THE OHIO STATE UNIVERSITY

College of Veterinary Medicine

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30 April 2020

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

RE: Annual Report for Canine Research Funds

Dear Ms. Zhan:

Please find enclosed the annual report describing the research performed by The Ohio State University College of Veterinary Medicine with the support of the Canine Research Fund. As you know, ten cents of each one year, thirty cents of each three year, and one dollar of each permanent Ohio county dog license fee is set aside in a fund to support small canine research grants, which are administered by The Ohio State University College of Veterinary Medicine. Details of the grant review process are provided in the report. Included in this annual report are 8 final and 20 interim progress and new project reports of research ranging from different types of cancer to improving techniques on joint and bone repair.

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 Green

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
2019

Submitted to:
The Ohio General Assembly

May 2020

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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 $125,000-$140,000. The money in its entirety is assigned to The Ohio State University College of Veterinary Medicine for distribution as small grants to College faculty.

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

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

Each grant will be reviewed by two council members. The reviewers will provide a written critique of each grant and, in open session, will share that critique with the rest of council. The critiques of each grant will be distributed to the principal investigator of each grant for their information. Council members who have a conflict of interest or who are directly involved in implementation of the grant are excused from the proceeding during that grant's review. Upon completion of the oral critique and following discussion by the entire council, each council member will assign a score of 1 to 10, where 1 is the perfect score. At the end of the proceedings, all grants will be ranked by their average score for the Councils review and recommendation on funding. Typically grants receiving a score of greater than 5 are not funded. Grant funding is capped at $25,000 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

FINAL REPORT (lay report) to the Ohio General Assembly	
Title	Comparison of preoperative analgesic protocols and evaluation of chronic neuropathic pain state in dogs undergoing TPLO
Principal Investigator (PI)	Nina Kieves
Co-PIs/Co-Is	Turi Aarnes, Stephen Jones, Alexandra Kalamaras, Sarah Moore

Introduction:

Neuropathic pain is a complex, chronic pain state caused by malfunction of the somatosensory nervous system. The hallmarks of neuropathic pain are hyperesthesia and allodynia (abnormal increased sensitization to pain), which manifest as a lowered sensory threshold (ST) for response to mechanical, thermal, or other sensory stimuli. Recently, a method to evaluate ST in dogs using an electronic von Frey anesthesiometer has been validated. This technique is able to distinguish dogs with normal sensing function from those with diminished sensation (hyposensate) and those with hyperesthesia (neuropathic pain) caused by various conditions. In a pilot study of client-owned dogs with naturally occurring osteoarthritis, we have also demonstrated a lower ST consistent with a neuropathic pain state.

Approach:

The goal of this study is to compare perioperative utility of preoperative opioid administration alone (Treatment 1) versus lumbosacral epidural analgesia (Treatment 2) versus direct femoral and sciatic nerve blockade (FSNB) (Treatment 3) to prevent the development of a chronic neuropathic pain state. We evaluated each of these interventions in the context of surgical repair for spontaneous cruciate ligament (CCL) rupture in client-owned dogs. We hypothesized that preoperative opioid medication alone will provide equivalent immediate postoperative analgesia when compared to the other two treatment groups; however, the incidence of neuropathic pain as evidenced by diminished ST would be lower in dogs that received an epidural or FSNB compared to those who receive opioids alone.

Results:

Forty-five dogs were enrolled (n=15/group). There were no significant differences among treatments for age, weight, sex distribution, surgical limb, meniscal status, or contralateral cruciate status or for ST at any time point nor across time within groups, though there was a trend for affected limbs to have a diminished ST compared to control non-operated limbs. Sedation scores for Treatment 1 were higher than Treatments 2 & 3 ($p=0.0098$) across time, and all sedation scores decreased over time. The average pain scores using 2 validated pain scales were lower for Treatment 3 than Treatments 1 & 2 across time ($p=0.0139$ and 0.0024 respectively). No dogs required rescue analgesia at any time point. No significant differences were seen in gait analysis between groups at any time point.

Relevance & Impact to Canine Health:

Validating the use of ST to evaluate neuropathic pain in dogs with orthopedic disease will allow future study of treatment of neuropathic pain that will be translational to a public health issue facing a significant portion of the population. While we saw a trend towards a difference in ST in operated limbs, no significant difference was seen. It is possible that with larger sample size, and longer follow up (i.e. > 8 weeks) we may have seen a difference.

In regards to immediate post-operative evaluation of pain and sedation, dogs in treatment group 3, had significantly lower pain scores than the other treatments. As no dogs in any treatment groups required rescue analgesia it is likely that all protocols provided adequate short-term perioperative pain management. Additionally, treatment group 1 had significantly higher sedation scores than the other groups at all time points assessed expect 24 hours post-operatively. This should be considered when making anesthetic plans for cases where higher levels of sedation may be contraindicated.

Conclusions:
Based on the current study, perioperative analgesic protocol does not appear to significantly impact long-term ST in dogs with spontaneous CCLR. Although all treatments appeared to provide adequate analgesia, femoral-sciatic blockade presented the best combination of analgesia and less sedation in the post-operative period.

Publications/Presentations/Grant Submissions:
This data was presented as an abstract at the 47th Annual Veterinary Orthopedic Society Conference February 2020. A portion of the data (immediate 24 hour post-operative data) has been submitted for publication in the **Veterinary Anesthesia** and **Analgesia** journal. Data related to the ST will be submitted separately to Veterinary Record by summer 2020. Finally, data has been submitted for abstract presentation at the 2020 ACVS Surgery Summit.

FINAL REPORT (lay report) to the Ohio General Assembly	
Title	Validation of PRMT5 as a candidate therapeutic target in canine lymphoma
Principal Investigator (PI)	William C. Kisseberth
Co-PIs/Co-Is	

Introduction:

Lymphoma is a common, highly malignant, cancer in dogs. Although it is generally initially highly responsive to combination chemotherapy with traditional cytotoxic drugs, remission times are short and cures infrequent. New treatment strategies are clearly needed. PRMT5 is a cellular enzyme whose expression is commonly dysregulated in cancer cells. New drugs developed to inhibit the activity of this enzyme show promise as anticancer therapies. In this set of experiments, we determined the prevalence and relevance of PRMT5 expression in a large set of lymphoma biopsy samples collected from affected dogs. We then characterized the molecular and cellular consequences of changes in PRMT5 expression in cancer cells *in vitro*. We then tested the ability of the new PRMT5 inhibitor drug, PRT220, to kill canine lymphoma cells, testing this drug on both dog lymphoma cell lines and cells collected directly from affected dogs. Finally, we sought to characterize changes in whole-genome chromatin accessibility in the presence and absence of PRMT5 inhibition and evaluate the effect of PRMT5 inhibition on global (whole transcriptome) gene expression.

Approach:

For this study, the goals were to determine that the drug "target" (the cellular enzyme PRMT5) is present in canine lymphoma and characterize in what types of lymphoma it is expressed and where it is localized in the cell. To do this we performed immunohistochemistry (a special stain) for PRMT5 on over 300 previously characterized canine lymphoma samples. The second goal was to determine the effects of an anti-PRMT5 drug, PRT220, a PRMT5 small molecule inhibitor, on canine lymphoma cells. To do this we treated canine lymphoma cell lines and lymphoma cells collected directly from canine patient tumors and treated them with different PRMT5 inhibitor drugs. After treatment, we are characterizing the effects of the drugs on canine lymphoma cell cytotoxicity (ability to kill the cells), apoptosis (programed cell death), target (histone and protein) methylation, and changes in gene expression.

Results:

We determined that PRMT5 is expressed in varying amounts in most canine lymphomas, with approximately half of tumors having high expression. In canine lymphoma, PRMT5 is expressed primarily in the cell cytoplasm, but occasionally it is present in the nucleus, particularly in specific subtypes of T cell lymphoma. We demonstrated that PRMT5 inhibition leads to growth suppression and induction of apoptosis in canine lymphoma cell lines CLBL-1, 17-71, OSW, and primary patient lymphoma cells in a time and/or dose-dependent manner, while selectively decreasing symmetric dimethylarginine (SDMA) marks on H4R3. Further, we found that canine lymphoma cell lines have varying sensitivity to PRMT5 inhibitors. For the PRMT5 inhibitor PRT220, the diffuse large B-cell (DLBCL) cell line was most sensitive, inhibiting cells in the high nanomolar range. The T-cell line OSW had intermediate sensitivity, demonstrating activity in the micromolar range. The B-cell lymphoma cell line was relatively resistant. Treatment of lymphoma cells collected directly from patient tumors also demonstrated sensitivity to PRMT5 inhibitors, generally of approximately similar sensitivity as OSW, i.e. micromolar range. We utilized Assay for Transposase-Accessible Chromatin, using sequencing (ATAC-seq) to determine global chromatin structure (euchromatin vs. heterochromatin) that occurred with PRMT5 inhibition. The canine B-cell lymphoma cell line 17-71 was treated with the selective PRMT5 inhibitor, PRT220. Results of the ATAC-seq experiment showed 953 individual regions with changes to chromatin accessibility with inhibition of PRMT5 resulting in altered expression 4,395 genes based on Affymetrix gene expression arrays.

Relevance & Impact to Canine Health:

Lymphoma is a common, highly malignant, cancer in dogs. Specific breeds are at greater risk for developing lymphoma (e.g. Golden Retrievers, Boxers); however, lymphoma occurs in all breeds and in mixed breed dogs. Lymphoma represents about 7-24% of all canine neoplasia and 83% of hematopoietic malignancies. Although lymphoma is generally initially highly responsive to combination chemotherapy with traditional cytotoxic drugs (e.g. CHOP-cyclophosphamide, doxorubicin, vincristine, prednisone), the median survival time is only about one year and cures are rare. Little progress or improvement has been made over the past 20 years in treating canine lymphoma with these drugs. New treatment strategies are clearly needed. This research is highly relevant in that it addresses an unmet need for a common cancer of dogs, i.e. a new therapeutic target for lymphoma, and further enhances the preclinical development of PRMT5 inhibitor drugs being developed by OSU investigators. Results of this study provide the necessary supporting evidence for PRMT5 inhibition as a relevant therapeutic target for canine lymphoma and provide justification for a clinical trial in dogs with lymphoma.

Conclusions:

We have validated PRMT5 as a relevant target for therapy in canine lymphoma and the use of canine lymphoma cell lines cell lines for preclinical evaluation of new therapies targeting PRMT5. Demonstration of PRMT5 target modulation and in vitro/ex vivo antitumor activity provides experimental evidence for justifying the further clinical development of these drugs, or analogs, for treating dogs with lymphoma and facilitating the preclinical development of PRMT5 inhibitors for treating cancer in dogs and people.

Publications/Presentations/Grant Submissions:

1. Renaldo K, Sloan S, Chung JH, Chung J, Courtney L, Shilo K, Kisseberth W, Baiocchi R. Exploring PRMT5 as a potential therapeutic target in canine lymphomas. *Advances in Veterinary Medicine*, College of Veterinary Medicine, The Ohio State University, 2019. Poster presentation.

2. Renaldo K, Sloan S, Chung JH, Courtney L, Shilo K, Kisseberth W, Baiocchi RA. Exploring PRMT5 as a potential therapeutic target in canine lymphomas. American Society of Hematology Annual Meeting. San Diego, CA (2018). Poster presentation.

3. Renaldo KA, Courtney LE, Shilo K, Baiocchi RA, Kisseberth WC. Validation of protein arginine methyltransferase 5 (PRMT5) as a candidate therapeutic target in the canine model of non-Hodgkin lymphoma. *Proceedings: AACR Annual Meeting 2018*; April 14-18, 2018; Chicago, IL. Poster presentation.

4. Renaldo KA, Courtney LE, Shilo K, Baiocchi RA, Kisseberth WC. Validation of PRMT5 as a candidate therapeutic target in the canine model of non-Hodgkin lymphoma. *Advances in Veterinary Medicine*, College of Veterinary Medicine, The Ohio State University, 2018. Poster presentation.

