn, R. Mrofchak, R. Madan, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, VL Hale. How variable is the urinary microbiota of healthy dogs over time? April 2023. Ohio State University College of Veterinary Medicine Research Day – *Columbus, OH*.
- Poster: A. McGlynn, R. Mrofchak, R. Madan, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, VL Hale. How variable is the urinary microbiota of healthy dogs over time? August 2022. National Veterinary Scholars Symposium – *Minneapolis, MN*.
- Poster: R. Madan, R. Mrofchak, C. Madden, A. McGlynn, S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. May 2022. *Longitudinal Examination of Urine Cultures from Healthy Dogs.* Purdue Applied Microbiome Symposium – *West Lafayette, IN*.
- Poster: A. McGlynn, R. Mrofchak, R. Madan, C. Madden, S. Justice, A. Rudinsky, J, Hokamp, V.L. Hale. May 2022. *Urine Trouble: Examining urine pH, specific gravity, and urine protein over time in dogs.* Purdue Applied Microbiome Symposium – *West Lafayette, IN*.
- Poster: R. Madan, R. Mrofchak, C. Madden, A. McGlynn, S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. April 2022. *Longitudinal Examination of Urine Cultures from Healthy Dogs.* OSU Spring Undergraduate Research Festival.
- Poster: A. McGlynn, R. Mrofchak, R. Madan, C. Madden, S. Justice, A. Rudinsky, J, Hokamp, V.L. Hale. April 2022. *Urine Trouble: Examining urine pH, specific gravity, and urine protein over time in dogs.* OSU College of Veterinary Medicine Research Day.
- Poster: A. McGlynn, R. Mrofchak, R. Madan, C. Madden, A. Rudinsky, J. Hokamp, V.L. Hale. August 2021. Examining urine pH, specific gravity, and urine protein over time in healthy dogs. National Veterinary Scholars Symposium.
- Ryan Mrofchak's Master's Defense: An Analysis of Canine Urine: Microbiota, Methods, and Changes in Health and Disease. April 2021.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Understanding and stopping persistent Ancylostoma caninum egg shedding in chronic shedders
Principal Investigator (PI)	Antoinette Marsh
Co-PIs/Co-Is	Jeanette O'Quin; Stephen Horvath
Interim or Final	Final

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

Approach:
This study involved privately-owned adult Greyhounds (including recent racetrack dogs) or other breeds presenting with persistent *A. caninum* egg shedding despite prior deworming. Eggs were collected at the start for genetic analysis. The dogs received a combination treatment protocol comprised of the original combination therapy (topical moxidectin, followed by oral pyrantel and febantel within 24 hours) or a modification combination therapy (topical moxidectin, followed by oral pyrantel within 24 hours and three days of oral fenbendazole). At about 14 days post-treatment, a fecal examination monitored for parasite eggs and for egg count reduction. Dogs remained on the monthly combination treatment protocols and fecal egg monitoring until they ceased shedding detectable eggs or for six months whichever occurred sooner. We then recommended the dogs remained on the monthly topical moxidectin maintenance treatment and periodically be checked or when fecal consistency changed.

Results:
Greyhound dogs that received the original combination therapy (topical moxidectin, followed by oral pyrantel and febantel within 24 hours) cleared and showed better response than the modification combination therapy (topical moxidectin, followed by three days of oral fenbendazole). In two cases the Greyhound dogs did not clear and required protocol modification (off-label drug use). In a non-greyhound breeding facility, the dogs immediately cleared with just the moxidectin treatment. In this latter kennel, three of the dog hookworm strains were characterized as fenbendazole resistant.

Relevance & Impact to Canine Health:
Fenbendazole resistant hookworms are present in Ohio and are present in non-greyhound dogs (first report to date). As Ohio contains many commercial dog breeders who use both pyrantel and fenbendazole as part of their puppy deworming program, initiation of fecal parasite monitoring in puppies (5-8 weeks of age) should be done to ensure puppies are not being sold that are shedding drug resistant hookworms. Puppies are being sold and transported across state lines. Outwardly these puppies may appear healthy, yet their feces could contain transmissible stages of this parasite.

Conclusions:
Dogs with positive hookworm fecal samples should be treated and re-tested at 10-14 days to ensure clearance of hookworm shedding. Centrifugation fecal flotation rather than passive flotation should be done to detect hookworm eggs. Fecal egg count reduction testing should be done on any hookworm positive dogs. Dogs that fail to clear or show significant reduction in fecal egg counts following treatment with topical moxidectin should be considered potentially infected with multi anthelmintic drug resistant hookworms and should be reported to the drug manufacturer. The project results also provided justification for surveying Ohio Commercial Dog Breeders and their knowledge about canine hookworm transmission. A majority of the respondents did not know or could not identify the multiple ways that canine hookworm transmission occurs.

Scientific Publications/Presentations/Grant/Training Submissions:
Marsh and Lakritz. 2023 Reflecting on the past and fast forwarding to present day anthelmintic resistant Ancylostoma caninum-a parasite of increasing concern we neglected to forecast. Int J Parasit: Drug and Drug Resist. 2023, 22:36-43.

Randolph, Winston, Bremer, and Marsh. 2022. Evaluation of a triple dewormer protocol to treat canine hookworms, presented at the American Association of Veterinary Parasitologists, Snowbird Utah (oral presentation & published abstract). Resident received student travel award for presentation.

Marsh. 2024. Hooks are everywhere… Don't go barefoot at the beach. Midwest Veterinary Conference, Columbus, OH (oral presentation and published proceedings). Attendees: DVMs and RVTs, approximately 50.

Hegde, S. Veterinary Students-Summer Research Scholars Program-Professional Students, Ohio State University, summer 2022 and 2023, support for project supplies and reagents required.

Presentation and published abstracts:
Hegde and Marsh. 2023. *In vitro* growth of *Ancylostoma caninum* supported by heat-inactivated bacteria, presented at the American Association of Veterinary Parasitologists, Lexington, Ky (oral presentation & published abstract). Student received student travel award for presentation.

Hegde and Marsh. 2023. Chemosensory behavior towards urocanic acid and inhibition of larval development by emodepside of *Ancylostoma caninum*. Veterinary Scholars Summer Research Program (poster-national meeting and presentation at OSU CVM)

Extension educational outreach
Attendance and educational booth at the Ohio Forum for Companion Animals (OFCA) in Millersburg, Ohio. Feb 24, 2024. Connected with approximately 70 individuals who were directly involved in Commercial Dog Breeding in Ohio. Educational presentation on canine parasites including canine hookworms and answered questions from the attendees.

Attendance and educational booth at 9th Annual Ohio Dog Breeding Symposium, Walnut Creek, Ohio. September 28, 2023. Connected with approximately 250 individuals who were directly involved in Commercial Dog Breeding in Ohio. Educational presentation on canine parasites including canine hookworms and answered questions from the attendees.

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

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

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

- **Aim 1**: **Determine the lowest volume of healthy canine urine that reliably generates valid shotgun metagenomic sequencing reads.** We will extract 5 different volumes of urine (5ml, 3ml, 1ml, 500ul, 200ul) across 7 different dogs using a single extraction method. We will measure total and bacterial DNA, total number of sequencing reads, proportions of host, microbial, and contaminant reads, and microbial community taxonomic and functional diversity and composition to assess the microbiome results by urine volume.

- **Aim 2**: **Identify an optimal host DNA removal method for shotgun metagenomic sequencing of canine urine by comparing results across 5 methods.** Based on Aim 1, we will select a standard urine volume for all extractions conducted in Aim 2. Urine samples from 7 different dogs will be aliquoted into 5 different host decontamination methods including: Molzym Molysis, NEBNext Microbiome DNA Enrichment, QIAamp DNA Microbiome Kit, Zymo HostZERO, and propidum monoazide. We will compare total DNA, bacterial DNA, and microbial community composition and diversity across these methods.

Results:
Aim 1, the urine volume experiment, has been analyzed and was presented at an Infectious Diseases Institute Works in Progress seminar by graduate student Zachary Lewis. For Aim 2, total and bacterial DNA have been analyzed and compared across all methods and were selected for presentation by undergraduate Angela Scott at the Denman Undergraduate Research Forum. Microbial community profiling (16S rRNA) and shotgun metagenomic sequencing has now also been completed and analyzed for Aim 2 and Zach Lewis is drafting this manuscript as part of his Master's thesis. The manuscript will be submitted to a journal for review by July of 2024.

