THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Office of Research and Graduate Studies

ANNUAL CANINE RESEARCH REPORT

FOR
2018

Submitted to:
The Ohio General Assembly

May 2019

CONTENTS OF REPORT

CANINE RESEARCH FUND

Description
The Canine Research Fund 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.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	*Clostridium difficile* in dogs: risk factors for colonization, infection and owner transmission
Principal Investigator (PI)	Greg Habing
Co-PIs/Co-Is	Jason Stull

Introduction:
Clostridium difficile is an important bacterium in human and dog health. It is the cause of over 14,000 human deaths each year in the United States and has been suggested to be an important cause of disease in dogs. It is thought that dogs may get and/or give *C. difficile* from/to people. Various toxins are produced by *C. difficile* that appear to be associated with illness; *C. difficile* stains with one or more of these toxins is termed toxigenic and is likely to cause illness in people, while nontoxigenic *C. difficile* strains do not contain these toxins and thus do not cause illness in people. The importance of *C. difficile* toxingenic status on dog health is unclear.

Using a survey of dog-owners with and without *C. difficile* infections and testing of fecal samples from them and their dogs, this project aimed to identify dog and person factors that affect the occurrence of *C. difficile* in dogs, dog illness from *C. difficile*, and determine the role of dogs in *C. difficile* transmission. Findings will assist in determining the importance of *C. difficile* for dog health and ways for protecting dog and human health.

Approach:
Dog-owners tested for clinical *C. difficile* infection at The Ohio State Wexner Medical Center were recruited from May 2016 through June 2017. Fecal samples from owners (1 sample) and up to two dogs (2 samples each) per owner were requested and cultured for *C. difficile*. A 5-minute questionnaire was used to capture important owner, household, and dog information at time of dog owner recruitment (time point 1; T0). A follow-up survey and requested dog fecal samples were used to assess changes in fecal *C. difficile* carriage and dog-owner practices approximately two months after initial survey (time point 2; T1). Molecular analysis (standard microbiological and PCR-based assays targeting the *C. difficile* triose phosphate isomerase (*tpi*) and toxin genes *tcdA* and *tcdB*) was used to characterize *C. difficile* samples (e.g., carrying disease-causing toxins). Results were analyzed to determine risk factors for dog *C. difficile* carriage, dog infection, and suggested dog-owner transmission. Analysis focused on the prevalence of *C. difficile* in human and dog feces, distribution of unique subtypes in each population and indistinguishable dog-owner pair subtypes. Fisher Exact tests were performed to identify univariable associations between dog *C. difficile* recovery and potential dog and human risk factors.

Results:
A total of 32 human patients and their 45 dogs were enrolled. All completed a survey and returned at least one dog fecal sample. Two fecal samples were returned from most dogs at enrollment (T0). Additional samples from 23 of the 45 dogs (51%) were provided at a later timepoint (T1), a median of 53 days (range: 41-295) after T0. Over the study, *C. difficile* was confirmed in 28% (9/32) of the human patients and 20% (9/45) of the dogs. Toxigenic *C. difficile*, positive for both *tcdA* and *tcdB* (4/9) or positive for only *tcdA* (3/9), were the most common toxin profiles in people, while toxigenic and nontoxigenic isolates were equally frequent in dogs.

Gastrointestinal illness (e.g., recent vomiting or diarrhea) was not reported in any of the dogs from which *C. difficile* was recovered. Close contact with the enrolled household dog was reported by most human participants [e.g. at least weekly dog licks person's face (18/45) or hands (26/45); never licks face (12/45) or hands (5/45)]. No significant husbandry, owner or dog factors were associated with *C. difficile* presence in the tested canine feces (Fisher Exact test; all $P > 0.3$).

C. difficile was identified in 53% (17/32) of the tested households (dog, human, or both), with toxigenic isolates in 10 households. Canine *C. difficile* shedding was intermittent (across consecutive samples and time points). No potential household transmission events were identified, with all human-canine pairs within a household exhibiting different toxin profiles. No long-term canine colonization was detected.