5. Courtney LE, Renaldo KA, Shilo K, Baiocchi RA, Kisseberth WC. Validation of PRMT5 as a candidate therapeutic target in the canine model of non-Hodgkin lymphoma. *2017 Merial National Veterinary Scholars Symposium*; National Institutes of Health, Bethesda, MD, August 3-5, 2017. Poster presentation.

6. Renaldo KA, Shilo K, Baiocchi RA, Kisseberth WC. Validation of PRMT5 as a candidate therapeutic target in canine B-cell lymphoma. *OSUCCC - James 18th Annual Scientific Meeting.* Columbus, OH, 2017. Poster presentation.

7. Renaldo KA, Courtney LE, Shilo K, Baiocchi RA, Kisseberth WC. Validation of protein arginine methyltransferase 5 (PRMT5) as a candidate therapeutic target in the canine model of non-Hodgkin lymphoma. *Proc Veterinary Cancer Society*, 37th Annual Conference, Portland, OR, 2016. Poster presentation.

FINAL REPORT (lay report) to the Ohio General Assembly

Title	Computed Tomography Quantification of Airflow Resistance Before and After Nasal Turbinectomy
Principal Investigator (PI)	Eric T. Hostnik, DVM, MS, DACVR
Co-PIs/Co-Is	Kathleen Ham, DVM, MS, DACVS (No longer at Ohio State University)

Introduction:

Selective breeding has produced brachycephalic breeds (Bulldog, French bulldog) with shortened skull features resulting in excessive tissue within the air passages that cause partial airway obstruction. Currently, surgical procedures are aimed at removing excessive tissue at the nasal opening and back of the throat. Visual examination of the nose and mouth is the current standard of care for surgical planning; however, intranasal structures are not visualized. We aim to use CT to characterize each dog's airway anatomy before and after surgery using a new surgical approach aimed at removing obstructing nasal turbinates. The CT scans will be used to generate computer- based models that allow quantification of airway resistance. Surgery for this disease has been performed for decades, this is the first attempt to quantify and objectively assess the effect of a new surgical method. We aim to quantitatively assess the effect of turbinate laser ablation in brachycephalic dogs.

Approach:

Privately owned purebred Bulldogs and French bulldogs are be recruited for this study from clients of The Ohio State University Veterinary Medical Center. Bulldogs and French bulldogs will be recruited due to the prevalence of brachycephalic obstructive airway syndrome (BOAS) within the breeds. Computed tomography examinations are performed using conscious sedation. The dogs are not intubated during the CT scan.

The patient will then be prepared for surgery with endotracheal intubation. The dogs will undergo holoium:YAG (yttrium-aluminum-garnet) turbinate ablation following the CT study. Guidance of the nasal turbinate ablation will be performed by assessment of the CT scans, endoscopic evaluation of the nasal turbinates, and direct visualization. Routine ventral wedge alarplasty and partial staphylectomy will be performed concurrently with the laser turbinate ablation. Computed tomography scans will be repeated at approximately 21 days and then between 90 – 110 days with similar settings as described above. The process used for model construction and meshing for CFD is outlined in previous study Hostnik 2017 (Vet Radiol Ultrasound. 2017 Sep;58(5):542-551. doi: 10.1111/vru.12531. Epub 2017 Jul 17).

Descriptive statistics including the median and standard deviation with 25% and 75% interquartile values will be calculated for the airway resistance by the operators and examined for normality using inspection of scatterplots and the Kolmogorov-Smirnov test. The measured resistance for pre- and post-surgical intervention will be compared using a paired t-Test or Wilcoxon signed rank test depending on if the data is normally distributed.

Results:

Three dogs have completely gone through the surgery and follow up procedures. Data collection has been stalled because the surgeon that was part of the project is no longer at Ohio State University. The surgeon left for a position at Michigan State University. The intention is to train and involve a newly hired faculty surgeon, Dr. James Howard, to replace Dr. Ham. Dr. Howard recently successfully passed his boards and is transitioning into the role as faculty. We are aiming to coordinate time with Dr. Ham for him to travel to Michigan in order to observe/train with the original surgeon for consistency.

The training of Dr. Howard was not possible and did not occur. There is currently no surgeon within the Ohio State University College of Veterinary Medicine that is performing this procedure.

Relevance & Impact to Canine Health:
Brachycephalic obstructive airway syndrome commonly affects popular breeds like the Bulldog and French bulldog. These are widely popular breeds and clinical disease related to airway incompetence is a very common problem – the project has direct relevance to many dogs. There is a wide spectrum of affliction between each individual dog with brachycephalic airway syndrome though the treatment is relatively uniform.

We have developed a standardized approach to evaluating the upper airway of these dogs using CT without the need for orotracheal intubation and anesthesia. Computed tomography, acquired under sedation, is used to generate a three-dimensional reconstruction of a finite element model to quantitatively assess the degree of airway resistance. The modeling and quantitative aspect of this investigation is a novel technique in veterinary medicine and to our knowledge we are currently the only group investigating clinical intervention of airway disease using this method. We adapted methods used for respiratory evaluation in human medicine to fit our canine patients. In a pilot study, we used CFD to evaluate the effect of alarplasty and partial staphylectomy on airway resistance of the nasal passage that showed an overall reduction in airway resistance in six Bulldogs at a significant level of alpha equaling 0.08. We are capable of quantitatively assessing therapeutic interventions in this disease. We can statistically compare and evaluate conventional and new surgical treatment strategies.

A technique using laser ablation of nasal turbinates has been described, though it has not been adapted into common practice. The technique was assessed by endoscopic visualization of the tissue and evaluation using cross-sectional CT; however, no quantitative value has been used to assess the effectiveness of this surgical intervention. We aim to use CFD to statistically evaluate the impact of laser ablation of turbinates on the airway resistance within the nasal passage. The potential benefit to our program is therefore substantial both for the evaluation of BOAS, but even for other body systems that can build upon the synergy of cross-sectional imaging and mathematical modeling to better understand disease and intervention in veterinary patients.

Conclusions:
No conclusions.

The project will not be completed due to a lack of surgery faculty to collaborate in this project. I recommend termination of this project and the funds be reallocated to future Canine Grants.

Publications/Presentations/Grant Submissions:
N/A

FINAL REPORT (lay report) to the Ohio General Assembly

Title	Serum cytokine concentrations in dogs with multicentric lymphoma before and after doxorubicin treatment
Principal Investigator (PI)	Dr. Megan Brown, DVM, MS, DACVIM (Oncology)
Co-PIs (if applicable)	Dr. Brittany Evans, DVM Dr. Joelle Fenger, DVM, PhD, DACVIM (Oncology)

Introduction (250 word limit):

Cellular senescence is phenomenon in which cells lose their ability to divide and can occur as a result of normal cellular aging or external insults such as exposure to chemotherapy drugs. Originally thought to protective against tumor formation, cellular senescence can also have detrimental effects in the context of cancer. This is partially due to senescent cells secreting cytokines, or signaling molecules, that promote inflammation and contribute to chemotherapy resistance, toxicity and a poorer outcome in cancer patients. Since little is known about cellular senescence in dogs, we proposed a pilot study to measure cytokine concentrations in dogs with lymphoma before and after chemotherapy (doxorubicin) treatment, as a marker of induction of cellular senescence. This project will provide a foundation for the understanding of cellular senescence across species as well as identify possible therapeutic targets for reducing side effects and improving outcome in dogs and humans with cancer undergoing chemotherapy.

Approach:

Sixteen dogs with diagnosis of lymphoma undergoing a CHOP or doxorubicin single agent chemotherapy protocol were recruited from the Integrated Oncology Service. Each dog had a definitive diagnosis of lymphoma in addition to complete bloodwork to evaluate general health and ability to receive chemotherapy treatment. Informed written consent was obtained prior to enrollment. For measurement of cytokine levels, dogs had baseline blood collection prior to starting the protocol and again before (0h) and 3-, 6- and 24-hours after doxorubicin (week 7 or 9) administration. A final blood draw will be performed one week after doxorubicin treatment. Owners also filled out a quality of life survey at screening, week 9 and week 10. Blood samples were processed and cytokine (IL-6, MCP-1) were measured via canine ELISA. Cytokine concentrations were compared from baseline, before and after doxorubicin treatment. We hypothesized that serum cytokines concentrations in dogs with lymphoma would significantly increase after doxorubicin administration due to induction of cellular senescence. Additional aims included comparing cytokine concentrations between naïve and treated dogs and determining if changes in cytokines correlated with response to chemotherapy treatment or toxicity.

Results:

All patients have been enrolled and data collection and analysis has been completed. Our results are as followed:

IL-6: IL-6 concentrations were unchanged from baseline to 0-hour but significantly decreased 1 week post doxorubicin, compared to 0-6 hour and 24-hour time points.

MCP-1: MCP-1 concentrations significantly decreased from baseline to 0-hour. Compared to 0-6 hour, MCP-1 concentrations transiently increased at 24-hour and decreased at 1 week post doxorubicin.

Changes in IL-6 and MCP-1 concentrations did not correlate with response to treatment or chemotherapy toxicities.

Relevance & Impact to Canine Health:

Cancer is now the leading cause of death in older dogs, with approximately half of dogs that live to 10 years of age or older dying of cancer. In total, an estimated 4 million dogs develop cancer each year and not surprisingly, cancer remains a top health concern for dog owners. Much of this trend can be attributed to changes in veterinary medicine, such as improvement in preventative care, increased availability of veterinary care, including specialty care, and a growing bond between pet owners and their pets. As a result, many petowners are seeking out advanced diagnostics and treatments for their pets.

Canine lymphoma is one of the most common neoplasms seen in dogs. Despite the prevalence of this disease, no significant advances in treatment or outcome have occurred in decades. The mulit-agent CHOP chemotherapy protocol remains the standard of care for most canine lymphoma patients with continued disappointing outcomes and lack of disease cure. This goal of this pilot study is to begin to understand the mechanisms of cellular senescence in dogs with lymphoma undergoing chemotherapy with a larger goal of understanding how cellular senescence contributes to chemotherapy resistance and toxicity. Given the similarities between canine and human lymphoma, data generated from this pilot study has the potential to inform future studies in both humans and dogs with lymphoma.

Conclusions:

To the investigator's knowledge, this is the first veterinary study to examine the effects of chemotherapy on cytokine concentrations in dogs with lymphoma. We hypothesized that serum IL-6 and MCP-1 levels would increase following doxorubicin chemotherapy as a result of induction of cellular senescence. While our study failed to support this hypothesis, changes in IL-6 and MCP-1 concentrations observed in our study suggest that serum cytokines are influenced by disease status and treatment, among other factors. Additionally, changes in IL-6 and MCP-1 concentrations did not correlate with chemotherapy toxicities and response to therapy although few toxicities were documented in our study and non-responders were removed from study. Further investigation of these and other cytokines to serve as biomarkers for induction of senescence, toxicity or response to treatment is recommended.

Publications/Presentations/Grant Submissions:

This manuscript was submitted for publication to the Journal of Veterinary Internal Medicine (Manuscript ID: JVIM-20-177) on 4/17/20. This manuscript serves to fulfill the requirement of Dr. Brittany Evan's combined residency/master's degree as well as the American College of Veterinary Internal Medicine (ACVIM) requirements for board certification

FINAL REPORT (lay report) to the Ohio General Assembly	
Title	Influence of neo-adjuvant steroid administration on histologic margins in canine cutaneous mast cell tumor
Principal Investigator (PI)	Vincent Wavreille
Co-PIs/Co-Is	Joelle Fenger, Ryan Jennings

Introduction:

Mast cell tumors (MCT) are the most common skin cancer in dogs. Surgery with a wide margin to include normal surrounding tissue is the most commonly prescribed treatment for canine MCT. Surgery requiring the excision of large volumes of cutaneous and subcutaneous tissue can be associated with pain and discomfort, and an increased risk for surgical wound complications. Several studies have assessed the use of pre-operative corticosteroids to reduce the overall size of MCTs. However, the effect of corticosteroid-mediated tumor size reduction to the histological quality of the surgical margins has not been established.