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

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

Conclusions:

- Based on 16S rRNA sequencing, healthy canine urines samples >1 ml were most effective for consistent profiling of the urobiome
- We shotgun sequenced the Zymo Gut Standard, a positive control containing 21 known taxa, at ten-fold dilutions ranging from 0.07 to 7 ng/ul. This yielded 11 total taxa. Taxa at low abundances were not detected at any dilution indicating that even with moderate DNA concentrations, rare taxa recovery via shotgun metagenomics is challenging.
- Healthy dogs varied in the amount of total and bacterial DNA present in their urine.
- QIAamp Bacteremia, which lacked a host DNA removal step, maximized total and bacterial DNA yields. However, no bacterial DNA was detected in a sample spiked with host cells indicating that this kit may not be effective for bacterial DNA in high host biomass samples.
- NEBNext Microbiome yielded total but not bacterial DNA indicating that this kit may not be effective for extracting bacterial DNA from low biomass urine samples.
- Molzym MolYsis and QIAamp DNA Microbiome demonstrated effective host DNA removal in low biomass urine samples.
- QIAamp DNA Microbiome and Propidium Monoazide demonstrated effective host DNA removal in samples with high host biomass.
- The QIAamp DNA Microbiome kit recovered the greatest numbers of microbial taxa while effectively reducing host cells/DNA, indicating that this method may be optimal in the presence of samples that contain high host cell burden.
- Shotgun metagenomic profiling at the read level was feasible and dog but not extraction method had a greater impact on overall urobiome profile; although microbial community variations by extraction method were still detectable.
- MAG assembly is feasible from urine samples, but does not capture the true urobiome diversity observed in 16S or metagenomic read level analyses.

Publications/Presentations/Grant Submissions:

Presentations

- (Submitted) J.M. Mason, Z.J. Lewis, A. Eleftheriou, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, D. Dhawan, B. Husbands, D. Knapp, V.L. Hale. April 2024. Fecal and urine microbiota of companion dogs vary by breed and sex. Midwest Microbiome Symposium – West Lafayette, IN.
- (Submitted) Poster: Z.J. Lewis, A.Scott, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. May 2024. Urobiome 101: Optimizing DNA extraction and host cell depletion to enable shotgun metagenomic studies on the urine microbiome. Midwest Microbiome Symposium – West Lafayette, IN.
- (Submitted) Poster: Z.J. Lewis, A.Scott, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. April 2024. Urobiome 101: Optimizing DNA extraction and host cell depletion to enable shotgun metagenomic studies on the urine microbiome. Ohio State University Microbial Communities Symposium.

- J.M. Mason, Z.J. Lewis, A. Eleftheriou, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, D. Dhawan, B. Husbands, D. Knapp, V.L. Hale. April 2024. Fecal and urine microbiota of companion dogs vary by breed and sex. College of Veterinary Medicine Research Day.
- Z.J. Lewis, A.Scott, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. April 2024. Urobiome 101: Optimizing DNA extraction and host cell depletion to enable shotgun metagenomic studies on the urine microbiome. Ohio State University College of Veterinary Medicine Research Day.
- Poster: Z.J. Lewis, A. Scott, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. October 2023. Technical difficulties: Standardizing methods for characterizing the canine urobiome. Veterinary Cancer Society Annual Conference - *Reno, NV*.
- Poster: Z.J. Lewis, A. Scott, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. August 2023. Technical difficulties: Standardizing methods for characterizing the canine urobiome. Bladder Cancer Advocacy Network Think Tank, Urothelial Carcinoma across Species Translational Summit - *Washington D.C*.
- Poster: Z.J. Lewis, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. May 2023. Urine limbo: determining minimum urine volume for effective characterization of canine urinary tract microbiota. Midwest Microbiome Symposium: Creating and Responding to Change – *Columbus, OH*.
- Z.J. Lewis, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. April 2023. Urine limbo: determining minimum urine volume for effective characterization of canine urinary tract microbiota. Ohio State University College of Veterinary Medicine Research Day.
- Z.J. Lewis, C. Madden, S.S. Justice, A. Rudinsky, J. Hokamp, V.L. Hale. March 2023. Urine limbo: determining minimum urine volume for effective characterization of canine urinary tract microbiota. Ohio State University Infectious Diseases Institute Works in Progress Seminar.
- A. Scott, C. Madden, S. Faith, J. Hokamp, S. Justice, A. Rudkinsky, V.L. Hale. March 2022. How Low Can You Go: Optimizing microbial DNA extraction in low biomass urine samples from health dogs. Denman Undergraduate Research Forum.

Submissions

- V. Hale, S. Justice, A. Rudinsky, B. Husbands were awarded funds for a clinical trial involving the canine urine microbiome: Can probiotics improve clinical outcomes in bladder cancer?: Addition of probiotic *Escherichia coli Nissle 1917* to a vinblastine / piroxicam protocol for the treatment for urothelial carcinoma in dogs
- V. Hale received an NIH K08 in September 2023. The grant is relevant to the urine microbiome and included preliminary data generated as part of this canine project.
- V. Hale received an Ohio State University Presidential Postdoctoral Scholar's Program Training Grant to support postdoctoral researcher Audra Crouch for two years as she expands work on urine metagenomics
- Pending (Submitted April 2024): Bladder Cancer Advocacy Network Research Innovation Award, PI: V. Hale. Title: Polycyclic aromatic hydrocarbons and bladder cancer: What's microbial metabolism got to do with it? This proposal included preliminary data generated from this canine grant.
- Pending (Submitted Jan 2024): Comprehensive Cancer Center Pelotonia Junior Investigator Award, PI: V. Hale. Title: Exploring the estrogenic potential of polycyclic aromatic hydrocarbon metabolism by the urobiome and its role in bladder cancer. This proposal included preliminary data generated from this canine grant.

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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	**Association of microscopic endometrial lesions observed in intraoperative c-section uterine biopsies to infertility in the bitch**
Principal Investigator (PI)	Christopher Premanandan
Co-PIs/Co-Is	Gail McRae, Marco Coutinho Da Silva, Erin Runcan
Interim or Final	Final

Introduction:
Endometrial biopsies have been used historically as a diagnostic tool as part of breeding management in some domestic species. In the mare,the findings in an endometrial biopsy can relate to the ability of that mare to carry a pregnancy to term. The value of the endometrial biopsy in the canine has not been fully established.

Approach:
In this study, the presence of inflammation, fibrosis, and endometrial cysts were chararacterized in full-thickness endometrial biopsies from subfertile bitches and compared to the same findings in fertile bitches. The hypothesis was that subfertile bitches will have more inflammation, fibrosis, and endometrial cysts. Full-thickness uterine biopsies were taken at the hysterotomy site at time of c-section for control cases (n=103), and previous submissions were collected from The Ohio States Reproductive Service as subfertile samples (n=263). The cases were then blinded, randomized, and evaluated. Chi-square or Fisher's exact tests were used to compare categorical variables between control and experimental samples. Wilcoxon rank sum test was used to compare ages between the groups.

Results:
Overall inflammation was observed more frequently in the subfertile group when compared to the control group. Lymphoplasmacytic inflammation was observed more frequently in the subfertile group when compared to the fertile group. Periglandular fibrosis and endometrial cysts were not significantly different between the two groups.

Relevance & Impact to Canine Health:
While canine endometrial is often assessed microscopically to investigate infertility, the significance of lesions observed is not certain. The first step in determining the significance of this diagnostic test is to determine the lesions that observed more frequently in a population of subfertile bitches when compared to a population of fertile bitches. This information can be used to more accurately guide clinicians when assessing cases of canine infertility.

Conclusions:
Despite limitations due to the retrospective nature of this study, some interesting conclusions can be made. Most notably, lymphoplasmacytic inflammation within the canine endometrium and in the deeper tissues of the uterus may be an indicator of subfertility. In addition, full-thickness uterine biopsies may be required to detect deeper lesions associated with subfertility. This study also demonstrated that the presence of fibrosis and single endometrial cysts can be observed in fertile bitches as defined by this study. Prospective studies utilizing entire uterine samples combined with complete reproductive histories will be necessary to further refine the usage of uterine biopsies as a prognostic tool for canine infertility.