Relevance & Impact to Canine Health:
C. difficile (including toxigenic strains) was frequently identified in the feces of diarrheic human patients and their healthy dogs. While recent history of taking an antibiotic and development of diarrhea are strongly associated with development of *C. difficile* illness in people, the results from our work suggest this is not the case in dogs. No identifiable risk factors were associated with canine *C. difficile* colonization (presence). We did not detect any household *C. difficile* transmission events, for which the dog, person or a shared source was identified. Our results lend evidence to the growing body of research suggesting that *C. difficile* (regardless of toxin profile) is largely asymptomatic in dogs and even under high infection pressure (e.g., living and having close contact with an infected person), human-dog transmission is uncommon. These findings have important implications for dog owners diagnosed with *C. difficile*, suggesting that the important human-animal bond can be maintained (e.g., close contact between dogs and the infected person) and specific prevention measures to protect dogs from *C. difficile* infection are not necessary. Similarly, dogs identified with *C. difficile* colonization (without clinical illness) are unlikely to pose a large health risk to their owners, and specific prevention measures to protect owners are unlikely to be necessary.

Conclusions:
Although *C. difficile* was cultured with similar frequency in dogs and people, we identified important differences in the epidemiology of *C. difficile* between these groups. Although an important disease-causing bacterium in people, our study suggests *C. difficile* does not serve this role in dogs. No dogs in our study showed signs of illness, regardless of toxigenic *C. difficile* presence. Furthermore, as we did not identify household transmission events, infected people do not appear to be a critical source for infection in dogs. We were unable to identify the source for *C. difficile* in the study dogs. It is likely a multitude of sources may be involved (e.g. food, water, environment). Future work should focus investigation on these additional sources. As small sample sizes may have affected our ability to identify significant risk factors or transmission events, future work may be warranted to utilize other population sources to increase sample numbers and allow for detection of smaller effects of *C. difficile* presence on dog health and human-dog transmission.

Publications/Presentations/Grant Submissions:
Results from this work were presented at the 15th International Symposium of Veterinary Epidemiology and Economics (2018; Chiang Mai, Thailand).

A manuscript of this work is in development.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Pharmacokinetics, pharmacodynamics, and sedative effects of orally, intramuscularly, and intravenously administered dexmedetomidine in dogs
Principal Investigator (PI)	Richard Bednarski, DVM
Co-PIs (if applicable)	

Introduction:
For some dogs a veterinary facility can cause unwanted anxiety, fear, and aggression. In these situations, dogs are often maximally restrained for intramuscular injection of sedative drugs. This can imprint a negative impression on the dog for subsequent veterinary visits and can be stressful to the dog and handler. Orally administered sedation can be a useful alternative requiring significantly less physical restraint. We propose to compare the sedation, recovery and blood plasma drug levels in dogs that receive sedation after intramuscular or oral dexmedetomidine. In a pilot study we have orally sedated aggressive dogs using this drug. Results have been encouraging. The goal of the study is to demonstrate that oral administration of dexmedetomidine is a viable alternative to intramuscular injection of this drug, providing a painless, low stress, positive experience for dog and handler.

Approach:
Parenteral sedation of aggressive, scared, or injured dogs requires significant restraint that can be stressful and dangerous for the dog and personnel. Orally (PO) administered sedation is a potential alternative because it requires less restraint for administration and is painless, reducing the stress of the dog and handler. Intravenously (IV) or intramuscularly (IM) administered dexmedetomidine, a potent alpha-2 adrenoreceptor agonist, is used commonly for sedation and analgesia in dogs. Clinically, we have administered dexmedetomidine PO to aggressive dogs with good effect. We propose to evaluate and compare the quality of sedation and plasma concentrations of PO and IM administered dexmedetomidine in dogs, and to determine and compare the bioavailability of these routes, which requires intravenous administration for these calculations. This study will determine the absorption of the injectable formulation of dexmedetomidine following PO administration to dogs, as well as how rapidly it is distributed and eliminated. In addition, the sedative efficacy of IM and PO administration of dexmedetomidine will be evaluated. Our findings will provide veterinarians with information addressing sedation in dogs using PO dexmedetomidine, including onset and duration of action, level of sedation, and potential side effects. This knowledge will be beneficial for increasing veterinarians' ability to administer PO dexmedetomidine safely and effectively.