Approach:

The proposed study tried to identify the efficacy of corticosteroids steroids in reducing microscopic MCT tumor burden, and in turn, the effect of steroid-associated tumor reduction in the context of surgical margin planning.

Results:

Preliminary data generated from the trial patient biopsies demonstrated that adequate numbers of mast cells were not present. This precluded analysis of these samples. Additional sections were examined and unfortunately these did not identify mast cells. This is an unanticipated limitation of the study design.

In addition, patient enrollment was lower than anticipated: it remains challenging to meet patient inclusion criteria at OSU (a tertiary referral institute).

Relevance & Impact to Canine Health:

Based on the the aforementioned challenges & the preliminary data generated from the first patient biopsies, the decision was made to close the study.

Conclusions:

Due to the challenges mentioned, this study was closed.

Publications/Presentations/Grant Submissions:

N/A

FINAL REPORT (lay report) to the Ohio General Assembly

Title	Interrogating the expression and function of *WWOX* in canine mast cell tumors
Principal Investigator (PI)	Joelle M. Fenger, DVM, PhD, DACVIM (Oncology)
Co-PIs/Co-Is	

Introduction:

Mast cell tumors (MCTs) are the most common skin tumor in dogs. Their behavior varies considerably with some dogs developing benign tumors cured with surgery alone, to aggressive, highly metastatic MCTs that are refractory to multimodal therapy. Tumor suppressor genes regulate processes related to cellular division and replication. When these genes are mutated, lost, or their functions are impaired, cells can progress to cancer. Our laboratory has been studying the biological functions of the tumor suppressor gene *WWOX* that is frequently deleted in human cancers and plays a central role in repairing DNA damage. In this project, our team is investigating how *WWOX* functions in normal and malignant mast cells in hopes of gaining a better understanding of how *WWOX* deficiency promotes the aggressive behavior of canine MCTs. The objectives of this study are to 1) define the expression of the tumor suppressor gene, *WWOX* in primary canine mast cell tumors (MCTs), malignant canine mast cell (MC) lines and normal canine bone marrow-cultured mast cells (cBMCMCs); and 2) manipulate the expression of Wwox in malignant MCs to begin to define the functional consequences of Wwox loss on DNA damage repair and survival in response to DNA damaging agents. Data generated from these studies will further our understanding of the role *WWOX* plays in canine MCTs and will serve as a foundation upon which potential novel targets for therapeutic intervention can be identified, with the ultimate goal of improving outcomes in dogs with MCTs.

Approach:

To more fully characterize Wwox protein expression in spontaneous canine MCTs, we generated a tissue microarray (TMA) consisting of X primary MCTs (X low-grade MCTs, X high-grade MCTs) with matched normal skin (source of normal mast cells) and performed immunohistochemical staining/scoring for Wwox. In conjunction with our TMA, we have performed real time PCR and Western blotting to evaluate Wwox transcript and protein expression in primary canine MCTs, malignant canine mast cell lines, and normal cBMCMCs. To assess the consequences of Wwox deficiency on malignant mast cells, we have generated canine mast cell lines expressing anti-*WWOX* lentiviral shRNA constructs and studied the impact of *WWOX* knockdown on mast cell viability and clonogenic survival following treatment with DNA damaging agents (ionizing radiation).

To more critically evaluate the effects of *WWOX* loss in normal mast cell biology, we have generated a conditional transgenic mouse model that allows for *WWOX* deletion specifically in mast cells and basophils (*CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg mice). Using this model, the consequences of *WWOX* deletion can be evaluated directly in mast cells and basophils *in vivo* and i*n vitro* without the confounding influence of altered Wwox expression in other tissues.

Results:

To determine whether Wwox is dysregulated in malignant canine MCTs, we evaluated Wwox protein expression in primary MCTs, malignant MC lines, and normal cBMCMCs and found that canine BMCMCs express high levels of Wwox transcript compared to mast cell lines and tumors. We further demonstrated by Western blotting and immunohistochemistry that Wwox protein is reduced in primary MCTs and that Wwox expression is significantly lower in high-grade MCTs compared to low-grade MCTs.

The C2 canine MC line that expresses high basal levels of Wwox was transduced with scramble or anti-*WWOX* shRNAs and the BR canine MC line that expresses low level of Wwox was transduced with control vector or *WWOX* overexpression vectors. Wwox knockdown or overexpression was confirmed by real time PCR and Western blotting. We found that Wwox knockdown enhanced clonogenic survival in the C2 MC line following ionizing radiation; however, this appeared to be a cell-intrinsic effect as Wwox overexpression was not associated with changes in cell viability in BR MC line.

To critically evaluate the effects of *WWOX* loss in normal mast cell biology, we generated a transgenic mouse model of mast cell-specific *WWOX* deletion (*CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg mice). We validated that our *CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg mice

function in a tissue-specific manner and confirmed that Wwox expression is significantly decreased in BMCMCs generated from *CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg compared to *WWOX*$^{fl/fl}$-Tg mice. Histologic analysis of *WWOX*$^{fl/fl}$-Tg and *CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg mice is currently underway to better characterize the consequences of *WWOX* deletion on mast cell maturation and resident tissue mast cell numbers.

Relevance & Impact to Canine Health:

Mast cell tumors (MCT) are the most common skin tumor diagnosed in dogs. The clinical behavior of MCTs varies considerably from benign tumors which can be cured by surgical removal alone, to highly aggressive, metastatic tumors that are refractory to multi-modal therapy. Our laboratory has been studying the biological functions of the tumor suppressor gene *WWOX* that is frequently deleted in human cancers and plays a central role in repairing DNA damage. We found that Wwox expression is reduced in primary canine MCTs and malignant MC lines compared to normal cBMCMCs. Furthermore, we found that Wwox expression is significantly lower in high-grade MCTs compared to low-grade MCTs, suggesting that dysregulation of Wwox may contribute to the aggressive biological behavior of MCTs. Our *in vitro* data demonstrate that knockdown of *WWOX* in the C2 MC line enhances clonogenic survival following treatment with ionizing radiation, supporting a role for Wwox in mediating DNA damage repair. To study the role of *WWOX* in normal mast cells, we have developed a mouse model that selectively deletes *WWOX* in mast cells to study how this gene affects mast cell behavior. Our team has begun to dissect the role of *WWOX* in normal and malignant mast cell biology in hopes of gaining a better understanding of how Wwox dysregulation promotes the development and progression of MCTs. Information gained from this study will serve as a platform for better understanding how WWOX dysregulation promotes the aggressive behavior of MCTs and identify new targets for treatment.

Conclusions:

Our findings demonstrate that dysregulation of the tumor suppressor gene *WWOX* is a frequent event in spontaneous canine MCTs. Furthermore, we found that Wwox expression is significantly lower in high-grade MCTs compared to low-grade MCTs, suggesting that loss of Wwox may contribute to the aggressive biological behavior of MCTs. Our *in vitro* data demonstrate that knockdown of *WWOX* in the C2 MC line enhances clonogenic survival following treatment with ionizing radiation, supporting a role for Wwox in mediating DNA damage repair. To better study the role of the tumor suppressor gene *WWOX* in normal and malignant mast cell biology, we have developed a transgenic mouse model allowing for mast cell-specific deletion of *WWOX* (*CPA3*-Cre*; Wwox*$^{fl/fl}$-Tg mice). We validated that our *CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg mice function in a tissue-specific manner and confirmed that Wwox expression is significantly decreased in BMCMCs generated from *CPA3*-Cre*; WWOX*$^{fl/fl}$-Tg compared to *WWOX*$^{fl/fl}$-Tg mice. Data generated from this study will further our understanding of the role Wwox plays in canine MCTs and will serve as a foundation upon which potential novel targets for therapeutic intervention can be identified, with the ultimate goal of improving outcomes in dogs with MCTs.

Publications/Presentations/Grant Submissions:

This grant has supported Rebecca Makii's primary research project in the combined DVM/MS Graduate training program offered through the Graduate Program in Comparative and Veterinary Medicine at the OSU College of Veterinary Medicine. Ms. Makii is committed to a career in comparative and translational oncology and this body of work will serve as the basis for her MS thesis project and training in Comparative and Veterinary Oncology.

Publications:

Makii B, Cook H, Louke D.S., Jennings R, Premanandan C, Breitbach J, Fenger JM. Characterization of Wwox expression and function in canine mast cell tumors and malignant canine mast cell lines. Submitted to BMC Veterinary Research (April 2020).

Presentations:

Makii R, Cook H, Louke D, Fenger JM. Interrogating the role of Wwox in canine mast cell tumors and cell lines. [Poster Presentation – Ms. Makii]. American College of Veterinary Pathologists 2019 Annual Meeting. San Antonio, TX. November 9-13, 2019.

Makii R, Cook H, Louke D, Fenger JM. Interrogating the role of Wwox in canine mast cell tumors and cell lines. [Poster Presentation – Ms. Makii]. Proceedings of the 2019 National Veterinary Scholars Symposium. Worcester, MA. July 25-28, 2019.

FINAL REPORT (lay report) to the Ohio General Assembly	
Title	Plasma Cytokeratin 18 and fecal Alpha1 Proteinase Inhibitor levels in dogs with appendicular osteosarcoma before and after treatment with carboplatin
Principal Investigator (PI)	Adam Rudinsky
Co-PIs/Co-Is	Kate Taikowski, Joelle Fenger, Emma Warry

Introduction:

Chemotherapy induced gastrointestinal (GI) toxicity occurs commonly, due to the indiscriminant nature of chemotherapy in targeting rapidly dividing cells. Consequences include; poorer patient outcomes, negative impact on patient quality of life, and increased treatment costs. Damage caused by chemotherapy manifests as mucositis, and cause a variety of clinical symptoms depending on the segment of the tract which is affected. Common findings include apoptosis of enterocytes and compromise to intestinal permeability. Measurement of Cytokeratin 18 (CK18), an intracellular structural protein released during epithelial apoptosis, and Alpha1-Proteinase Inhibitor (α1-PI) in feces provides a mechanism for evaluating the intestinal mucosa. Thus, these biomarkers may help identify and predict patients at risk for GI mucositis.

Approach:

Specific Aims: (1) Measure and compare plasma CK18 levels prior to amputation to those collected prior and after carboplatin. (2) Measure and compare fecal α1-PI levels prior to amputation to those collected prior and after carboplatin. (3) Determine if changes in plasma CK18 and fecal alpha-1 protease levels following a single dose of carboplatin correlate with the development of clinical gastrointestinal toxicity. Methods and Procedures; In this prospective clinical trial dogs with a confirmed histopathologic diagnosis of osteosarcoma, who have undergone an amputation and will be receiving adjuvant carboplatin will be enrolled.

Expected Outcome: We expect plasma CK18 and fecal alpha-1 protease levels to significantly increase following carboplatin administration due to damage inflicted by carboplatin on the gastrointestinal tract. Significance: This pilot study will identify biomarkers that can be used to detect damage to the GI tract following carboplatin. In addition, this project will correlate gastrointestinal epithelial damage, as measured by CK18 and alpha-1 protease, to the clinical manifestation of chemotherapy induced GI mucositis.

Results:

The development of GI toxicosis in this study population was minimal. Mean CK18 levels were not significantly different before or after chemotherapy treatment (11.66 ± 6.52 ng/ml, 13.58 ± 8.33 ng/ml, 12.03 ± 6.57 ng/ml, mean +/- SD respectively, $p = 0.23$). Mean A1AT levels were not significantly different before or after treatment (18.28 ± 7.38 ng/ml, 15.03 ± 7.76 ng/ml respectively, $p = 0.13$).

Relevance & Impact to Canine Health:

The prevalence of cancer is increasing in companion animals, with at least 4 million dogs developing cancer per year. A Morris Animal Foundation Animal Health Survey in 1998 found that cancer was a leading cause of death in dogs, and a survey in 2005 found that cancer is the largest health concern cited among pet owners. Additionally, more owners are actively pursuing advanced diagnostics and treatment for their pets. Appendicular osteosarcoma is the most common primary bone tumor in dogs. Standard of care includes limb amputation followed by chemotherapy to address metastatic disease. While chemotherapy is generally well tolerated in companion animals, many dogs will experience gastrointestinal toxicity. This toxicity is often mild and self-limiting, but may have a significant impact on quality of life. Additionally, in veterinary medicine it is likely that we underestimate the frequency and severity of gastrointestinal toxicity due to limitations in patient/physician communication.