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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs
Principal Investigator (PI)	Audrey Wanstrath, DVM, MS, Dipl.ACVS-SA
Co-PIs/Co-Is	Nina Kieves, Turi Aarnes, Rikki Horne, Phillip Lerche, Juli DiMichele
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 up to 45 dogs with unilateral cranial cruciate ligament rupture (similar to an ACL tear in humans). Dogs will be treated by tibial plateau leveling osteotomy (TPLO), which is the current gold standard surgical therapy. Dogs will be randomly assigned to 1 of 3 groups: gabapentin only, carprofen only, or gabapentin + carprofen treatment. All investigators will be blinded to each dog's treatment group. While in hospital, dogs will be examined regularly for pain using the Glasgow Composite Pain Scale and treated with injectable rescue medication if perceived to be painful. Dogs will be discharged from the hospital 2 days after surgery and will receive their assigned pain medication(s) regularly for 2 weeks post-operatively, during which time owners will have access to an oral rescue medication if they feel their dog is painful. In addition to pain scoring, we will also walk the dogs on a pressure sensing mat - an objective measure of lameness - pre-operatively, 2-days post-operatively, and at 2-weeks post-operatively. Additionally, we will be testing drug levels in blood samples to ensure that the orally provided medications are reaching therapeutic levels. Based on pain scale and lameness evaluations, we will determine if there is a difference in post-operative pain between the 3 medication treatment groups and will be able to determine if gabapentin provides measurable pain relief.

Results:
We are in the final stages of enrollment of cases. The data from the cases completed thus far was evaluated by a statistician to determine if we had enough cases to find a significant difference between groups. There was no difference identified so we will continue to enroll cases with the goal of getting close to 45 cases enrolled.

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. Once completed, a manuscript will be prepared for submission to a veterinary journal.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	A pilot study on the role of *Staphylococcus pseudintermedius* toxins and virulence regulators in canine pyoderma
Principal Investigator (PI)	Lorch, Gwendolen
Co-PIs/Co-Is	Cole, Lynette Diaz-Campos, Dubraska Diaz Vergara, Sandra Montgomery, Christopher 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:

We will define the role of toxin expression in the severity of skin infections (pyoderma) and the relationship between the immune response and 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 a board-certified veterinary dermatologist. 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:

Twelve healthy dogs and 13 dogs with pyoderma were enrolled. A total of 34 *S. pseudintermedius* isolates were obtained from the colonization or infections sites of dogs. There were 23 different multilocus sequence types (MLST), of which 14 were new MLST types. The most common *agr* group was group II (n=16), followed by group IV (n=7), group III (n=6), and group I (n=5). Nine of 34 isolates were methicillin-resistant *S. pseudintermedius* carrying *mecA* gene. Two isolates carried *expA*, 7 carried *expB*, and 20 carried *siet*. Analysis for other exotoxins was pending.

Twelve serum samples from healthy dogs and 13 paired serum samples from dogs with pyoderma were obtained. Analysis of the toxin expression via quantitative real-time polymerase chain reaction and toxin production via high-performance liquid chromatography in the bacterial isolates and the level of antibody in the serum is 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 against bacterial toxins and to define the association of antibody level with disease severity, which is critical for future vaccine development.

Conclusions:
The conclusion is pending.

Publications/Presentations/Grant Submissions:
A manuscript will be forthcoming.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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 continues to slowly enroll patients with 13/16 patients enrolled at this time. Thus far, we have had a very low number of metastatic lymph nodes in the population. Fortunately, all have imaged well and we have been able to create a robust OCT training image library of metastatic and non-metastatic nodes. Initial imaging findings were recently reported by Yi-Fan Shen (DVM Class of 2025) in his poster presentation at the 2024 CVM Research Day.

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

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

Publications/Presentations/Grant Submissions:
No publications have been submitted.
A retrospective analysis of outcome in 99 patients with OMM who either underwent surgery alone or surgery with cervical lymphadenectomy was submitted to Veterinary and Comparative Oncology in December 2022. This manuscript was rejected and is under author revisions for resubmission.

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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Evaluation of SpO2 to FiO2 ratio with PaO2 to FiO2 ratio in dogs receiving high flow nasal cannula oxygen therapy for hypoxemic respiratory failure
Principal Investigator (PI)	Jiwoong Her
Co-PIs/Co-Is	Edward Cooper, Page Yaxley
Interim or Final	Interim

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

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

Results:
The study targeted to obtain 240 data collection. So far, targeted data is collected and the correlation analysis is completed. The results indicated that PF ratio and SF ratio are well correlated. Further analysis is ongoing.

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

Conclusions:
Conclusion will be drawn based on the analysis of final results.

Publications/Presentations/Grant Submissions:
This project is funded by OSU College of Veterinary Medicine Office of Research and Graduate Studies Canine Grant.

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

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

Approach:
We initially identified 25 dogs diagnosed with presumed EPAC in the The Ohio State University College of Veterinary Medicine Anatomic Pathology autopsy and surgical biopsy service archives; of these cases, upon further review 16 dogs had a confirmed diagnosis of EPAC. Beyond these cases at OSU, we have recruited collaborators from 5 institutions, and from these we have confirmed the diagnosis of EPAC in 36 additional cases, resulting in a current count of 52 cases undergoing analysis. Our original goal was to include at least 50 total cases in the study, and so we have exceeded that goal currently. In this study, we will 1) characterize unique microscopic features from these tumors in order to develop guidelines for microscopic subtypes of EPAC; 2) develop novel biomarkers for each subtype in order to further aid in diagnosis and 3) correlate these microscopic subtypes and biomarkers to clinical information (example: breed, age, sex, survival rate) for each dog. We hypothesize that a combination of unique microscopic features and novel biomarkers will allow for identification of distinct subtypes of canine EPAC. In turn, by correlating microscopic and biomarker features with clinical data, we predict that we will identify links between these distinct subtypes and clinical outcomes for canine patients.

Results:
To date, we have completed initial histologic evaluation and subtyping for all 52 cases, and have completed in-depth histologic and immunohistochemical analysis on 27 cases; completion of these aims for the remaining 25 cases is ongoing. Clinical data has been collected for a subset of cases and is currently being completed for remaining collaborator cases. Histologic subtypes characterized include acinar-non-hyalinizing (29/52), acinar-hyalinizing (18/52), mixed (3/52), and ductal (2/52). Beyond subtype, additional histologic features characterized included degree of differentiation, cell death, mucin production, fibrosis, and acinar-to-ductal cell transition (metaplasia). Across all subtypes of those cases evaluated to date, mucin production has been associated directly with differentiation, while fibrosis was inversely associated with differentiation. Next, we successfully developed 3 new biomarker tests that identified acinar cells, and tested each case for labeling for these biomarkers in addition to 2 ductal biomarkers. Acinar cell biomarkers were most strongly positive in acinar-hyalinizing cases, which were the most well-differentiated tumors. Loss of acinar cell biomarkers was most common in poorly-differentiated areas of tumors. Regardless of subtype, in the cases currently evaluated, the majority of tumors showed some degree of positive labeling for ductal biomarkers, which was interesting given the lack of overt ductal histologic appearance. Last, clinical information has

been currently gathered for the initial 16 cases; senior dogs (11/16 dogs >10 years old) were overrepresented. Clinical signs, breed, sex, and location of the tumor was variable, and metastatic disease was common (11/16 cases), with the liver being the most commonly affected organ.

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

In addition to the impact this study will have on canine health, this study will also serve as a critical step in career development for Dr. Schreeg and Dr. Jeong, two veterinary pathologists who are passionate about understanding and, in turn, combatting pancreatic disease and cancers in dogs as well as other veterinary patients. Important career development steps have already occurred for both of these individuals as a result of this study (see below).

Conclusions:
While conclusions are currently limited due to in progress evaluation of half of the study cases, we can draw some preliminary conclusions. First, currently it appears that acinar EPAC subtypes are most common in dogs; this is in contrast to humans, and it is possible that dogs may serve as a natural model for the acinar EPAC subtype. Further, irrespective of subtype, the degree of differentiation was related to the degree of mucin production and the degree of fibrous tissue deposition in the tumor, suggesting that these may be important features to further evaluate for prognosis. Second, we have been able to confirm that 3 novel biomarkers (trypsin, amylase, and lipase) are helpful markers for identification of neoplastic acinar cells in dogs. Further, despite the lack of ductal subtypes, many EPAC tumors were positive for ductal biomarkers; this may suggest that transformation of acinar cells to ductal cells may promote cancer development.