Results:
Data collection and analysis is complete. The manuscript detailing the IV and PO data has been submitted for publication to the American Journal of Veterinary Research and is currently under review. The manuscript detailing the IM data is currently in progress and will be submitted to Journal of Veterinary Pharmacology and Therapeutics.

Relevance & Impact to Canine Health:
The research was undertaken to evaluate how the drug dexmedetomidine is absorbed after oral administration to determine if it can be used to provide sedation in dogs.

Conclusions:
Dexmedetomidine concentrations from plasma samples from dogs that received intravenous, intramuscular, and oral dexmedetomidine are currently being evaluated.

Publications/Presentations/Grant Submissions:
None at this time.

PROGESS REPORT (lay report) to the Ohio General Assembly

Title	*Akkermansia muciniphila* effect on intestinal microbiome in healthy dogs post antibiotic treatment
Principal Investigator (PI)	Adam Rudinsky
Co-PIs (if applicable)	Maria Jugan, Chen Gilor, Joshua Daniels, Jan Suchodolski, Joerg Steiner

Introduction:
Akkermansia muciniphila is a bacterium that normally resides in the intestines and contributes to mucosal health. Antibiotic-associated diarrhea (AAD) is an expensive, ongoing disease in human and veterinary medicine, with potential life-threatening complications due to bacterial translocation after antibiotics adversely affect the normal gastrointestinal bacteria (dysbiosis). Recently, probiotic administration was evaluated in humans with AAD with promising results and an ability to stabilize and minimize the effect on the gastrointestinal bacteria. With this generous grant, we have been studying the effect of the probiotic *Akkermansia muciniphila* in dogs treated with metronidazole or amoxicillin-clavulanic acid.

Approach:
Eight healthy research dogs were included in the placebo-controlled, repeated measures study. Dogs received the sequential antibiotic treatments with wash-out/rest periods in between: metronidazole (12.5 mg/kg POq12h) for 7 days (day 8-14) followed by 7 days (day 15-21) of omeprazole (1 mg/kg PO q24 hours) with either *A. muciniphila* (1-5 x 1010 CFU/ml in sterile PBS; 109 CFU/kg PO) or vehicle (equal volume PBS PO). The study was then repeated with Clavamox.

Fecal samples were collected throughout the study after natural voiding and the DNA extraction was performed using a QIAamp stool DNA kit (Qiagen). *A. muciniphila* was quantitated by in-house PCR in fecal samples from the above days using a previously described protocol. Fecal microbiome analysis and sequencing was performed on the above days using Illumina based on V4-16S rRNA sequence for total bacteria assessment at AnimalBiome, with qPCR validation and calculation of dysbiosis index in the Gastrointestinal Laboratory at Texas A&M University.

Results:
The microbiome results underwent statistical analysis in conjunction with AnimalBiome bioinformaticians and were completed/returned to OSU in February 2019. The sequencing results revealed good quality data, repeatability, and adequate sample volume for microbiome analysis allowing confidence in interpretation.

Initial data available demonstrated successful administration of the probiotic, improved subjected fecal scores, and improved dysbiosis index when antibiotics were given followed by the probiotic. *A. muciniphila* was identified by fecal DNA PCR in all dogs during the study indicating successful administration. It was detected at post-supplementation time-points on day 21 (concentration: 101-102 molar copies/µL), day 91 (concentration: 1-102 molar copies/µL), and day 125 (concentration: 101-102 molar copies/µL). No *A. muciniphila* was detected in any fecal sample at baseline periods (days 7, 43, 76, 112) or after antibiotic supplementation (days 15, 50, 84, 119). Fecal score was worse following metronidazole treatment compared to amoxicillin-clavulanate ($p<0.05$), and there was trend toward improved fecal scores following Akkermansia. Dysbiosis index showed a trend towards a protective effect of *A. muciniphila* administration after antimicrobials. Primary impact to the total microbiome was dictated by antimicrobial exposure and to a lesser extent the protective effect of *A. muciniphilia*.