Chemotherapy induced GI toxicity may impact patient outcome secondary to treatment delays, dose reductions and decreased dose intensity. Given the similarities in gastrointestinal physiology between humans and dogs, we are

hopeful that the data generated from this pilot study will improve our knowledge and understanding of chemotherapy induced GI toxicity in dogs.

Conclusions:

Plasma CK18 and fecal A1AT concentrations were not clinically useful biomarkers for the detection of GI toxicosis secondary to carboplatin treatment. Further prospective evaluation of CK18 and A1AT as biomarkers of drug-induced GI toxicosis is warranted in a larger cohort of dogs receiving cytotoxic chemotherapy with more severe adverse events.

Publications/Presentations/Grant Submissions:

Presentation Target: Abstract Accepted - ACVIM June 2020 Meeting – Baltimore Maryland

Publication Target: Manuscript Submitted for Publication – Journal of Veterinary Internal Medicine

Grant Target: No current plans to submit on this topic due to negative results in this study. In the interim, plan to gather additional preliminary data to better design next phase of the study (e.g. targeted analysis of severely affected dogs).

FINAL REPORT (lay report) to the Ohio General Assembly	
Title	Pilot Study: Serum vitamin C levels in dogs with non-septic and septic critical illness
Principal Investigator (PI)	Adam Rudinsky
Co-PIs/Co-Is	Daniel Gordon, Karina Creighton, Julien Guillaumin

Introduction:

The mortality rate in sepsis ranges from 20-80%, and even higher in dogs with severe sepsis and septic shock. In humans, vitamin C levels are low in patients with severe sepsis and septic shock. The benefits of vitamin C supplementation in humans has been well documented, including reduction in time on life-supporting medications (e.g. vasopressors) and improvement in 28-day mortality rates. Vitamin C is not considered an essential nutrient in dogs as it is produced in the liver. To date, vitamin C levels have not been measured in critically-ill dogs. The goal of this study is to measure vitamin C levels in patients with severe sepsis and septic shock, and compare those levels to patients presenting in circulatory shock. We hypothesize that patients in circulatory shock will have low circulating levels of vitamin C, and that patients with sepsis will have lower levels of vitamin C than patients with other causes of shock.

Approach:

This study was aimed at testing the hypothesis that critically ill dogs, both in circulatory shock and with sepsis will have a reduction in vitamin C levels compared to healthy dogs. Understanding vitamin C status, would provide foundational data to further study the effects of vitamin C on survival in severely ill dogs.

Aim 1: To investigate the patient populations in which vitamin C levels are affected. Plasma vitamin C levels were measured in dogs using high-performance liquid chromatography. Various patients in circulatory shock (severe dehydration, acute blood loss, GDV) compared to patients with sepsis (septic peritonitis, pneumonia, urosepsis) were assessed to establish which patient populations are most affected. This helped determine whether patients with a septic process have a greater reduction in vitamin C levels compared to those in shock secondary to a non-septic process.

Aim 2: To investigate whether vitamin C levels are within or below reference range between study populations. Reference ranges for plasma vitamin C have been established by Wang et al. (2001). Comparison to these reference ranges and the established control group (healthy dogs) will determine statistical and clinical significance of the values.

Results:

The differences in vitamin C status in this study population was minimal. Mean vitamin C levels were not significantly different between health controls (5.82 +/- 0.40 SD), circulatory shock dogs (4.61 +/- 1.69), and septic dogs (7.79 +/- 4.74 SD) ($p = 0.31$). Individual comparison between healthy and circulatory shock ($p = 0.31$), healthy and sepsis ($p = 0.97$), circulatory shock versus sepsis ($p = 0.47$) were not significantly different. Mean vitamin C levels were not significantly different at admission, day 1 of hospitalization, or day 2 of hospitalization in any group ($p = 0.14$). There was no correlation between vitamin c levels and disease severity scoring systems ($p =0.57$).

Relevance & Impact to Canine Health:

This canine grant targets diseases that are amongst the most deadly in veterinary medicine. Specifically, severe sepsis and septic shock are associated with mortality rates as high as 60-90% in dogs.This pilot study provides, for the first time, information upon vitamin C status in affected cases based on the promising data in human medicine for supplementation of vitamin C in severe sepsis and septic shock. By investigating plasma vitamin C levels in our canine patients we have been able to determine if vitamin C is an area that should be further investigated as a life saving measure for dogs.

Furthermore, dogs as well as other large animal species, are considered a useful animal model for sepsis in people. This accents the human-animal bond and one health concepts showcased in this translational research. Due to the devastating nature of sepsis in people and in dogs and the large number of individuals it affects in intensive care units,

the results of this pilot study could have lead to significant advancements to both sides of this relationship through mutually beneficial research.

Conclusions:

Plasma vitamin C concentrations were not correlated to clinical illness. Furthermore, there were no significant differences between healthy dogs and ill dogs. Based on this data, further exploration of vitamin C dysregulation is not indicated. However, individual dogs did have very low vitamin C levels in the critically ill groups. Further investigation into the specific commonalities between the dogs with low vitamin C is indicated. Unfortunately, this pilot study was not large enough to determine specific features of those cases.

Publications/Presentations/Grant Submissions:

Presentation Target: Abstract Accepted – OSU CVM Research Day – Columbus Ohio

Publication Target: There are two manuscripts derived from this grant. The first manuscript, "Vitamin C in health and disease: a companion animal focus" was accepted to Topics in Companion Animal Medicine (Friday March 27th, 2020). The second manuscript, "Pilot study: vitamin C status in dogs with critical illness and sepsis" was recently submitted for in Journal of Veterinary Emergency and Critical Care in April 2020 and is pending review.

Grant Target: No current plans to submit on this topic due to negative results in this study. In the interim, plan to gather additional preliminary data to better design next phase of the study (e.g. targeted analysis of severely affected dogs with vitamin C deficiency).

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

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

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

Results:
Data collection will be concluded in 2020

Relevance & Impact to Canine Health:
Results of this study will be directly applied to patient care and can provide useful information for future research. This tool may provide the clinician the ability to detect patients that may require additional analgesia before surgical pain is inflicted. In addition, this index may provide immediate feedback to the operator and can be used as a valuable teaching tool for residents and veterinary students

Conclusions:
Data collection not completed yet.

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

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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

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

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

Result:
Data collection will be concluded in 2021

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

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

Conclusions:
Data collection not completed yet.

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

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

Introduction:
Soft tissue sarcoma (STS) is a common cancer in dogs, especially Labrador and Golden Retrievers. In the proposed study we will analyze STS tumors from 96 dogs to identify copy number alterations, i.e. alterations in chromosome/gene number, to identify the most common STS alterations in these breeds. Based on what is known about STS in humans, tumor samples that have a relatively normal genome structure likely will carry a translocation-mediated gene-fusion (i.e. rearranged chromosomes). We will then perform RNA sequencing (RNAseq) of 20 of the tumors normal ploidy (relatively normal genomes) to determine which genes have fused and validate the candidate translocations using PCR or Southern blotting. If successful, this study will have high impact, establishing whether translationally relevant translocations/driver-gene fusions exist in canine STS and will provide important data for identifying and developing new therapies.

Approach):
In this study we propose to integrate genetic determinants of germ line STS-risk with analysis of additional dimensions of germ line risk and somatic alterations. Specifically, we will define the somatic copy number alterations (i.e. changes in the number of chromosomes) in 96 STS tumors from Labradors and Golden Retrievers. By using an advanced canine genomic platform (test), we will identify the most common STS gene alterations and "hotspots" for structural gene mutation in these breeds. This analysis will establish which samples have a normal genome structure, presumably carrying a translocation-mediated gene-fusion (the type more common in pediatric STS in people) that drives that STS. We will then conduct RNAseq of 12 of the tumors with normal ploidy and thus presumed to carry a translocation-mediated gene fusion that drives that STS. Candidate translocations will be validated by PCR or Southern blotting. This analysis will reveal the identity of the most common fusions in Labrador/Golden Retriever STS.

Results:
The tumor samples required for this study have been identified in the OSU CVM Biospecimen Repository and Colorado State University veterinary tumor bank. Although additional samples were identified and requested from the Canine Comparative Oncology Genomics Consortium (CCOGC), they are not releasing specimens to investigators for an indeterminate period of time. However, we have identified a sample set of DNA samples from retriever dogs at Cornell University that may be suitable control for a genome-wide association study (GWAS).

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

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

Publications/Presentations/Grant Submissions:
This study is still ongoing.

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Analgesic effects and tolerability of tapentadol in combination with NSAIDs in dogs with appendicular osteosarcoma
Principal Investigator (PI)	Dr. Megan Brown, DVM, MS, DACVIM (Oncology)
Co-PIs/Co-Is	Dr. Bianca Alva, DVM; Dr. Turi Aarnes, DVM, MS, DACVAA; Dr. Nina Kieves, DVM, DACVS, DACVSMR, CCRT; Dr. Vincent Wavreille, DVM, MRCVS, DACVS

Introduction:

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

Approach:

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

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

Results:

Since the trial opened in November of 2019, two patients have enrolled and completed the study. Four additional patients have been screened but were deemed ineligible for enrollment. An additional patient was set to enroll but could not due to hospital operations changing as a result of the COVID-19 pandemic. Due to low enrollment numbers, the financial incentive for participation in the study was increased.

Relevance & Impact to Canine Health:

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

Conclusions:

Once normal hospital operations resume, enrollment will continue and is expected to continue through fall of 2021. Data analysis and preparation will occur at the end of 2021/beginning of 2022 with an anticipated manuscript submission by April of 2022. Loss of recruitment and enrollment during the COVID-19 pandemic may impact final study numbers.

Publications/Presentations/Grant Submissions:

Data collection not yet completed.

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

Introduction:

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

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

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

Approach:

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

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

All 30 Labrador Retrievers will undergo electrodiagnostics, kinematic MR and CT imaging of the lumbosacral vertebral column under general anesthesia.

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

Results:

At this point four affected and three normal dogs have participated in the study. No initial morphologic or morphometric assessments have been made, though clear differences between neutral and kinematic positioning have been found.
Hypothesis: On MRI we expect to see a more severe reduction in the width, height and area of the lumbosacral vertebral column as well as intervertebral neurovascular foramina on extension in dogs clinically affected with DLSS compared with those not affected. We also expect to see a reduction in area of the neurovascular foramina on parasagittal views in addition to transverse views. When performing electrodiagnosics, we expect the evoked MEP in the semimembranosis/semitendinosis muscles to not be significantly different between groups. We expect a significant delay in MEP latency in the cranial tibial and coccygeus muscles in the affected group compared to non-affected group.

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

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

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

Publications/Presentations/Grant Submissions:
No publications or presentations have yet been made. Additional funding was provided by a grant through the Gray Lady Foundation.

PROGESS REPORT (lay report) to the Ohio General Assembly

Title	Assessment of regional intestinal perfusion by thermal imaging during foreign body surgery
Principal Investigator (PI)	Dr. Ed Cooper
Co-PIs/Co-Is	Dr. Tencate, Dr. Yaxley, Dr. Mcloughlin, Dr Guillaumin

Introduction:

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

Approach:

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

Using the thermal imaging camera, the pre-surgery image 1 was taken, centered on the foreign body. Surgeons were asked to point to the oral side as a reference point. The data was saved on the device's memory card. Sublingual microcirculation data was collected concurrently using the Microscan™ to obtain five, 20-sec videos, which were stored for later quantitative vascular analysis. The patient's vital parameters and ambient room temperature were also record at the time of imaging. Surgery location, type and foreign-body type were recorded. Post enterotomy or resection anastomosis, the affected intestinal loop, centered on the surgical incision site, was imaged using the thermal imaging camera (image 2). Sublingual microcirculation data, vital patient parameters and ambient room temperature were recorded as described above, at the time of imaging.