Publications/Presentations/Grant Submissions:
The initial data on the 16 confirmed canine EPAC cases was presented as a poster by Dr. Jeong at the Annual Meeting for American College of Veterinary Pathologists (ACVP), an international meeting for advancement of veterinary pathology. In addition, this study has served as a springboard for Dr. Schreeg to be invited as a contributing author to a chapter on exocrine pancreatic neoplasia in Tumors in Domestic Animals. Initial formulation of this data into a manuscript for submission to Veterinary Pathology is ongoing. Further, this study is setting the foundation for 1) preparation of a review article on EPAC in veterinary patients and 2) preparation for studies investigating similar features and biomarkers of EPAC in feline and equine patients.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Analysis of detection and genotyping of *Giardia*, an elusive zoonotic protozoa
Principal Investigator (PI)	Antoinette Marsh
Co-PIs/Co-Is	
Interim or Final	Interim

Introduction:
There is increasing evidence that *Giardia* occurs frequently in domestic dogs. This is a potential public health concern since some genotypes are zoonotic. *Giardia* is the one of the most common enteric parasites of dogs. Prevalence rates vary and are influenced by the sampling strategies and diagnostic methods. The diagnostics of *Giardia* are problematic because of their size and difficulty in visualizing the parasite on a fecal examination. Antigen based assays are expensive and are not the first line of screening for wellness checks or for gastrointestinal diagnostics. At the OSU CVM, the primary diagnostic test used is the centrifugation fecal flotation. In 2019 and 2020, the prevalence of *Giardia* detected in VMC dogs was 6.67% and 7.6% respectively.

Approach:
We genotyped banked and newly acquired *Giardia* isolates from dogs seen at the VMC. At project start, we had 36 *Giardia* samples banked (from the preceding 16 months) from dogs seen at the VMC (dogs seen for either wellness and clinic visits associated with gastrointestinal signs). We anticipated that genotypes associated with human infections would be detected in dogs along with a majority of D-assemblages primarily associated with dog infections. These results will assist in addressing risk factors associated with dogs shedding *Giardia* and the likelihood of having an assemblage capable of being transmitted to humans.

Results:
Of 59 positive *Giardia* fecal samples, 26 (44%) were specifically D (dog-specific) assemblage. The two cat-derived samples did not have the D assemblage. Nearly 56% of the *Giardia* strains from small animal testing were not D-assemblage. This is surprising and more investigation is needed. This suggests that dogs are transiently passing or infected with potentially other assemblages that are zoonotic (A or B) or ones that are not associated with human infections (C, E, G, G and H). As part of the samples collected in this study, a DVM student visited 6 Ohio commercial dog kennels, collecting >10 samples per kennel. At all 6 kennels as least one sample collected was positive for *Giardia* cysts and one kennel had 50% of the samples collected demonstrated *Giardia* cysts.

Relevance & Impact to Canine Health:
As Ohio contains many commercial dog breeders who use a variety of drugs as part of their puppy deworming program to target *Giardia*, initiation of fecal parasite monitoring in puppies (5-8 weeks of age) should be done to ensure puppies are not being sold that are shedding this parasite. Using the rotational drugs in their deworming protocols and the potential high transmission will select for drug resistant strains (as seen for hookworms). Puppies are being sold and transported across state lines. Outwardly these puppies may appear healthy or slightly ill, yet their feces could contain transmissible stages of this parasite. Based on our results, 6 out of 6 kennels had detectable *Giardia*. There is a strong likelihood that dogs in these facilities have *Giardia* although clinical signs may not be obvious. Also while with a commercial dog broker, puppies may be in mixed housing groups or close contact which can also lead to transmission of agents between dogs, and the parasites acquired while in a pet store just prior to new ownership.

Conclusions:
Giardia is a potential zoonotic parasite of dogs. Asymptomatic dogs can be a source of infection for other dogs, susceptible hosts, including humans, depending on the assemblage the dog is shedding. Environmental or water contamination serves as an ongoing source of exposure as the cysts can survive for months. Some dog day care environments pose a greater risk for dogs particularly when an asymptomatic shedder brings it into the facility. *Giardia* is commonly found in dogs housed within animal shelters or in research dogs. By evaluating and determining the *Giardia* assemblage, we can better understand the zoonotic risk and provide this information to veterinarians in this region.

Publications/Presentations/Grant Submissions:

Marsh, Leutenegger, Lozoya. More than a whiate box: Molecular diagnostic parasitology testing incorporated into student training, presented at the American Association of Veterinary Parasitologists, Lexington, Ky (oral presentation & published abstract); approximately 40 in attendance.

Zentkovich. Veterinary Students-Summer Research Scholars Program-Professional Students, Ohio State University, summer 2023, support for project supplies and reagents required.

Presentation and published abstracts:
Zentkovich, O'Quin, Wittum and Marsh. 2023. Prevalence of enteric pathogens in dog breeding facilities (poster presentation).

Extension educational outreach:
Attendance and educational booth at the Ohio Forum for Companion Animals (OFCA) in Millersburg, Ohio. Feb 24, 2024. Connected with approximately 70 individuals who were directly involved in Commercial Dog Breeding in Ohio. Educational presentation on canine parasites including canine *Giardia* and answered questions from the attendees.

Attendance and educational booth at 9th Annual Ohio Dog Breeding Symposium, Walnut Creek, Ohio. September 28, 2023. Connected with approximately 250 individuals who were directly involved in Commercial Dog Breeding in Ohio. Educational presentation on canine parasites including canine Giardia and answered questions from the attendees.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Surgical margin assessment for canine soft tissue sarcoma with enhanced optical imaging and deep learning
Principal Investigator (PI)	Laura Selmic
Co-PIs/Co-Is	Ping Zhang
Interim or Final	Interim

Introduction:
Surgery is the most common treatment used for skin cancer in dogs. A pathologist determines whether surgery has removed all cancer cells many days after the procedure. We need rapid and accurate testing during surgery to detect residual cancer to decrease cancer recurrence and the need for more surgery or treatments. Polarization-sensitive optical coherence tomography (PS-OCT) is an imaging technology that uses light waves to generate high-resolution images of the microscopic structure of tissues. Many images are generated when the tumors are scanned after removal. We recently completed a study using PS-OCT in dogs with soft tissue sarcoma. The proposed study will focus on the use of artificial intelligence for PS-OCT image interpretation to determine if the tumor was completely removed. This project will open the door to veterinarians having the technology for accurate real-time surgical margin assessment to minimize the need for additional treatments and decrease tumor recurrence.

Approach:
The *purpose of this study* is to develop and compare the accuracy of deep learning (DCNN) for determining surgical margin status following STS resection in dogs, based on spectral domain-OCT (SD-OCT) images alone, and PS-OCT and SD-OCT images. The *central hypothesis* of this study is that the DCCN trained using PS-OCT and SD-OCT images will have a **higher accuracy for determining surgical margin status** compared to the DCNN trained on SD-OCT images alone. Our hypothesis is based on the high accuracy of PS-OCT for differentiation of tissue types in human breast cancer, and our own preliminary experience using DL for assessment of OCT images. If this approach shows promise this will likely offer valuable and complementary real-time information to guide canine and feline cancer treatment. The specific aims of the proposal are:

Aim 1: Develop and compare the accuracy of separate DCNNs for determining surgical margin status based on SD-OCT images alone, and SD-OCT and PS-OCT images of surgical specimens following STS resection in dogs and compare the accuracy of these DCNN.

Aim 2: Determine if the addition of PS-OCT metrics [including retardation, optical axis, and degree of polarization uniformity (DOPU)] to DCNN improves accuracy compared to the DCNN trained with SD-OCT and PS-OCT images.

Results:
We have a PhD student working on this project from the Department of Computer Science and Engineering. The student received training on OCT and PS-OCT image interpretation. We have an image data set of SD OCT and PS-OCT images, and he has created a DCCN models to differentiate cancerous tissue images from non-cancerous images. Initial results are promising, next we will test models within the different combinations to assess our study aims. We have a biweekly team meeting to discuss progress and address questions.