Relevance & Impact to Canine Health:
Although the exact incidence of AAD is unknown in dogs, the rate is suspected to be similar to humans, with side-effects dependent upon the antibiotic class. Dogs experience side-effects to a similar spectrum of antibiotics as humans. Amoxicillin-clavulanate results in gastrointestinal side-effects in approximately 25% of humans, and microbiome shifts have been noted in dogs receiving ampicillin, which would likely translate to a potentiated penicillin. Furthermore, although metronidazole is the most commonly used antibiotic for chronic enteropathies in dogs, decreased microbiome diversity and microbiome shifts have been noted. Based on these results and based on data from other models, we expect that following antibiotic therapy there would be shifts toward gram negative microorganism populations and decreased microbiome diversity. Concurrent administration of the mucosal protective probiotic, should lessen the dysbiosis and overall impact on the gastrointestinal bacteria. These results would help establish *A. muciniphila* as a target drug, with potential for use as a probiotic to treat AAD in canine medicine in further studies.

Conclusions:
Akkermansia muciniphilia provided a protective effect following antimicrobial exposure. The effect was noted in specific microbial strains, overall microbiome health, as well as clinical outcome measures including gastrointestinal permeability and fecal quality.

Publications/Presentations/Grant Submissions:

Once final results are acquired, the following plan will be updated as needed. Tentatively, our group is planning the following strategy:

Data Presented: ACVIM June 2018 Meeting – Seattle Washington
Publication – Manuscript 1: AJVR, published (Am J Vet Res. 2018 Aug;79(8):884-892.)
Publication Target – Manuscript 2: PLoS One, expected submission July/August 2019

Grant Target: The results of this study will serve as preliminary data for grant targets of other gastrointestinal epithelial injury models (e.g. chemotherapy). Primary target for follow-up studies to be the Veterinary Cancer Society Grants.

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FINAL PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Pharmacokinetics of intranasal and intravenous naloxone hydrochloride in dogs
Principal Investigator (PI)	Dr. Turi Aarnes
Co-PIs (if applicable)	

Introduction:
Naloxone is an opioid-antagonist utilized in veterinary medicine for opioid reversal. Opioid overdose is an unfortunately common occurrence in people, and animals have been treated for inadvertent opioid ingestion in homes where pets are present. Additionally, working dogs and their handlers (police, military, search/rescue) have been treated for opioid intoxication (heroin, fentanyl) in the course of routine service. Treatment of opioid ingestion in these dogs has been empirical through the administration of intravenous (IV) or intramuscular naloxone. However, an intranasal (IN) formulation of naloxone is available for humans for bystander administration. The use of the IN naloxone formulation had not been investigated in dogs. This study determined the pharmacokinetics and clinical effects of IV and IN naloxone in adult dogs. This study will provide veterinarians and first responders with information addressing the efficacy of intranasal naloxone administration in dogs, a potentially useful route of administration for non-veterinary personnel.

Approach:
The objective of this study was to determine the pharmacokinetics of IN and IV naloxone in adult purpose bred dogs. The study was a one-drug trial utilizing two different routes of administration, with all dogs receiving each treatment in a blinded two-way cross over design. Dogs received IV naloxone (0.04 mg/kg) and IN naloxone (4 mg total dose) and blood samples were collected. Behavioral and physiologic variables (heart rate and respiratory rate) were evaluated following administration of naloxone. We hypothesized that IN naloxone will be well absorbed in dogs and will have high bioavailability, similar to that in humans. Our aims were to determine the pharmacokinetics of IN and IV naloxone, to compare pharmacokinetic parameters after IN and IV naloxone, and to determine absolute bioavailability of IN naloxone by comparing the ratio of AUC_{IN} to AUC_{IV} (dose normalized).