Results:

The study is actively enrolling patients, results are not available at this time.

Relevance & Impact to Canine Health:

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

This study proposes a more objective intestinal viability assessment technique, which can be utilized to help with intraoperative determination of intestinal perfusion and viability. To date, the use of thermal imaging for assessment of intestinal perfusion has not been done in dogs with naturally occurring GI foreign bodies. If shown to be reliable, this technique could provide objective, real-time information about local perfusion and tissue viability. Thermal images are an easy and non-invasive imaging modality that can be used during foreign body surgeries and has the potential to provide additional objective information. The combination of subjective and objective assessment can strengthen the decision making for the most appropriate surgical technique and hopefully lower the risks of intestinal surgical site dehiscence, reducing the number of surgical complications.

Conclusions:

Conclusions are not available at this time due to current active enrollment of study subjects

Publications/Presentations/Grant Submissions:

OSU Intramural Canine Grant submission and acceptance. No publications and presentations to date due to current active enrollment of study subjects

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

Introduction:

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

Approach:

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

Results:

This is a two-year study and we have completed one year of research activities including completion of sample collection. We exceeded our projected sample accrual (n=35 samples from dogs with TCC and 35 samples from dogs without TCC) and were able to sequence (16S rRNA – short read sequencing) samples from a total of 61 dogs with and without bladder cancer. Sequencing results are being processed now and should be returned to us within a few weeks. We will analyze these results to characterize the urine and stool microbial communities in dogs with and without bladder cancer. We will also use these results to guide our selection of optimal samples for shotgun metagenomic sequencing. Shotgun metagenomic sequencing is deeper sequencing that will allow us to compare all of the microbial genes present in dogs with and without TCC to determine if the microbial communities in dogs with bladder cancer have an increased potential to process chemicals like tobacco smoke or pesticides. In preparation for metagenomic sequencing, we have also established a collaboration with Nationwide Children's Hospital Institute for Genomic Medicine and begun protocol development on low biomass library preparation. Urine has few cells, and thus a low concentration of DNA. Standard protocols for extracting DNA from urine may cause DNA loss. Specialized protocols are under development to prepare samples with low DNA concentrations for metagenomic sequencing.

Relevance & Impact to Canine Health:

Over 40,000 cases of canine TCC are diagnosed each year, and there are some breeds, including Scottish terriers, Shetland sheepdogs, West Highland white terriers, beagles, and wire hair fox terriers that exhibit a much higher risk of developing bladder cancer. Elucidating factors that contribute to the development, progression, mitigation, or prevention of bladder cancer is especially important for these breeds. Additionally, canine and human bladder cancers are quite similar in histological appearance and behavior; both maintain a sex predilection (humans: males; canines: females); and the BRAF (V600E) mutation present in 85% of canine TCCs is also common in many human cancers. Dogs

are considered a relevant and valuable model for human bladder cancer. As such, our study is well aligned with the purpose of the OSU CVM canine funding in that, it is "for the research and study of diseases of dogs" and for research that will "provide information applicable to the prevention and treatment of both human and canine illnesses."

Conclusions:

Conclusions will be shared once sequencing data is processed and at the end of this two-year study.

Publications/Presentations/Grant Submissions:

Our work this far has resulted in the following:

- **Pending grant submission:** Morris Animal Foundation Established Investigator Canine Proposal. *Investigating the canine bladder cancer microbiome and metabolome in relation to polycyclic aromatic hydocarbons.* Submitted March 2020. PI: Vanessa Hale.
- **Pending grant submission**: National Institutes of Health R21. *Examining microbial-derived polycyclic aromatic hydrocarbon metabolism in bladder cancer.* Submitted April 2020. PI: Vanessa Hale
- **Accepted Presentation** (cancelled): American Society for Microbiology (ASM) Microbe Conference, June 2020. *Urine and Stool Microbiota in Dogs with Bladder Cancer.* Authors: Vanessa L. Hale, Ryan Mrofchak, Morgan V. Evans, Christopher Madden, Deepika Dhawan, Nicholas Chia, Deborah W. Knapp
- **Planned Presentation** (cancelled): Research Day. *Comparison of DNA Extraction Method for obtaining microbial DNA from Canine Urine*. Graduate Student: Ryan Mrofchak

PROGESS REPORT (lay report) to the Ohio General Assembly

Title	Pilot study on the effects of intra-articular allogeneic stem cell therapy for the treatment of osteoarthritis
Principal Investigator (PI)	Nina R. Kieves
Co-PIs/Co-Is	Jennifer Barret, Eric Hostnik

Introduction:

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

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

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

Approach:

Dogs with naturally occurring elbow dysplasia undergoing surgical treatment will be prospectively enrolled in this study with written informed owner consent and IACUC approval. At the time of surgery, dogs will be randomly assigned to one of two groups using a computer-generated randomization program. **Group 1** will receive an intra-articular injection of allogeneic stem cells suspended in autologous serum at the two-week post-operative exam, while **Group 2** will serve as a control and receive placebo injection of autologous serum alone at the two-week post-operative exam. Dogs will be re-evaluated with objective data being gathered at 3, 6, and 9 months post-operatively, including objective gait analysis, joint fluid analysis, and re-imaging via CT scan.

Results:

We are currently enrolling dogs for this study, and thus have no results to report.

Relevance & Impact to Canine Health:

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

Conclusions:

This study is ongoing.

Publications/Presentations/Grant Submissions:

This study is ongoing.

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

Introduction:

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

Approach:

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

Results:

We collected 121 UPEC isolates from 110 unique dogs over the course of the study period. Of this sample, 88 unique dogs (80%) representing 99 UPEC isolates (81%) had a detailed history that allowed for retrospectively analysis of previous antimicrobial use in the past 30 days. Demographically, our sample consisted of 24.1% small breed dogs, 20.8% large breed dogs and 15.4% mixed breeds, 19% spayed females, 26.7% intact females, 19% castrated males and 16.7% intact males, with an average and median age of 7.8 and 8 years, respectively. Using phenotypic data we found that among all factors evaluated, current antimicrobial use and a history of antimicrobial use were the strongest predictors for dogs with an antimicrobial resistant UPEC infection. Dogs with multidrug resistant UPEC UTI were more likely to have a history of current antimicrobial use and a 30-day history of antimicrobial use. Within our sample, we found 44 dogs that we could estimate the number of antimicrobial prescribed in the past thirty days. If dogs were prescribed greater than 1 antibiotic in the past thirty days their odds of having a multidrug resistant UPEC UTI were increased with marginal significance. Dogs with recurrent UPEC infections were at increased odds of having a multidrug resistant isolate and a physiological comorbidity like a metabolic disease, chronic renal disease, bladder stones or urogenital cancer.

These increased odds were marginally significant. Whole genome sequencing of UPEC UTI is still underway. Approximately 50% of our targeted sample is either sequenced or awaiting post-sequencing processing.

Relevance & Impact to Canine Health:

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

Conclusions:

Current and previous antimicrobial use for any reason can result in the development of antimicrobial resistant UPEC, including multidrug resistant UPEC. Moreover, if a dog is treated with more than one antibiotic the risk of developing a multidrug resistant infection marginally increase. These resistant and multidrug resistant UPEC may promote the development of recurrent UPEC UTI states. Using the whole genome sequence data we hope to integrate this data with virulence data, antimicrobial resistant genotypes and phylogenetic analysis to understand how antimicrobial use can influence pathotypes, virulotypes and resistance genotypes and how these findings influence recurrent UPEC UTI infections. Our data support the call for judicious use of antimicrobials in any situation and promotes the establishment of antimicrobial stewardship programs in veterinary medicine.

Publications/Presentations/Grant Submissions:

We plan to submit this data as preliminary data for Morris Animal Fund and American Kennel Club funds during their next funding cycles. We anticipated presenting this data at the 2020 Ohio State University College of Veterinary Medicine Research Day, but this presentation was cancelled because of the SARS-Cov-2 pandemic. We expect to present our data at the Conference for Researcher Workers in Animal Diseases (CRWAD). Following our whole genome sequencing data analysis, we will pursue scientific publication.

PROGESS REPORT (lay report) to the Ohio General Assembly

Title	Canine glioma as a model for testing MKlp2 inhibition in human glioblastoma
Principal Investigator (PI)	Sarah A. Moore
Co-PIs/Co-Is	Morgan S. Schrock

Introduction:

Glioblastoma (GBM) is one of the most fatal human cancers. Most patients with this brain tumor live only 14 months after diagnosis despite aggressive surgery, radiation and chemotherapy. A major challenge in developing new treatments has been the lack of a robust animal model that accurately predicts toxicity and efficacy of new drugs. Currently, novel chemotherapy drugs are tested in laboratory mice prior to moving into human clinical trials, however, 95% of novel cancer treatments show great promise in mice, but fail in the first phases of FDA human cancer clinical trials. In this proposal, we outline work that will begin investigating a novel treatment for GBM, C38—a small molecule drug inhibitor of the protein MKlp2. Being mindful of the limitations of mouse models, we also plan to test this drug in a new animal model: pet dogs with naturally-occurring canine glioma. Currently, pet dogs with glioma receive treatment that ranges from symptom management, to radiation, surgery, and/or chemotherapy. We seek to test compound 38 in pet dogs as a step in between standard mouse experiments and human clinical trials, where dogs will be enrolled in a veterinary clinical trial. Inclusion in the trial would help cover the cost of standard treatments while also providing access to compound 38. The efficacy and toxicity results would greatly inform human clinical trials, while also financially assisting with treatment for enrolled pet dogs.

Approach:

We have preliminary data that indicates MKlp2 helps cells divide properly. We have shown that inhibiting MKlp2 with C38, stops cancer cell growth in human and canine GBM cells. Because the function for MKlp2 has not yet been reported, we sought to continue our work understanding MKlp2 function, but also to prepare for testing this drug in laboratory mice (the required standard) and in an emerging animal model: pet dogs with glioma as part of a veterinary clinical trial. Therefore, the first goal of this grant was to continue studying MKlp2 function in cellular division, while the second goal was to develop new samples of canine glioma to test the efficacy of C38 *in vitro*. Although there are three established canine GBM cell lines, all three lines are grade IV astrocytoma and were established over twenty years ago with methods that are now known for causing genetic changes. Our novel lines would represent the spectrum of glioma types (astrocytoma, oligodendroglioma) and grades (I – IV), serving as a better representation of the true distribution of canine glioma treated clinically. Upon receipt of glioma cells (post surgery or humane euthanasia), we will inject cells into the subcutaneous tissue of immunocompromised mice, where the cells engraft and grow in an *in vivo* environment, greatly limiting the accumulation of genetic changes. We will test the efficacy of C38 in these new canine glioma samples to generate preliminary data for a veterinary clinical trial.

Results:

We were selected for funding in the summer of 2019. The initial fall months of 2019 were dedicated to preparing and getting approval for our mouse IACUC protocols and establishing additional collaborations for receipt of canine glioma cells. Our initial proposal outlined collaboration with Dr. Tim Bentley (Purdue University), who resects approximately eight glioma cases/ year and estimated he could provide 4-5 glioma samples annually for our studies. Because we know that not all glioma cells will engraft into mice (~50% engraftment rate), we sought to acquire more glioma samples and established a new collaboration with Drs. Mike Olin (glioblastoma scientist) and Liz Pluhar (veterinary neurosurgeon), both at the University of Minnesota, to obtain ten glioma samples (5 oligodendroglioma, 5 astrocytoma) for our studies. We plan to receive and engraft these upon return to work once COVID-19 restrictions are lifted.

Because our grant did not become active until December 18, 2019, we have only had our funds for four months, including March, where our activities became greatly restricted due to COVID-19 precautions. Nevertheless, we received one glioma sample (high grade oligodendroglioma), which we were able to grow as neurospheres in culture. Two mice were injected with the cells and are currently being monitored for growth (first engraftment can take up to 6 months to grow). We have also made significant progress uncovering the role of MKlp2 in cellular division. We have

discovered that MKlp2 is necessary for chromosomes to move around the cell in preparation for division and we have begun to work out how MKlp2 regulates this process.