Relevance & Impact to Canine Health:
Soft tissue sarcoma is a common malignant canine skin tumor, where surgical excision is considered the frontline treatment. This tumor type presents unique therapeutic challenges related to its locally invasive biological behavior. Local disease control in the form of complete surgical margins is still one of the most important factors in determining prognosis and recommendations for adjunctive therapies. In veterinary medicine, histopathologic margins for small and moderate sized tumors are generally assessed by cross sectioning (radial method). In this method, the specimen is cut through the center of the tumor on the short axis and then each section is cut again perpendicular to the original cut along the long axis. Using this type of sectioning results in **less than 1% of the total histopathologic margin being**

evaluated. Assessment of such as small portion of the surgical margin makes missed incomplete or "dirty" margins a real possibility, especially for tumors such as STS, which often have invasive and asymmetric growth. Failure to recognize incomplete margins can result in **inappropriate treatment recommendations and missed opportunities for local disease control**. Unrecognized incomplete margins may also be a possible contributor to the phenomenon of local recurrence rates despite clean margins. When incomplete margins are present this is concerning for residual microscopic tumor in the patient so for STS either revision surgery or radiation therapy is often recommended depending on the tumor location and amount of resectable tissue. Utilizing these adjunctive therapies for STS can result in a long-term favorable prognosis for the patient.

Conclusions:
We have made good progress and have built and are validating the DCCN to assess the images. In the next 6-month period we will work to assess the accuracy of the DCNN for margin assessment for canine STS.

Publications/Presentations/Grant Submissions:
Manuscript being drafted. Presidents research excellence grant received by Ping Zhang for continuing this work in humans. Planned AKC CHF and NIH R01 grant submissions later in 2024.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Feasibility and agreement study between ICTL and ICGL for sentinel lymph node mapping in dogs with visceral tumors
Principal Investigator (PI)	Janis Lapsley
Co-PIs/Co-Is	Laura Selmic, Eric Green, Megan Schreeg
Interim or Final	Interim

Introduction:
Lymph node (LN) evaluation is crucial to assess if a tumor has already spread beyond the local site. The presence of neoplastic cells in LN(s) may have important ramifications on a patient's survival time and may affect the clinicians' recommendations for adjunctive treatment. Identification of which LN is draining lymph from the tumor (sentinel LN) can be challenging as this may not be the closest LN to the tumor. Different techniques have been used to identify the sentinel LN, but to our knowledge, none were applied to gastrointestinal tumors in dogs and only two papers described the technique for pulmonary LN mapping in normal dogs. The goal of this study is to evaluate the ability of a preoperative (indirect computed tomography lymphangiography [ICTL]) and an intraoperative (indocyanine green [ICGL]) technique to identify the sentinel LN and compare the agreement between the two techniques in dogs with primary pulmonary or gastrointestinal tumors.

Approach:
The purpose of this study is to evaluate the feasibility of preoperative indirect computed tomography lymphangiography (ICTL) and intraoperative indocyanine green lymphangiography (ICGL) to identify the sentinel lymph node in pulmonary and gastrointestinal tumors as well as evaluate the agreement between the two techniques. Identification of the sentinel lymph node should allow more accurate staging of the patient and possibly decrease patient's surgical morbidity. The use of two different techniques will increase the ability to identify all sentinel lymph nodes, as suggested by prior studies. The central hypothesis of our study is that both ICTL and ICGL would have a high identification rate of sentinel lymph nodes and that ICGL would detect more lymph nodes than ICTL. This hypothesis is based on previous canine studies on oral tumors, as ICTL for canine pulmonary and gastrointestinal tumors has not been performed.

Aim 1: Investigate the feasibility of ICTL and ICGL in detecting the sentinel lymph node(s) in dogs with naturally occurring primary pulmonary and gastrointestinal tumors

Aim 2: Report the agreement between ICTL and ICGL in detecting sentinel lymph node(s)

Results:
No results are currently available. Starting enrollment for this study was delayed due to transfer of the grant to the new PI (Dr. Lapsley) and availability of the ICG contrast. Additionally, when we did start enrollment in the fall of 2023, price increases across the VMC have greatly impacted the intended scope of the project. In order to obtain a useful amount of information, we have limited patient enrollment to dogs with GI tumors at this time and aim to enroll 4-6 dogs with GI tumors, budget allowing. Thus far we have enrolled 2 patients and continue to seek additional patients for enrollment.

Relevance & Impact to Canine Health:
Cancer is a common problem in old dogs, and it is estimated that one in four dogs will be affected by it in their lifetime. Primary pulmonary and gastrointestinal tumors are uncommon in dogs. The reported median survival time (MST) for pulmonary and intestinal tumors is approximately 1 year. Multiple studies have shown that the presence of metastatic lymph nodes with lung tumors has a negative impact on survival time, with a reduction in survival from 456 to 167 days. Similarly, a study on intestinal tumors reported a MST of 15 months for patients without lymph node metastasis, compared to 3 months for the one with lymph node metastasis. Preoperative CT is a more sensitive test to identify potential lymph node metastasis in dogs with primary pulmonary neoplasia compared to radiographs. Unfortunately, the sensitivity of CT in identifying neoplastic lymph nodes is only 83%, and a more recent study detected a relatively high presence of neoplastic cells in lymph nodes apparently normal on CT and at surgery. Comparable information is not available for gastrointestinal tumors, but as the most commonly reported tumor in both locations is a carcinoma,

the metastatic behavior could be assumed to be similar for tumors in these two locations. Currently, when dealing with primary pulmonary and gastrointestinal tumors, veterinary surgeons are unsure of which lymph node to biopsy if no abnormalities are seen on preoperative imaging. As ICGL imaging becomes more readily available in veterinary hospitals, this modality can help to identify lymph nodes of interest and thus improve patient staging and help inform prognosis.

Conclusions:
This project aims to investigate an emerging diagnostic imaging and LN mapping tool, ICG, in order to better serve our patients.

Publications/Presentations/Grant Submissions:
No publications have been submitted as of yet. We plan to submit results to either Veterinary and Comparative Oncology or Veterinary Surgery.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Establishing the role of the virulome and resistome in *Enterococcus*-associated urinary tract infections
Principal Investigator (PI)	Wittum, Thomas E.
Co-PIs/Co-Is	Ballash, Gregory A; Diaz-Campos, Dubraska
Interim or Final	Interim

Introduction:
Urinary tract infections (UTIs) are one of the most common infections in small animal medicine, affecting approximately 15-20% of dogs at least once in their lifetime. Among the notable causes of UTIs, *Enterococcus* spp. are of particular concern because they cause up to 10% of first time UTIs, but nearly 25% of recurrent UTIs. Moreover, while the pathogenesis of *Enterococcus* in humans has been worked out and requires catheterization, the mechanism for canine *Enterococcal* UTI remains unknown as it does not require catheterization. One potential mechanism for their success is their capacity to form biofilms in the lower urinary tract where they resist treatment and incite recurrent UTI that increase morbidity and frequency of long-term sequelae like renal scarring. Despite this recent observation, the mechanisms that *Enterococcus* use to cause UTI and persist in the canine bladder are largely unknown. We hypothesize that dogs with comorbidities and previous antimicrobial use will be more likely to develop *Enterococcus* UTI that form biofilms, and these strains will harbor a diverse and robust virulome and resistome that facilitates colonization of the urinary tract.

Approach:
Enterococcus isolated from dogs with clinical UTIs will be phenotypically assessed for their capacity to form biofilms and resist common antibiotic therapy using a standard crystal violet absorbance assay and broth microdilution method, respectively. In addition, these isolates will be sequenced using next-generation whole genome sequencing technologies to identify whole genome-based relatedness, antimicrobial resistance genes, and virulence genes necessary to form biofilms and cause UTIs. Patient demographic and clinical history will be collected via retrospective medical record review for each of the UTI isolates. These data will be aggregated to identify patient profiles that are at risk for developing *Enterococcus* UTIs that are antimicrobial resistance or biofilm forming. Moreover, we will compare the whole genome sequences developed in this study to lab and probiotic strains of *Enterococcus* and human UTI-associated *Enterococcus* to identify virulence profiles that facilitate UTI and biofilm formation in canine patients.