Results:
IN administered naloxone was well absorbed after a short lag time (mean ± SD, 2.3 ± 1.4 minutes). Mean maximum plasma concentration following IN and IV administration was 9.3 ± 2.5 ng/mL and 18.8 ± 3.9 ng/mL, respectively. Mean time to maximum concentration following IN administration was 22.5 ± 8.2 minutes. Mean terminal half-life after IN and IV administration was 47.4 ± 6.7 minutes and 37.0 ± 6.7 minutes, respectively. Mean bioavailability of IN administered naloxone was 32 ± 13%. There were no notable changes in dog behavior, heart rate, or respiratory rate following naloxone administration by either route.

Relevance & Impact to Canine Health:
Use of a naloxone atomizer for IN naloxone administration may represent an effective alternative to IV administration in emergency situations involving opioid exposure.

Conclusions:
Naloxone was rapidly absorbed in the dogs of the study reported here, with clinically useful bioavailability following IN administration by use of a commercially available naloxone atomizer. Plasma half-life was similar following IV and IN administration. Given the absence of noted adverse events and the need for minimal restraint associated with IN administration in the dogs in the present study, the naloxone atomizer may represent an effective alternative to IV administration in emergency situations involving opioid exposure.

Publications/Presentations/Grant Submissions:
An abstract was presented at the American College of Veterinary Anesthesia and Analgesia annual meeting in September 2018. The manuscript detailing the results of the study has been accepted for publication in the American Journal of Veterinary Research.

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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Safety and efficacy of platelet-mimicking nanoparticles to reduce bleeding time in dogs
Principal Investigator (PI)	Julien Guillaumin, Docteur veterinaire
Co-PIs/Co-Is	Patrick Satchell, Page Yaxley, Anirban Sen Gupta

Introduction:
Platelets are essential components of clotting to stop bleeding. There are many situations in dogs where platelets are lacking or not working, which put them at risk for massive bleeding and death. Unfortunately, platelet transfusion is difficult in veterinary medicine, because of the lack of an appropriate natural product. Therefore, dogs with low platelets suffer devastating consequences, and may even die.

Approach:
The issue with lack of appropriate platelet product can be resolved by a synthetic product that mimics platelets. Such a product is being developed using nanoparticles at Case Western Reserve University. The goal of our study is to evaluate the safety and applicability of the synthetic platelet particles in normal dogs.

Results:
Six purpose-bred dogs received 3 different doses of synthetic platelets in a cross-over design with a wash-out period of 1 week minimum. Platelet function assays, coagulation assays and general biochemist try and complete blood count were assessed. There were no changes in all parameters assessed. There were no adverse reactions to the synthetic platelets.

Relevance & Impact to Canine Health:
These findings in dogs are important pilot data to test those synthetic platelets in a patient population in needs of platelet transfusion.

Conclusions:
These findings in dogs can not only lead to a synthetic platelet applicable veterinary medicine, but may subsequently revolutionize platelet transfusions in humans.

Publications/Presentations/Grant Submissions:
Presentation Proposal Submitted – April 2019
Publication Submission Date – Excepted Summer/Fall 2019
Department of Defense Grant Submission – Expected Fall 2019/Spring 2019

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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Microsensitivity of Freeze-Thaw-Cycled Canine Plasma
Principal Investigator (PI)	Page Yaxley
Co-PIs (if applicable)	Kathy Gerken, Julien Guillaumin, Ed Cooper, Josh Daniels

Introduction:
Fresh frozen plasma and frozen plasma are used to replace coagulation factors and protein in veterinary patients. In clinical practice, sometimes plasma units are thawed, but then discarded because of a change in patient status, death, or complications with the actual plasma unit. It has been previously studied that the coagulation factors and hemostatic proteins remain stable once these units have been thawed and then frozen again for use at a later date. It has not been studied if these units develop any bacterial contamination due to the change in temperature and re-storage. The goal of this study is to determine if bacterial contamination as a result of thawing and refreezing is a substantiated complication that would prevent the future use of these units in the clinical patient.

Approach:
A preliminary study has been performed to determine if canine plasma can serve as a medium for bacterial growth. The results showed that plasma is a medium for growth. Despite some inhibition of growth, the preliminary study also concluded that retrieval of bacteria is possible following a freeze thaw cycle.