Relevance & Impact to Canine Health:

While we propose to study canine glioma to enhance drug testing for human GBM, this work will provide potential benefit to pet dogs as well. The possibility to participate in a veterinary clinical trial would offer owners who cannot afford the standard medical treatment financial assistance that would help them provide more medical care for their pets. In addition, the incorporation of a novel treatment would provide an alternative treatment option for canine patients which are refractory to the standard treatment regimen. Therefore, obtaining unique canine glioma samples and testing the efficacy of a novel antimitotic will greatly benefit comparative oncology and inform veterinary treatment as well.

Conclusions:

Although we cannot yet draw conclusions on our work establishing the novel canine glioma patient-derived lines, we are encouraged by the successful *in vitro* growth of our first sample and anticipate the predicted 50% engraftment rate for future samples. Our findings regarding the basic biology of MKlp2 and its role in chromosome movement during cellular division underscore the potential usefulness of C38 as a chemotherapy drug, particularly for highly aggressive cancers that grow quickly and divide often. We have confirmed the efficacy of C38 in human patient-derived lines and plan to evaluate the drug in combination with radiation, standard treatment in humans, prior to testing in mice.

Publications/Presentations/Grant Submissions:

We submitted an abstract to present this project at the College of Veterinary Research Day in April. Unfortunately, like many other conferences, the gathering was canceled due to COVID-19 precautions. We also submitted an abstract for a grant proposal ($15,000) to the OSU College of Medicine Department of Radiation Oncology for their Basic Seed Grant. The purpose of these additional funds is to pay for experiments that are necessary to move forward with testing a 3rd generation MKlp2 inhibitor in laboratory mice. We need to use the 3rd generation drug because it is more potent and therefore has the best chance of being effective in mice, dogs, and humans. We anticipate submitting a manuscript for publication by the end of the year reporting MKlp2 involvement in chromosome movement during cell division.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Efficacy of a commercially available LH surge detection strip in the bitch
Principal Investigator (PI)	Erin Runcan
Co-PIs/Co-Is	Marco Coutinho da Silva

Introduction:

Determining the ideal time for breeding is important to dog breeders when a male is unavailable, or in cases of infertility or limited semen. Many methods exist to determine the best day for breeding to maximize pregnancy, but these tests are not always accurate. Blood hormone testing can be performed to estimate ovulation, but requires multiple veterinary visits. Because of these limitations, various at-home tests are available to help predict the day of ovulation, however very few are actually validated for use. One such test, an "LH surge test strip," claims to change color when exposed to vaginal fluid at the time of the hormonal surge which triggers ovulation. Our research will determine if these strip tests are accurate by comparing them to validated hormonal blood tests.

Approach:

Client-owned bitches (n=11; ten experimental, one control) will be brought in five days after the onset of heat. Blood will be drawn daily and serum saved for hormonal testing. At each daily visit, a "LH surge test strip" will be exposed to the vaginal fluid and evaluated for color change. Per the manufacturer's recommendation, any degree of color change (purple spots, bands, or complete color change) should be regarded as "positive" with no color change indicating a "negative" sample. A glucose reading of vaginal fluid will also be made. Once ovulation is detected via serum hormone testing, luteinizing hormone (LH) testing will also be performed on stored serum. LH levels will be determined using a commercial cage-side assay as well as sent to Colorado State University's Veterinary Endocrinology Laboratory to determine qualitative values. The sensitivity, specificity and accuracy of the LH test strips will be determined against the true serum levels of LH.

Results:

To date, a total of three bitches have been enrolled in the study, and one of the three has completed the sampling schedule and two are ongoing. An additional three patients are scheduled to be enrolled once they come into season in the following one to two weeks.

Relevance & Impact to Canine Health:

If proven to be accurate, this at-home LH test strip may provide an inexpensive way for breeders to determine the optimum time for breeding. This could greatly decrease the need for multiple costly veterinary visits and the stress of handling and daily blood draws for the bitch.

Conclusions:

At this time, conclusions are still pending final statistical analysis of data. Study is ongoing.

Publications/Presentations/Grant Submissions:

Study is ongoing.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms
Principal Investigator (PI)	Laura E. Selmic
Co-PIs/Co-Is	Josie Dornbusch, Ryan Jennings, Vincent Wavreille

Introduction:

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

Approach:

The *purpose of the proposed study* was to assess the accuracy of OCT for assessment of surgical margins for resected canine skin and subcutaneous tumors. The specific aims are as follows:

Aim 1: Compare normal and abnormal histological features with OCT images for surgical margins from resected cutaneous and subcutaneous tumor specimens in dog. These OCT images and corresponding correlations with histopathology will form a training set for observers.
Rationale: To optimize OCT imaging of surgical margins of different tumor types, evaluation of a training set of surgical margins of resected tumors is needed to enhance OCT imaging protocols and to establish future image evaluation criteria of the surgical margins for different skin and subcutaneous tumor types. By comparison of OCT images to histopathologic sections evaluation methodology and image feature criteria for identifying incomplete margins will be developed.

Aim 2a: Determine the diagnostic accuracy of OCT for assessment of surgical margins for resected canine cutaneous and subcutaneous tumors.
Aim 2b: Determine the frequency that OCT and standard pathology assessment detect incomplete margins for the same canine cutaneous and subcutaneous tumors.
Rationale: Evaluation of new methods of surgical margin assessment need to involve both assessment of the diagnostic test characteristics (sensitivity and specificity) and comparison to standard methods for evaluation of clinical utility. Further investigation of novel surgical margin imaging techniques is needed to improve speed and accuracy of detection of incomplete margins to guide individualized treatment recommendations to help reduce treatment associated morbidity and impact on the patients.

Results:

Enrollment has exceeded expectations and we have enrolled 38/40 dogs for aims 1 and 2. We have completed the comparisons between the normal and abnormal features. We have used these images to train the investigators performing the imaging. To date enrolled cases have comprised predominantly of malignant tumors including mast cell tumor and soft tissue sarcoma. Following enrollment of the last two cases in the next six months we will perform the data analysis and complete the manuscript writing. We don't anticipate that the stop to clinical research with COVID 19 will affect our ability to complete this project by the two year timeline we proposed.

Relevance & Impact to Canine Health:

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

Conclusions:

Evaluation of OCT is needed to assess its accuracy for detection of incomplete margins for companion animal tumors following resection. Real-time imaging of surgical margins of specimens in pathology could increase the accuracy of determination of completeness of surgical resection and guide treatment recommendations in an effort to reduce local recurrence rates, morbidity and mortality. If promising, future studies will be planned to evaluate the effect of OCT imaging on treatment outcomes. This cutting-edge technology and its advantages have the potential to revolutionize veterinary surgery for specialist surgeons, general practitioners and pathologists, to guide and improve surgical resection and histopathological assessment of many tumor types in companion animals.

Publications/Presentations/Grant Submissions:

We were awarded a grant (02758) by the American Kennel Club, utilizing our preliminary data from this project. This grant is an extension of the current project and investigates the impact of thorough OCT surgical margin assessment.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs
Principal Investigator (PI)	Selena Tinga
Co-PIs/Co-Is	Turi Aarnes, Stephen Jones, Nina Kieves, Phillip Lerche, Carolina Ricco Pereira, Audrey Wanstrath

Introduction:

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

Approach:

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

Results:

The study's IACUC has been approved and at this stage we are awaiting re-opening of the hospital (closed for elective procedures due to COVID19) to begin case enrollment. Once case enrollment begins, we hope to have the data collected within 12-18 months.

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:

Project is still in progress.

Publications/Presentations/Grant Submissions:

Project is still in progress.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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

Introduction:

Appendicular osteosarcoma (OSA) is locally aggressive and has a very high tendency to spread. Limb amputation followed by chemotherapy has been considered the standard of care treatment. Numerous limb salvage techniques have been described in dogs but the use of these techniques, including radiation therapy (RT), has been restricted due to high complication rates. The OSA lesion compromises the structural integrity of the affected bone, explaining the high incidence of pathological fractures encountered in dogs following RT. To prevent this complication, open surgical stabilization has been performed with RT but this approach was also associated with a very high complication rate (94%). Application of a stabilizing implant via a minimally invasive approach to reduce soft tissue damage and associated complications is available and has been used in people with success.

This proposal will evaluate the clinical outcome of a limb salvage technique using the combination of RT and this novel implant.

Approach:

We design a pilot study including 6 client-owned dogs diagnosed with appendicular OSA. Dogs will be staged routinely Extension of the lesion will be assessed before treatment using a computed tomography (CT) scan of the affected limb. Radiation therapy will be delivered in 3 sessions and placement of the intramedullary implant will be performed 24h after the last radiation treatment. Chemotherapy will be started 2 weeks after surgery. The dog's owner will complete a quality of life questionnaire multiple times during the study (at the initial appointment and then every 2 months). A gait analysis will be performed before the treatment and every 2 months during the follow-up to assess the limb function. Radiographs of the affected limb will be performed every 2 months.

Results:

We **enrolled 3 cases**. No complications were recorded in these cases. All of them started chemotherapy in time (2 weeks post-surgery). We are performing the follow-up of these 3 patients. Because of the COVID-19, no new cases have been seen since the beginning of the "emergency only" policy.

Relevance & Impact to Canine Health:

We expect the surgical procedure to be well-tolerated, have **a low complication rate** and result in good to excellent quality of life and limb function. We anticipate local tumor control and disease-free intervals comparable to other limb sparing studies.

Conclusions:

So far, the results are very encouraging.

Publications/Presentations/Grant Submissions:

We are planning to submit an abstract to ACVS for an oral presentation in October this year (2020).

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

Introduction:

Ursodiol is an FDA approved naturally occurring bile acid that is used to treat a variety of liver and gastrointestinal diseases. Ursodiol is routinely administered in veterinary medicine; however, it is unknown how this drug impacts the canine intestinal ecosystem. Evidence is mounting that bile acids, such as Ursodiol, can alter the gut microbial composition and host physiologic response during health and disease. Our study aims to determine how oral administration of Ursodiol 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:

The *overall objective* of this study is to determine the impact of Ursodiol on the canine intestinal ecosystem. Based on our preliminary work with Ursodiol in conventional mice, our *central hypothesis* is that oral administration of Ursodiol will alter the canine intestinal ecosystem, specifically the fecal microbiota and bile acid. The *rationale* of the proposed work is that Ursodiol is routinely and liberally administered to canines, however the ramifications of how this drug impacts the canine intestinal ecosystem is unknown. We plan to accomplish our objective for this study by pursing the following Specific Aims:

Specific Aim 1: Determine if Ursodiol administration modulates the canine fecal microbiota. The working hypothesis is that Ursodiol will modulate the canine fecal microbial community structure. Client-owned healthy dogs will be administered Ursodiol for 21 days at a clinically relevant dose. Fecal microbial community structure will be evaluated at baseline, serially during Ursodiol administration, and following discontinuation of Ursodiol.

Specific Aim 2: Determine if Ursodiol administration modulates the canine fecal bile acid pools. The working hypothesis is that Ursodiol will modulate the canine fecal bile acid metabolome. Client-owned healthy dogs will be administered Ursodiol for 21 days at a clinically relevant dose. The fecal bile acid pools will be evaluated at baseline, serially during Ursodiol administration, and following discontinuation of Ursodiol.

Results:

Project is still in progress; we were in the recruitment phase of this experiment when the OSU VMC Clinical Trials Office (CTO) suspended research due to COVID -19. resume We will recruitment once the CTO reopens for clinical trials.