Results:
As of April 8th, 2024, we have collected clinical and epidemiological information, conducted biofilm and phenotypic antimicrobial resistance assays, and sequenced 95% of the isolates in our *Enterococcus* library. In addition, we have randomly selected 250 *Enterococcus* from human urinary tract infections and identified probiotic and laboratory strains of *Enterococcus* for comparative genomic use. From this preliminary data, most canine UTI-associated *Enterococcus* carry resistance to at least one of the common antibiotics used to treat these infections. Similarly, 75% of the isolates can form a biofilm and 25% of isolates can form a moderate to strong biofilm community. A comparison of biofilm formation to antimicrobial resistance suggests that *Enterococcus* that are resistant to ampicillin and tetracycline do not commonly form moderate to strong biofilms. *Enterococcus* that form moderate to strong biofilms are at 38% fewer odds of forming a biofilm for each antibiotic they are resistant to. These findings are similar to another common uropathogen, *Escherichia coli*. A preliminary investigation of the virulome and resistome identified a negative correlation, where those *Enterococcus* that carry fewer resistance genes tend to carry more virulence genes and vice versa. These findings are similar to those previously reported in *E. coli* causing urinary tract infection. These findings suggest two broad classifications of uropathogens – low virulence/high resistance and high virulence/low resistance that colonize the urinary space in companion animals. Comparative genomics are underway to identify virulence and resistance genes underlying these associations.

Relevance & Impact to Canine Health:
Urinary tract infections are one of the most common infections affecting canine patients. While a standard dose of antibiotics should resolve uncomplicated infections, UTIs caused by Enterococcal spp. are causing frequent UTI recurrence. Repeated UTIs result in prolonged morbidity, additional office visits, client dissatisfaction, and repeated antibiotic exposure that can promote the development of future resistant infections. How Enterococcal spp. cause these UTI in canines and why they are so different from humans is a fundamental knowledge gap in understanding this disease. Our proposed genomics survey of *Enterococcus* associated UTI will address some of these key knowledge gaps and identify virulence and resistance genes associated with common virulence and resistance phenotypes. In addition, we will identify known and novel virulence factors that contribute to the establishment of *Enterococcal* UTI and how those differ from *Enterococcus* associated UTIs in humans. In doing so, we will generate a foundational network on the phenotypic and genotypic predictors of *Enterococcus* UTI that lay the groundwork for future studies on pathogenic mechanism, diagnostic utilities, and predictors of disease.

Conclusions:
At this stage of our grant, it is difficult to make concrete conclusions. However, we expect to identify antibiotic resistant phenotypes and resistance and virulence genotypes that are associated with and might contribute to biofilm formation among *Enterococcus* spp. that cause UTIs. In addition, we will generate a clinical characterization of canine patients at risk of developing an *Enterococcus* UTI with the capacity to form recalcitrant biofilms. Lastly, we expect to identify individual and groups of virulence and resistance genes that contribute to *Enterococcus*-associated UTIs for both *E. faecalis* and *E. faecium*

Publications/Presentations/Grant Submissions:
We will use the remaining time on this grant (Dec. 2024) to finish the comparative genomic analysis. Upon completion we plan on submitting a publication to Microbiology Spectrum and American Journal of Veterinary Research. We will be presenting this data at the National Institute for Antimicrobial Resistance Research and Education in May of this year. We are using the current data to support a grant submissions to the American Kennel Club (May 15th, 2024), and as part of a R21 grant (Fall/Winter 2024).

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Investigating the Fecal Origins of Uropathogenic Escherichia coli in Canine Recurrent Urinary Tract Infections
Principal Investigator (PI)	Dubraska Diaz-Campos, DVM, PhD
Co-PIs/Co-Is	Nora Jean Nealon, DVM, PhD; Jenessa Winston, DVM, PhD, DACVIM (SAIM); Adam Rudinsky, DVM, DACVIM (SAIM); Joany Van Balen, DVM, PhD; Thomas Wittum, PhD
Interim or Final	Interim

Introduction:

Urinary tract infections (UTIs) are among the most common reasons why dogs and people are prescribed antibiotics. Recurrent UTIs require repeated, long-term use of antibiotics and contribute to multidrug resistance. Uropathogenic E. *coli* (UPEC) are the most common cause of UTIs in dogs and people. Canine and human research supports that UPEC originate from the gastrointestinal tract, and it serves as a reservoir for recurrent infections. However, this reservoir is not well-characterized in dogs, nor is the impact of antibiotic use on multidrug resistance within the gut microbiota. The goal of this study is to examine canine feces as a UPEC reservoir and compare long-term gut microbiota (including gut residing UPEC) responses to antibiotic treatment in dogs with and without multidrug resistant UPEC recurrent UTIs. The findings from this study will improve prevention and treatment of canine UTIs, enhance antibiotic stewardship, and have translational impact to improve human UTI management.

Approach:

We are using a combined culture-based and genomics approach to examine how antibiotic therapy and antibiotic resistance impact canine gut UPEC populations. All dogs will be recruited through the OSU Veterinary Medical Center following diagnosis of a recurrent UTI by their attending veterinarian. A diagnosis of a UPEC versus non-UPEC recurrent UTI as well as multidrug resistant versus non-resistant will be made by OSU's Microbiology service using urine from each patient.

Feces are the most common method for characterizing the canine gut microbiota. Owners will bring in a fecal sample from their pet before while on antibiotics for their UTI, and then one, four, and eight weeks after completing treatment.

Culture-based analyses will examine if each patient harbors antibiotic resistant bacteria in their feces and how these bacteria change over the course of antibiotic treatment for the recurrent UTI. Fecal samples collected by owners will be placed onto standard microbiology laboratory media prepared with and without antibiotics in the media. Growth of bacteria on plates with antibiotics will be used as an indicator of antibiotic resistance within the gut microbiota.

Genomics analysis will occur through the OSU Applied Microbiology Services Laboratory using a metagenomics approach, which establishes what bacteria are within the gut microbiota as well as identifies the genes they carry. Consequently, metagenomics is a powerful tool that will allow us to identify UPEC strains in canine feces as well as to determine if UPEC carry genes that can cause antibiotic resistance.

Results:

A. Culture-based analysis: A*ntibiotic resistance is prevalent in healthy canine feces:*

As proof of concept for the culture-based methods proposed in this study, Dr. Nealon has characterized the antibiotic resistance profiles of eighteen healthy, adult canine fecal donors screened through OSU's Companion Animal Fecal Bank. The feces from all dogs showed abundant bacterial growth when cultured onto routine microbiology media. All eighteen dogs demonstrated resistance to at least one antibiotic, where more than 50% of dogs sampled showed resistance to two or more antibiotics. In turn, we are confident that when applied to canine patients in this study, that our culture-based approaches will allow us to successfully detect antibiotic resistance within this population.

B. *Patient Recruitment and Clinical Metadata Collection:*

Efforts are underway for the recruitment of patients in this study. We have currently enrolled 13 dogs, 10 of which have completed the study as of 4/19/2024. Over 400 canine urine culture records have been screened for eligibility. All patient metadata, including signalment, antibiotic courses, urine culture susceptibility results, and comorbidities, have been documented by Dr. Nealon and will be helpful when interpreting phenotypic and genotypic resistome profiles in our future planned analysis. We plan to use this larger metadata archive to evaluate/update antibiograms for canine recurrent urinary tract infections in central Ohio, as well as to drive additional collaborative analyses that examine antibiotic use, prevalence of comorbid conditions, and other features of urinary tract infections in companion dogs examined at OSU.

C. Ongoing metagenomics analysis:
We anticipate that this analysis will occur during the late fall of 2024 through to the early spring of 2025.

Relevance & Impact to Canine Health:
This award has been used to help Dr. Nealon (postdoctoral fellow/co-investigator on this proposal) successfully secure extramural funding to expand on the metagenomic analysis started with this project. During March 2023, Dr. Nealon was awarded a two-year Morris Animal Foundation Research Training Fellowship (~$125,000 total award). This award, when combined with the support of the Canine Grant, will be instrumental in Dr. Nealon's career development as she transitions from a postdoctoral scholar into an independent investigator position. Dr. Nealon has actively been working under this fellowship since July 2023 and will continue to do so through July 2025.

Furthermore, this award is being used to further ongoing studies through OSU's Microbiology and Small Animal Internal Medicine services that examine the role of the beneficial microbe (probiotic) Escherichia coli Nissle on canine gut UPEC populations (PI Dr. Adam Rudisnky). Owners who successfully complete this study will be offered the opportunity to have their dog participate in an ongoing clinical trial assessing how oral supplementation with E. coli Nissle probiotic impacts levels of gut UPEC, to understand if this probiotic can be used as an effective and affordable treatment in the management of canine recurrent UTIs. Probiotic supplements have been ordered for eligible dogs as of March 2024, and 10 total dogs are currently eligible for enrollment in this study, which will collect feces 2-, 4-, and 8-weeks following E. coli Nissle supplementation.