Based on the preliminary data, thirty fresh frozen microunits underwent a freeze-thaw-cycle process. Six fresh plasma units were divided at collection using standard technique by the blood bank into 5 microunits. Each unit was cultured to rule out bacteremia, and serve as a negative control. Microunits were frozen at -20C for a minimum of two weeks. These were then thawed in a 37C rocking water bath. After the first thaw, the unit was spiked with a site coupler to replicate clinical practice. A 200 microliter sample was then collected and plated in duplicate on sheep blood agar plates. Each microunit remained at 4C (refrigerated) for four hours, then was refrozen at -20C for one week. Lastly, during the second (final) thaw, another 200 microliter sample was collected and plated. Culture plates were monitored daily for 72 hours. Bacterial colonies were speciated.

Results:
Preliminary data suggests that the freeze-thaw-cycle process itself may be prohibitive to bacterial growth. Lower numbers of bacterial colonies were observed in samples undergoing the freeze-thaw-cycle process compared to those allowed to proliferate in ideal conditions, however they were retrievable. Results from the primary study revealed no gross bacterial contamination at any time, and cultured negative after the first thaw. Growth of one colony on one plate was identified after the second thaw, which was later speciated to be *Staphylococcus epidermidis*. The single colony likely represents contamination, and does not contain aggressive virulence factors.

Relevance & Impact to Canine Health:
With the increasing costs and limited resources for attainment of blood products, the ability to prolong the availability of blood products already on hand that it is a stable and biosafe product, is of value to the veterinary patient and clinician alike. This project sought to incur evidence to show that these products are still safe and available for use after this cycle process, and enhance stewardship of their use.

Conclusions:
The freeze-thaw-cycle process does not appear to contribute to contamination of products in the preliminary study. Canine plasma is a viable growth medium for both gram positive and gram negative inoculums, however, the freeze-thaw-cycle process actually inhibits bacterial growth. The majority of fresh frozen plasma that underwent a freeze-thaw-cycle process, remained aseptic and viable for use. Findings should be taken in caution and best practice management and judgment with regard to use of freeze-thaw-cycled plasma should be employed before use in the clinical patient.

Publications/Presentations/Grant Submissions:
Manuscript in process with intended submission to JVECC 2019

THE OHIO STATE UNIVERSITY
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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Liponucleotide therapeutics for canine acute respiratory distress syndrome
Principal Investigator (PI)	Davis, Ian Christopher
Co-PIs (if applicable)	

Introduction:
Lung damage is a complication of conditions such as trauma and sepsis, viral and bacterial lung infections, and inhaled toxic gases in both companion animals and man. If lung damage is severe, it can progress to acute respiratory distress syndrome (ARDS), which requires Intensive Care Unit (ICU) care and is often fatal. Consequently, there is an urgent unmet need for new host-directed drugs that can prevent development of ARDS in at-risk human and veterinary patients or ameliorate ongoing ARDS, ideally irrespective of its underlying cause. Using a murine experimental model, we have identified a novel drug formulation (liponucleotides [lipoNTs]) with a high safety profile that can attenuate respiratory dysfunction in mice with ARDS. In this study, we are testing our lipoNT formulation in client-owned dogs with severe respiratory distress (mild ARDS) that are admitted to the small animal ICU at the Ohio State University Veterinary Medical Center (VMC). We are assessing the impact of treatment with either our drug or a placebo on blood oxygenation and severity of fluid accumulation in the lung (pulmonary edema) using standard clinical methods. If this trial is successful, it will set the stage for development of novel drugs to reduce ARDS mortality rates in both dogs and humans.