Relevance & Impact to Canine Health:

Ursodiol is routinely and liberally administered to canines, however the ramifications of how this drug impacts the intestinal ecosystem remains unknown. This study will provide valuable data on the impacts of Ursodiol, administered at a clinically relevant dose, on the canine intestinal ecosystem. Specifically, the proposed clinical trial will be 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 gastrointestinal diseases, intestinal pathogens, diabetes mellitus, chronic kidney disease (CKD), and obesity. To this effect, this study will be the first to integrate multi-omics approaches to evaluate the canine gut microbiota-bile

acid-host axis, which will provide a foundation for unraveling the complex intricacies of bile acid metabolism within the canine intestinal ecosystem with the ultimate goal of improving canine health and quality of life.
Conclusions: *Project is still in progress.*
Publications/Presentations/Grant Submissions: *Project is still in progress.*

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Utility of cardiac MRI to diagnose cardiac fibrosis in dogs with mitral valve disease: a pilot study
Principal Investigator (PI)	Randolph Winter
Co-PIs/Co-Is	William Clark, Jaylyn Rhinehart, Karsten Schober, Turi Aarnes, Eric Green, Daniel Addison, Harry Friel

Introduction:

Chronic valvular disease is a common heart disease in older dogs. Some dogs maintain a normal quality of life despite this disease, whereas others develop disease progression and heart failure. Ultrasound and cardiac biomarker measurements help to identify dogs with poor heart function. However in humans that are older or have chronic valvular disease, magnetic resonance imaging (MRI) studies demonstrate that patients with fibrosis (scarring) or poor blood flow to their heart muscles develop heart failure or death sooner compared to patients without these MRI findings. It is unknown if dogs have similar patterns of fibrosis or decreased blood flow, and results from this study will allow that determination to be made. MRI results will be compared to ultrasound and cardiac biomarker results, which will help better classify the cardiac health of dogs with chronic valvular disease and identify those that may benefit from early or novel therapeutic interventions.

Approach:

Six healthy dogs and 6 dogs with chronic mitral valve disease are scheduled to be recruited for this study. All dogs will undergo diagnostic testing to determine cardiac and systemic health, including x-rays of the heart and lungs, ultrasound of the heart, blood pressure measurement, and full bloodwork including cardiac biomarker measurement. After these tests, dogs will have anesthesia induced and have a cardiac MRI performed. With the data collected from the MRI, the degree and location of ischemia (poor blood flow) and fibrosis (scar tissue formation) within the heart muscle will be determined. Ultimately, this data will reveal if these conditions (ischemia and fibrosis of the heart muscle) are a prominent aspect of chronic mitral valve disease in the dog.

Results:

This is an ongoing study, so this is a preliminary report. Within the first year, we have had 2 healthy dogs and 4 dogs with chronic mitral valve disease complete this study. All data will undergo batch analysis at the conclusion of the study. All dogs have completed anesthesia and cardiac MRI with no immediate complications. No dogs with chronic mitral valve disease developed heart failure or any other cardiac-related complication during or after anesthesia. Three additional dogs were scheduled to complete this study (1 healthy dog, and 2 dogs with chronic mitral valve disease) by the end of April, but due to social distancing guidelines secondary to the covid-19 pandemic, these studies were delayed.

Relevance & Impact to Canine Health:

The results of this study will highlight an important gap in the collective knowledge of dogs with MMVD. Humans with similar types of heart disease have variable patterns of myocardial fibrosis and ischemia identified with CMR, and these findings are important for optimizing clinical management. If dogs with MMVD Stage B2 have myocardial fibrosis and ischemia identified with CMR, then this could have important implications for therapeutic management or prognosis.

Conclusions:

As this is a preliminary report with no initial data analysis, no conclusions can be reached, other than that this study has not caused any ill effects to its participants.

Publications/Presentations/Grant Submissions:

Extramural funding was sought and denied from the American College of Veterinary Medicine Cardiology Resident Research Grant. Submission for extramural funding is planned for the American Kennel Club this year.

PROGESS REPORT (lay report) to the Ohio General Assembly

Title	Alveolar type II (ATII) cell function in dogs with severe acute respiratory distress syndrome (ARDS)
Principal Investigator (PI)	Davis, Ian Christopher
Co-PIs (if applicable)	

Introduction:

Acute respiratory distress syndrome (ARDS) in man is characterized clinically by acute onset of severe hypoxemia accompanied by evidence of non-hydrostatic pulmonary edema and reduced lung compliance. There are more than 300,000 cases of ARDS in humans in the USA alone each year, resulting from both direct and indirect lung insults. Due to the ongoing SARS CoV-2 pandemic, this number is likely to increase significantly in the coming years. Data on incidence of ARDS in veterinary patients (VetARDS) are limited, but a best estimate is 5-10% of ill dogs.

Treatment options for ARDS are currently limited to protective mechanical ventilation, conservative fluid administration, and supportive care. Consequently, mortality rates in man remain at around 40%, and many survivors have prolonged and often debilitating lung dysfunction. Likewise, recovery rates from VetARDS are generally very poor. Indeed, the majority of dogs with VetARDS are euthanized at the owner's request. Hence, there is an urgent unmet medical need for new therapeutics that can prevent, retard, or ameliorate ARDS in both the veterinary and human medical fields, irrespective of its underlying etiology.

Alveolar type II (ATII) cells are essential to normal lung function and may be central to ARDS pathogenesis. However, the impact of ARDS on human ATII cell function *in vivo* is unknown, primarily because it is almost impossible to obtain any significant quantity of live ATII cells from ICU patients with ongoing ARDS as these subjects are too fragile. We propose that lung tissue from dogs euthanized for spontaneous severe VetARDS will provide a meaningful experimental surrogate.

Approach:

We will recruit 5 adult, otherwise healthy, client-owned dogs who are to be euthanized for severe spontaneous VetARDS, which will be diagnosed using the same clinical criteria as used in humans (primarily P_aO_2:F_iO_2 ratios and evidence of pulmonary edema on chest radiograph). Once a diagnosis of VetARDS has been made and owner consent obtained, we will collect venous blood (plasma and leukocytes) for CBC/diff and clinical chemistry. Additional plasma will be banked for later analysis of circulating inflammatory markers. We will then euthanize the dog, remove its lungs for pathologic assessment, and perform bronchoalveolar lavage (BAL). Plasma, lungs, and BAL fluid from a cohort of 5 normal dogs will be used as controls. We will select regions of grossly normal and grossly abnormal lung tissue for *ex vivo* and *in vitro* experiments. In all dogs, we will assess pathology at the gross level, by light microscopy for histopathology, and by electron microscopy to quantify effects of lung injury on ATII cell ultrastructure and mt morphology. BAL fluid total and differential leukocyte counts will be determined and remaining BAL fluid cells will be banked for downstream analysis (e.g., phenotyping by flow cytometry, metabolomics). We will directly isolate ATII cells from normal and VetARDS canine lungs by a digestion and negative selection method. These cells will be used to determine effects of ARDS on the ATII cell phenotype, mitochondrial energetics, and the ATII cell transcriptome, lipidome, and metabolome. Finally, we will generate precision cut lung slices to characterize the ARDS lung secretome.

Results:

Standardized protocols for tissue collection have been developed and a source of normal canine lungs identified (Matt Joseph, OSU Interventional Cardiology Cath Lab). All necessary reagents are in place and experimental methods have been validated. Unfortunately, however, the social distancing measures instituted in response to the ongoing SARS CoV-2 pandemic, together with the need to preserve PPE for essential healthcare workers, have made it impossible to begin experiments.

Relevance & Impact to Canine Health:

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

Conclusions:

All protocols are in place to facilitate rapid resumption of proposed studies as soon as work restrictions at OSU have been lifted.

Publications/Presentations/Grant Submissions:

This study was recently funded and is just getting started.

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

Introduction:

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

Approach:

To define the role of toxin expression in the severity of skin infections (pyoderma) and the relationship between the immune response to the presence of infection and recurrent infection, a clinical trial recruiting both healthy dogs and dogs with a first-time pyoderma will be conducted, in which dogs with pyoderma will be evaluated for the recurrent infection through monthly clinical follow-up until 6 months after the first infection. Bacterial and sera samples at the determined time will be collected. A thorough medical examination and scorings for clinical skin lesions and itch will be performed by board-certified veterinary dermatologists. We will characterize the gene expression of *S. pseudintermedius* pore-forming toxins and those regulating the toxin secretion (virulence regulators) in *S. pseudintermedius* clinical bacterial isolates from dogs using molecular techniques. The gene expressions will be correlated with the clinical lesion scores for pyoderma. To evaluate if the bacterial toxins will induce an antibody response in dogs during infections, we will compare the antibody levels between healthy dogs and dogs presented with a first-time pyoderma. To define the relationship of the immune response induced by bacterial toxins and the presence of recurrent infections, we will compare the antibody levels between dogs with a first-time pyoderma that do not develop a recurrent infection and dogs with recurrent infection during the 6-month follow-up period.

Results:

The results for this project are pending. Patient recruitment is postponed to late Spring, 2020 due to COVID-19. The study has all elements in place to start recruiting once the college returns to business as usual.

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

Conclusions:
The conclusions for this study are pending.

Publications/Presentations/Grant Submissions:
A $3000.00 CCTS voucher was awarded for a REDCap build and data management

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

Introduction:

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

Approach:

Blood and ticks will be obtained from 534 shelter dogs from five counties in southern Ohio. DNA will be extracted from blood for parallel screening for spotted fever group rickettsiae, *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Borrelia burgdorferi* using serology and PCR. Ticks will be examined under light microscopy to determine species before DNA extraction and PCR testing for pathogens. Sampling will be conducted over a period of 12 months to capture peak activity of all tick life stages. Data on tick species, tick abundance, and pathogen prevalence in ticks and dogs from this study will be compiled by county. Risk factors for tick-borne disease in shelter dogs will be assessed by univariate and multivariate logistic regression analyses. This data will be compared to publicly available county-level data on human tick-borne disease from the Ohio Department of Public Health, local vector control agencies, and the Centers for Disease Control and Prevention to determine the efficacy of shelter dogs as sentinels for human health risk.

Results:

This study began in March of 2020 and is still in progress.

Relevance & Impact to Canine Health:

The number of annual canine tick-borne disease reports in the United States is increasing, particularly in Ohio where the blacklegged tick (*Ixodes scapularis*) became established around 2012. Cases of canine Lyme Disease in Ohio have subsequently increased 10-fold from 825 in 2012 to 8,196 in 2019. Canine Lyme Disease most commonly causes recurrent lameness, joint inflammation, fever, lethargy, and sometimes kidney failure. In addition to Lyme Disease, *I. scapularis* can also transmit anaplasmosis and babesiosis. Two native Ohio ticks, *Dermacentor variabilis* and *Amblyomma americanum*, also vector spotted fever rickettsiosis and ehrlichiosis, respectively. In the past decade, cases of canine anaplasmosis have increased 4-fold and ehrlichiosis cases 7-fold, but there is no data on canine spotted fever rickettsiosis or babesiosis because they are not included in the current tick-borne disease assays. The lack of surveillance data on spotted fever rickettsiosis is especially concerning since infection with *Rickettsia rickettsia,* the etiologic agent of Rocky Mountain Spotted Fever (RMSF), can be fatal in up to 10% of canine cases. The results of this study will directly inform our understanding of the risk of tick-borne disease to domestic dogs in Ohio.

Conclusions:

This study began in March of 2020 and is still in progress.

Publications/Presentations/Grant Submissions:

This study began in March of 2020 and is still in progress. No publication, presentations, or grants have been submitted as of yet.

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

Introduction:

Obesity is a growing epidemic in companion animals. Obesity leads to physical impairment, comorbidities, and reduced quality of life and healthspan. Evidence is mounting that the microbes living in the gut contributes to obesity, and rational manipulation of this ecosystem may confer a health benefit. Our overall objective is to provide scientific and clinical assessment of the efficacy of fecal microbiota transplantation (FMT; transfer of feces from a healthy donor to a diseased recipient) as an adjunctive therapy for canine obesity management. We hypothesize that FMT will amplify weight loss compared to the use of standard dietary obesity management. To test this, we are conducting a 24-week clinical trial in client-owned obese dogs. Execution of the SLIM Study will provide scientific and clinical evidence for FMT efficacy in canine obesity management and facilitate rational manipulation of the intestinal ecosystem into a personalized medicine approach for dogs suffering from obesity.