Conclusions:
The objectives of this proposal were, in companion dogs with recurrent urinary tract infections, to examine the impact of antimicrobial therapy on UPEC fecal reservoirs using an integrated culture-based and metagenomic approach. To date, study objectives are being achieved and enrollment is progressing as anticipated. Dr. Nealon has been actively working with owners and the OSU Veterinary Microbiology Service to enroll patients, process samples, and compile valuable metadata that will assist not only with this analysis, but provide a database that can be leveraged for future collaborative projects, including those which can potentially involve veterinary students and other OSU research trainees and personnel. The investigator team looks forward to future analysis and outcomes for this project over the coming months and through early 2025.

Publications/Presentations/Grant Submissions:
Nealon, NJ et al. Investigating the Fecal Origins of Uropathogenic Escherichia coli in Canine Recurrent Urinary Tract Infections. ~$124,982.00. Morris Animal Foundation, Fellowship Training Grant. (6/2023-7/2025)
Nealon, NJ et al. Investigating antimicrobial resistant Escherichia coli in canine fecal donors and recipients. Grant submitted to L'Oréal USA For Women in Science. Submitted 2/2024, awards will be announced summer 2024/TBD. ~$59,018.30

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Effects of oral premedication with gabapentin and trazodone combination on the MAC of isoflurane in dogs
Principal Investigator (PI)	Brittney Carson
Co-PIs/Co-Is	Jake Aiello, Turi Aarnes, Mary McLoughlin, Audrey Wanstrath, Gianluca Bini
Interim or Final	Interim

Introduction:
Premedications are medications used prior to anesthesia that can help reduce the amount of inhalant anesthesia and limit the inhalant's negative side effects (e.g. low blood pressure). Trazodone and gabapentin are two commonly used oral medications in veterinary medicine, both of which cause sedation in dogs. When given by mouth as premedications, both trazodone and gabapentin have each been shown to reduce the amount of an inhaled anesthetic needed to prevent movement in previous studies.

Approach:
Six healthy dogs will be randomly assigned to be administered no premedication or oral trazodone (8 mg/kg) and gabapentin (20 mg/kg) two hours prior to induction of isoflurane anesthesia in a cross over design. Dogs will be anesthetized twice and receive both treatments during the study with a minimum of 7 days between treatments.

Results:
This study determined that oral administration with oral trazodone(8mg/kg) and gabapentin (20mg/kg) 2 hours prior to induction of anesthesia decreases the dose (MAC) of inhalant anesthesia by 33%. There were no impacts on vitals throughout the experiments with premedication or no premedication.

Relevance & Impact to Canine Health:
The goal of this study was to determine if trazodone and gabapentin given together as a premedication will reduce the amount of inhaled anesthesia required to prevent response to stimulation in anesthetized dogs; We learned that it does decrease this amount. Information obtained in this study will further veterinarians' understanding of the value of by mouth premedications for reducing inhalant anesthetic dosing. The amount of dose (MAC) reduction we observed in this study (33%) is more than the amount of dose reduction we expect with trazodone (17%) or gabapentin (20%) when used alone as oral premedications based on previous studies. When using this premedication, we can reduce the dose dependent negative effects of inhalant anesthesia.

Conclusions:
This combination of trazodone (used as an oral premedication in dogs decreases the dose of inhalant anesthesia when administered 2 hours prior to anesthesia induction. The amount of dose (MAC) reduction we observed in this study (33%) is more than the amount of dose reduction we expect with trazodone (17%) or gabapentin (20%) when used alone as oral premedications based on previous studies.

Publications/Presentations/Grant Submissions:
Manuscript is currently in progress with intended submission to the American Journal of Veterinary Research by the end of June 2024.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (non-technical report) to the Ohio General Assembly	
Title	Advancing Bordetella bronchiseptica Research through Pathomics
Principal Investigator (PI)	Kara Corps
Co-PIs/Co-Is	Rajendar Deora; Purnima Dubey; Giovanni Lujan; Samuel Neal
Interim or Final	Interim

Introduction:
Bordetella bronchiseptica (Bb), one of the causes of kennel cough and a model for whooping cough, continues to cause widespread respiratory disease in dogs, humans, and other mammals globally. It is critical to improve our understanding of how Bb causes disease to enhance preventative and therapeutic measures. We aim to use artificial intelligence (AI) to quantify the changes in the lungs of mice infected with Bb to study Bb infections in dogs. We then will use this novel data to determine predictors of a highly effective immune response, enabling us and other groups to design therapeutics to induce such an immune response. This approach may also provide a template for use in other infectious disease research.

Approach:
We will utilize a repository of mouse lung specimens from previous research on Bb infections to train AI-based algorithms to extract quantitative data from mouse lung specimens. This will be an iterative process where a pipeline of AI algorithms in Visiopharm will be trained to 1) extract relevant pathology data encompassing all expected pathologic changes, and 2) other findings of unknown diagnostic significance. These models will be thoroughly trained, tested, and validated to assure their accuracy. The data extracted by the pipeline will then be combined with other information about the mice including physiologic, diagnostic, and immunologic data. All of this information combined will be used to create predictive models of optimal immune responses and lung function. These models will enable our groups to better characterize known aspects of the diseases caused by Bb and begin discovering new, important information that could lead to better prevention and treatment strategies. These models will also ideally be applied to other mouse models of infectious bacterial lung diseases like whooping cough, as well as lung diseases caused by viruses.

Results:
This project is in its early stages. Relevant "tissue detection" and "tissue compartmentalization" models have been created utilizing 52 H&E-stained murine lung samples. The “tissue detection” model differentiates tissue from non-tissue, and it is performing well enough for us to move into the second phase. The “tissue compartmentalization” model is performing well but inconsistently identifying small structures, like capillaries. To resolve this, a higher magnification compartmentalization model is being developed to supplement the existing model through more fine-tuned training. A thorough “funneling strategy” to capture all desired pathologic changes (starting with large, more obvious ones and gradually moving to finer details) has been created and is in the process of being implemented to begin work on single cell identification.

Relevance & Impact to Canine Health:
Given the continued morbidity caused by Bb in the canine population despite widespread vaccination, this project is highly relevant and impactful to canine health. Our ability as veterinarians, public health officials, and dog lovers to provide our canine companions with long, healthy and happy lives is enhanced when we thoroughly understand and characterize illnesses that affect them. The project seeks to further our understanding of disease caused by Bb in a novel manner to strengthen our ability to understand the optimal immune response to Bb, which is critical to preventing an infection or fighting one off should it occur. Further, the project will allow for the assessment of the ability of AI trained by skilled lung infectious disease researchers and veterinary pathologists with expertise in novel digital pathology approaches to drive forward infectious disease research. We hope also to possibly provide a template for its implementation in future infectious disease research, including study of similar pathogens like *Bordetella pertussis*, the bacterium that causes whooping cough, and various viruses that cause respiratory diseases.

Conclusions:
Our preliminary models are promising and proceeding through training at a steady pace. Further model development should move quickly over the next year and allow for the incorporation of quantitative pathology data with other relevant data about the mice in multimodal predictive models. No unforeseen challenges have arisen that would hinder future progress, and our group is excited about adding higher numbers of samples to our training and testing sets to enhance model performance.

Publications/Presentations/Grant Submissions:
An NIH R21 grant proposal was submitted on February 16th, 2024, titled "Multimodal pathomics approach for quantitative pathology in murine infection models" with the goal of extending this approach to multiple respiratory pathogens. No publications or presentations have been made regarding the project given its infancy, but we anticipate beginning to present our work later this calendar year.