Approach:

The *overall objective* of this study 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. Based on our preliminary data, personalized obesity feeding plans are effective at achieving weight loss goals in canine patients. Our *central hypothesis* is that FMT will amplify weight loss compared to the use of standard dietary obesity management. The *rationale* of the proposed work is that this study will be the first robust clinical trial assessing the efficacy of FMT for obesity management in dogs. We plan to accomplish our objective for this project by pursing the following Specific Aim:

> **Specific Aim: 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.** The working hypothesis is that capsular FMT added to standard dietary obesity management will amplify weight loss compared to the use of dietary obesity management alone or with placebo. Client-owned obese, but otherwise healthy dogs will be prospectively enrolled in a randomized, double-blinded, placebo controlled, cross-over clinical trial. Throughout the 24-week clinical trial, serial monitoring of body weight, body condition score (BCS), activity, quality of life questionnaires, and metabolic markers (triglycerides, cholesterol, GLP-1, and adipokines) will be evaluated to assess clinical and owner perceived improvement.

Results:

Project is still in progress; we were performing to launch this study in April when the OSU VMC Clinical Trials Office (CTO) suspended research due to COVID -19. We will resume recruitment once the CTO reopens for clinical trials.

Relevance & Impact to Canine Health:

Canine obesity rates remain unacceptably high, with over 56% of dogs in the United States classified as overweight or obese. Obesity ultimately occurs from an imbalance between energy intake and energy expenditure. Although obesity is a preventable disease, this growing epidemic is the most common nutritional disorder in dogs and is associated with significant consequences. Dogs suffering from obesity experience a higher frequency of multiple devastating comorbidities including arthritis, endocrine dysfunction, cruciate ligament rupture, lower urinary tract disease, oral disease, diabetes mellitus, pancreatitis, and cancer. Furthermore, in a lifelong study in Labrador retrievers, even moderately overweight dogs were at greater risk for earlier morbidity and a shortened lifespan. Traditionally, standard of care obesity management incorporates two major principles: feed less and exercise more. Despite veterinarian recommendations regarding dietary intervention and exercise, obesity remains a major canine health concern. Aside

from these strategies, veterinarians are limited in providing other treatments to abate obesity and its devastating comorbidities, thus demonstrating the *critical need* to identify novel treatment strategies for obese patients. The SLIM study is significant because it will be the first to evaluate the efficacy of FMT as an adjunctive therapy for canine obesity management. This treatment is based on growing evidence that the gut microbiota plays an integral role in obesity. Success of this study will be of immediate benefit to obese dogs by providing a microbial intervention to augment current strategies for canine obesity management aimed at promoting weight loss, normalizing metabolic status, and improving quality of life.

Conclusions:

Project is still in progress.

Publications/Presentations/Grant Submissions:

Project is still in progress.

Grant Submission:

This randomized, double-blinded, placebo controlled, cross-over clinical trial will provide preliminary data to later utilize an integrated multi-omics approach to comprehensively evaluate and characterize the intestinal ecosystem throughout the trial via 16S rRNA gene sequencing (for microbial community composition) and global untargeted metabolomics (for microbial community function). This project was submitted to the American Kennel Club (AKC) for an Oak Grant, which was successfully funded ($94,989). Therefore, the SLIM project will include the integrated multi-omics approach outlined above.

FUNDING OF PROJECTS	
TITLE	**BUDGET**
Comparison of preoperative analgesic protocols and evaluation of chronic neuropathic pain state in dogs undergoing TPLO	$22,645
Validation of PRMT5 as a candidate therapeutic target in canine lymphoma	$21,484
Computed tomography quantification of airflow resistance before and after nasal turbinate laser ablation	$22,727
Serum cytokine concentrations in dogs with multicentric lymphoma before and after doxorubicin treatment	$22,100
Influence of neo-adjuvant steroid administration on histologic margins in canine cutaneous mast cell tumors	$22,698
Interrogating the expression and function of WWOX in canine mast cell tumors	$22,654
Plasma Cytokeratin 18 and fecal Alpha1 proteinase inhibitor levels in dogs with appendicular osteosarcoma before and after treatment with carboplatin	$15,776
Pilot Study: Serum vitamin C levels in dogs with non-septic and septic critical illness	$ 8,182
Perfusion index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs	$11,588
Pulse oximetry pleth variability index as a predicter of fluid responsiveness in dogs	$22,728
Germ line and somatic genetics of canine soft tissue sarcoma	$22,493
Analgesic effects and tolerability of tapentol in combination with NSAIDS in dogs with osteosarcoma	$22,575
Morphologic, morphometric and functional characterization of degenerative lumboscacral stenosis in Labrador Retrievers	$23,422
Assessment of regional intestinal perfusion by infrared thermography during foreign body surgery	$ 8,037
Characterizing the microbiome in dogs with and without bladder cancer	$22,682
Pilot study on the effects of intra-articular allogenic stem cell therapy for the treatment of osteoarthritis	$22,727
Effects of antimicrobial therapy on virulence and antimicrobial resistance of canine EPEC UTIs	$22,600
Canine glioma as a model for testing MKlp2 inhibition in human glioblastoma	$22,619
Efficacy of a commercially available LH surge detection strip in the bitch	$ 8,046
Optical coherence tomography for margin evaluation of canine skin and subcutaneous neoplasms	$15,255
Efficacy of gabapentin for the treatment of acute orthopedic surgical pain in dogs	$22,727
Use of radiation therapy and conforming intramedullary implant to treat canine appendicular OSA	$22,421
Impact of the secondary bile acid ursodeoxycholic acid (Ursodiol) on the canine gut microbiota and bile acid metabolome	$22,633

Utility of cardiac MRI to diagnose cardiac fibrosis in dogs with mitral valve disease: a pilot study	$22,727
Alveolar type II (ATII) cell function in dogs with severe acute respiratory distress syndrome (ARDS)	$27,000
A pilot study on the role of *Staphylococcus pseudintermedius* toxins and virulence regulators in canine pyoderma	$26,919
Molecular and serologic surveys of shelter dogs and their ticks as sentinels for tick-borne disease risk in Ohio	$21,020
Scientific and clinical assessment of fecal microbiota transplantation to enhance weight loss in obese dogs (SLIM pilot study)	$27,233

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			%		DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT		EFFORT ON PROJ.		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.*

			BREAKDOWN OF EACH TYPE OF TAG SOLD				
County	Invoice	Amt Paid $	1 - YR	3 - YR	PERMNT	Dangerous Dogs	KENNEL REG
Adams County Auditor	1	$719.00	6,875	66	8	0	37
Allen County Auditor	1	$1,627.30	15,493	150	23	0	100
Ashland County Auditor	1	$876.10	7,996	173	17	0	76
Ashtabula County Auditor	1	$1,054.00	9,419	274	28	0	19
Athens County Auditor							
Auglaize County Auditor	1	$940.90	8,553	232	16	0	0
Belmont County Auditor	1	$939.80	8,111	314	30	0	45
Brown County Auditor	1	$818.90					
Butler County Auditor	1	$3,085.90					
Carroll County Auditor	1	$816.90					
Champaign County Auditor	1	804.30	7765	61	8	0	15
Clark County Auditor	1	$2,169.60					
Clermont County Auditor	1	$1,759.40	16,155	296	53	0	21
Clinton County Auditor	1	$798.80	7,481	99	20	0	15
Columbiana County Auditor	1	$2,079.50	20,636	17	9	0	18
Coshocton County Auditor	1	$900.10	8,298	16	0	0	655
Crawford County Auditor	1	$860.60					
Cuyahoga County Auditor							
Darke County Auditor							
Defiance County Auditor	1	$756.50	6,888	142	19	0	61
Delaware County Auditor							
Erie County Auditor	1	$1,326.00	13,218	1	3	0	9
Fairfield County Auditor	1	$1,913.40	17,572	387	36	0	41

Fayette County Auditor	1	$421.30	3,928	73	6	0	6
Franklin County Auditor	1	$10,528.90	80,291	4,897	1,025	0	57
Fulton County Auditor							
Gallia County Auditor	1	$209.90	1,753	0	0	0	286
Geauga County Auditor	1	$1,160.70	11,273	58	16	0	122
Greene County Auditor	1	$2,405.20	20,809	759	92	0	46
Guernsey County Auditor	1	$675.40	6,204	107	13	0	99
Hamilton County Auditor							
Hancock County Auditor	1	$1,383.30	12,878	183	25	0	156
Hardin County Auditor	1	$720.80	7,139	16	0	0	21
Harrison County Auditor	1	$315.70					
Henry County Auditor	1	$634.70	6,174	25	5	0	48
Highland County Auditor	1	$515.00	4,645	110	14	0	35
Hocking County Auditor	1	$481.10	4,603	26	9	0	40
Holmes County Auditor	1	$1,042.90	10,021	10	0	0	378
Huron County Auditor	1	$1,079.20	10,358	81	17	0	21
Jackson County Auditor	1	$767.60	7,318	58	13	0	154
Jefferson County Auditor	1	$496.50	4,182	170	27	0	3
Knox County Auditor	1	$970.50					
Lake County Auditor	1	$2,702.40	24,813	475	76	0	26
Lawrence County Auditor	1	$820.80	8,042	41	4	0	3
Licking County Auditor	1	$3,021.90	30,095	33	0	0	25
Logan County Auditor	1	$638.20					
Lorain County Auditor	1	$2,707.80	24,365	527	91	0	222
Lucas County Auditor	1	$5,419.00	50,664	898	81	0	22
Madison County Auditor	1	$652.00	5,082	176	19	0	0
Mahoning County Auditor	1	$2,904.10					
Marion County Auditor							
Medina County Auditor	1	$2,366.90	19,841	832	125	0	82
Meigs County Auditor	1	$206.90	1,935	24	4	0	22
Mercer County Auditor	1	$413.50	4,009	18	0	0	72

Miami County Auditor	1	$1,838.90	16,001	518	81	0	24
Monroe County Auditor	1	$359.90	3,511	1	4	0	150
Montgomery County Auditor							
Morgan County Auditor	1	$273.10	2,451	58	8	0	26
Morrow County Auditor	1	$576.30	5,086	136	59	0	21
Muskingum County Auditor	1	$1,164.50	11,211	54	11	0	162
Noble County Auditor	1	$169.10	1,531	15	3	0	85
Ottawa County Auditor	1	$827.40	7,734	133	14	0	1
Paulding County Auditor	1	$336.00	3,054	51	12	0	33
Perry County Auditor	1	$595.60					
Pickaway County Auditor							
Pike County Auditor							
Portage County Auditor	1	$3,180.80					
Preble County Auditor	1	$361.20					
Putnam County Auditor	1	$691.80	6,400	4	2	0	486
Richland County Auditor	1	$4,890.20	18,322	0	0	0	580
Ross County Auditor	1	$1,406.30	13,936	2	2	0	101
Sandusky County Auditor	1	$1,192.90	11,316	124	19	0	51
Scioto County Auditor	1	$470.20	3,830	109	21	0	335
Seneca County Auditor	1	$1,064.90	9,601	156	38	0	200
Shelby County Auditor	1	$855.30	8,027	96	14	0	98
Stark County Auditor							
Summit County Auditor	1	$3,970.90	36,846	692	70	0	87
Trumbull County Auditor	1	$1,819.10					
Tuscarawas County Auditor	1	$1,605.10	15,003	203	37	0	69
Union County Auditor	1	$969.20	7,646	467	64	0	5
Van Wert County Auditor	1	$536.70	4,852	124	12	0	23
Vinton County Auditor							
Warren County Auditor	1	$2,862.20	23,531	1,220	141	9	21
Washington County Auditor	1	$1,126.90					
Wayne County Auditor	1	$1,853.30	17,344	167	44	0	248

Williams County Auditor	1	$545.20					
Wood County Auditor	1	$2,445.90	18,739	1,228	195	0	86
Wyandot County Auditor							
	Total:	**$107,898.10**	**760,854**	**17,383**	**2,783**	**9**	**5,950**
****NOTES:**							
Invoice 1 sent out							
Payment not yet received as of 4/29/2020							
Breakdowns of type of tags sold not provided as requested							