FUNDING OF PROJECTS	
TITLE	**BUDGET**
Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the canine gut microbiota and bile acid metabolome	$22,633
Pilot study on the effects of intra-articular allogenic stem cell therapy for the treatment of osteoarthritis	$22,727
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
Understanding and stopping persistent *Ancylostoma caninum* egg shedding in chronic shedders	$27,188
How low can you go?: Low volume, low biomass urine microbiome sequencing optimization	$27,271
Association of microscopic endometrial lesions observed in intraoperative c-section uterine biopsies to infertility in the bitch	$13,807
Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs	$22,727
A pilot study on the role of *Staphylococcus pseudintermedius* toxins and virulence regulators in canine pyoderma	$26,919
Evaluation of OCT for metastatic lymph node identification in dogs with oral malignant melanoma	$25,358
Evaluation of SpO2 to FiO2 ratio with PaO2 ratio in dogs receiving high flow nasal cannula oxygen therapy for hypoxemic respiratory failure	$17,180
Retrospective histologic and immunohistochemical characterization of canine exocrine pancreatic adenocarcinoma and correlation to clinical features and outcome	$22,520
Analysis of detection and genotyping of Giardia, an elusive zoonotic protozoa	$12,474
Surgical margin assessment for canine soft tissue sarcoma with enhanced optical imaging and deep learning	$27,273
Feasibility and agreement study between ICTL and ICGL for sentinel lymph node mapping in dogs with visceral tumors	$24,794
Establishing the role of the virulome and resistome in *Enterococcus*-associated urinary tract infections	$27,250
Investigating the fecal origins of uropathogenic *Escherichia coli* in canine recurrent urinary tract infections	$27,200
Effects of oral premedication with gabapentin and trazadone combination on the MAC of isoflurane in dogs	$5,870
Advancing Bordetella bronchiseptica research through pathomics	$30,000

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.*

	A	B	C	D	E	F	G	H
1				No. of Tags Sold				
2	**County**	**Invoice**	**Amt Paid $**	**1 - YR**	**3 - YR**	**PERMNT**	**KENNEL REG**	NOTES
3	Adams County Auditor	1	$705.60	6,328	150	27	8	
4	Allen County Auditor	1	$1,555.00	14,603	172	33	101	
5	Ashland County Auditor	1	$875.10	7,658	218	36	19	
6	Ashtabula County Auditor	1	$1,203.50	9,090	511	106	352	
7	Athens County Auditor	1						
8	Auglaize County Auditor	1	$898.60	7,516	320	51	0	
9	Belmont County Auditor	1	$698.10	5,494	280	56	87	
10	Brown County Auditor	1	$874.90	7,573	221	50	13	
11	Butler County Auditor	1	$5,617.00					INCLUDES BOTH 2022 AND 2023 TAG SALES
12	Carroll County Auditor	1	$763.60	7,076	101	24	17	
13	Champaign County Auditor	1	$706.50	6,499	105	15	151	
14	Clark County Auditor	1	$1,884.20	16,624	566	51	10	
15	Clermont County Auditor	1	$1,840.40					
16	Clinton County Auditor	1	$725.30	6,217	181	48	13	
17	Columbiana County Auditor	1	$2,081.00	17,210	707	147	20	
18	Coshocton County Auditor	1	$915.00	8,392	43	8	549	
19	Crawford County Auditor	1	$840.30					
20	Cuyahoga County Auditor	1						
21	Darke County Auditor	1	$1,189.50	11,107	138	31	64	
22	Defiance County Auditor	1	$707.70	6,229	177	24	77	
23	Delaware County Auditor	1	$2,010.10					
24	Erie County Auditor	1	$1,141.10	11,388	2	1	7	
25	Fairfield County Auditor	1	$2,250.70					
26	Fayette County Auditor	1	$378.50	3,186	120	23	9	
27	Franklin County Auditor	1	$9,161.00					
28	Fulton County Auditor	1	$738.00	6,349	235	28	46	
29	Gallia County Auditor	1	$122.10	1,063	15	11	3	
30	Geauga County Auditor	1						
31	Greene County Auditor	1	$2,469.26	18,932	1,220	208	26	
32	Guernsey County Auditor	1	$628.60	5,329	187	34	56	
33	Hamilton County Auditor	1						
34	Hancock County Auditor	1	$1,295.00	11,934	214	24	143	
35	Hardin County Auditor	1	$664.50					
36	Harrison County Auditor	1	339.3	3161	25	14	17	

	A	B	C	D	E	F	G	H
37	Henry County Auditor	1	$1,204.40	11,080	179	31	117	INCLUDES BOTH 2022 AND 2023 TAG SALES
38	Highland County Auditor	1	$1,142.30	9,526	354	69	145	INCLUDES BOTH 2022 AND 2023 TAG SALES
39	Hocking County Auditor	1	471.6	4086	156	13	32	
40	Holmes County Auditor	1	858.8	7278	19	1	1243	
41	Huron County Auditor	1	$1,062.00	9,560	241	32	17	
42	Jackson County Auditor	1	$839.00	6,328	145	30	135	
43	Jefferson County Auditor	1	$426.80	3,384	189	31	7	
44	Knox County Auditor	1	$955.00	7,981	294	62	67	
45	Lake County Auditor	1	$2,369.20					
46	Lawrence County Auditor	1	$618.60	5,845	63	15	2	
47	Licking County Auditor	1	$2,743.70	24,257	798	76	26	
48	Logan County Auditor	1	$591.60					
49	Lorain County Auditor	1	$2,413.30	21,354	614	92	17	
50	Lucas County Auditor	1	$4,560.60	38,298	1,762	200	22	
51	Madison County Auditor	1	$531.00	4,361	237	19	48	
52	Mahoning County Auditor	1	$2,833.70	24,819	764	99	241	
53	Marion County Auditor	1	$829.80	6,805	277	53	132	
54	Medina County Auditor	1	$2,264.30	16,764	1,208	188	75	
55	Meigs County Auditor	1	$216.30	1,754	88	13	15	
56	Mercer County Auditor	1	$382.50	3,720	21	0	42	
57	Miami County Auditor	1	$1,761.70	13,742	735	158	90	
58	Monroe County Auditor	1	$317.20	2,802	88	7	36	
59	Montgomery County Auditor	1	$5,019.40					
60	Morgan County Auditor	1	$265.30	2,138	78	12	161	
61	Morrow County Auditor	1	$544.60	4,578	192	28	12	
62	Muskingum County Auditor	1	$1,160.30	1,095,377	77	21	209	
63	Noble County Auditor	1	$132.60	1,190	13	2	77	
64	Ottawa County Auditor	1						
65	Paulding County Auditor	1	$388.30					
66	Perry County Auditor	1	$579.80					
67	Pickaway County Auditor	1	$1,403.20	9,838	536	214	446	INCLUDES BOTH 2022 AND 2023 TAG SALES
68	Pike County Auditor							**HAVE NOT RECEIVED 2022 AND 2021**
69	Portage County Auditor	1	$3,112.90	25,010	1,292	218	63	
70	Preble County Auditor	1	$668.90	5,992	79	35	110	INCLUDES BOTH 2022 AND 2023 TAG SALES
71	Putnam County Auditor	1	$737.30	6,619	98	20	260	
72	Richland County Auditor	1						

	A	B	C	D	E	F	G	H
73	Ross County Auditor	1	$1,275.80	12,075	6	1	655	
74	Sandusky County Auditor	1	$1,203.90	10,695	231	50	151	
75	Scioto County Auditor	1	$480.90	3,592	179	42	26	
76	Seneca County Auditor	1	$1,026.00	8,784	278	63	12	
77	Shelby County Auditor	1						
78	Stark County Auditor							**Have NOT received payment for 2020, 2021 and 2022**
79	Summit County Auditor	1	$3,455.10					
80	Trumbull County Auditor	1	$1,675.60	14,013	558	63	439	
81	Tuscarawas County Auditor	1	$1,569.20	14,065	258	77	83	
82	Union County Auditor	1	$782.60					
83	Van Wert County Auditor	1	$415.80	3,664	98	19	10	
84	Vinton County Auditor	1	$203.90	1,568	5	0	456	
85	Warren County Auditor	1	$2,697.40	19,882	1,548	243	18	
86	Washington County Auditor	1	$973.20	8,004	358	64	14	
87	Wayne County Auditor	1	$2,205.70	16,108	1,042	141	1,413	
88	Williams County Auditor	1	$536.50					
89	Wood County Auditor	1	$2,241.80	15,510	1,357	276	77	
90	Wyandot County Auditor	1	$453.80	3,497	270	22	11	
91								
92		**Total:**	**$109,882.66**	**1,704,901**	**22,693**	**3,850**	**9,000**	
93	**NOTES:							
94	**Payment still not received as of 4/26/24**							
95								
96	**Payment still not received for multiple years including 2023**							