Approach:
We are performing a randomized, placebo-controlled, prospective clinical trial in dogs admitted to the Ohio State University VMC small animal ICU for aspiration pneumonia. Defined enrollment criteria are used to optimize signal-to-noise ratio: these include specific age and weight ranges, evidence of moderate to severe hypoxemia, and the absence of significant chronic co-morbidities (endocrine, cardiovascular, or neurological disease) or aggressive behavior. Written informed consent is obtained from all owners prior to enrollment. Animals are randomized to placebo (i.v. bolus of saline every 12 hours) and lipoNTs (i.v. bolus of saline containing 5 mg/kg CDP-choline) treatment arms – clinicians and the PI will be blinded to randomization. With the exception of increased frequency of arterial blood draws (every 12 hours instead of every 24 hours) and administration of test agents, all patients will be managed according to current best practice standards of the attending clinician. We will assess effects of lipoNT treatment for up to 4 days on clinically-relevant primary and secondary indices of pulmonary function (vital signs, arterial O_2 saturation, arterial blood gases, plasma enzymes, and peripheral blood leukogram) in each patient as well as over the whole treatment group: this approach will also maximize statistical power with a small study population. Positive treatment outcomes include clinically-significant improvements in vital signs, short- or long-term increases in S_aO_2 of >2%, >50 mmHg increase in P:F ratios, improvements in ventilation/perfusion matching, and reduced lung infiltrates. Reductions in time to discontinue oxygen support and time to discharge will also be viewed as positive outcomes.

Results:
In collaboration with ICU clinicians and the VMC Clinical Trials Office, we made several modifications to the study protocol in order to ensure consistent enrollment and treatment of enrolled dogs. For example, we added aggression or difficulty with handling to exclusion criteria after an animal was enrolled that could not be consistently bled due to its aggressive behavior. Due to difficulty obtaining sufficient supplies of CDP-ethanolamine, we also modified the treatment regimen to use CDP-choline only. These changes to the study protocol necessitated some redesign of the REDCap database and required approval for the amended study from the Ohio State University Institutional Animal Care and Use Committee. We have recruited 10 patients that met the enrollment criteria so far. No treatment-related adverse events have been reported and all data from each patient has been added to the REDCap clinical database. After enrolling the first 8 patients we decoded the data. While it is not yet clear that liponucleotide treatment is effective, there is no evidence of either futility or harm. We have also been able to show that it is possible to conduct rigorous clinical trials of ARDS therapies in a veterinary ICU – to our knowledge this has been the first study of its kind in a veterinary medical center.

Relevance & Impact to Canine Health:
If successful, our studies will provide firm evidence that i.v. lipoNT treatment can reduce the severity of pulmonary dysfunction and hypoxemia in a clinically-relevant model of moderate, spontaneous ARDS in dogs. Since they could lead to new FDA-approved ARDS drugs, our findings will have the potential to transform critical care resulting in both improved survival and reduced health care costs for human patients and animal owners. Successful development of a new ARDS drug will also improve long-term quality of life for dogs that survive acute disease as ARDS survivors commonly exhibit prolonged and often debilitating lung dysfunction. Hence, our proposal is closely aligned with one goal of this program, which is to "research diseases of dogs that, by their nature, will provide information applicable to the prevention and treatment of both human and canine illnesses."

Conclusions:
Although we cannot yet determine the efficacy of lipoNT therapy for canine ARDS, we have established for the first time a working clinical trials protocol for dogs with ARDS and are successfully recruiting patients as they present to clinicians. During the second year of this project we have recruited an additional 5 dogs and are beginning statistical analysis to determine lipoNT efficacy in canine ARDS. We have also used this trial to establish a standard protocol for assessment of novel therapeutics in dogs with moderate to severe spontaneous ARDS. This will help us to establish the VMC ICU as a unique resource that can be used in future clinical trials in collaboration with physicians in the Ohio State University College of Medicine Division of Pulmonary, Critical Care, and Sleep Medicine – in fact, discussions with Dr. John Christman to initiate such a trial are ongoing. We believe that the ability to perform such trials will be of interest to both the scientific community (facilitating grant funding) and companies interested in developing new treatments for ARDS.

Publications/Presentations/Grant Submissions:
OSU Center for Clinical and Translational Sciences Veterinary Clinical Trials Office Voucher (supported by the National Center for Advancing Translational Sciences, National Institutes of Health; Award number UL1TR001070) – provide support for REDCap software usage.

PROGESS 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 direct femoral and sciatic nerve blockade (FSNB) versus lumbosacral epidural analgesia versus preoperative opioid administration alone to prevent the development of a chronic neuropathic pain state. We are evaluating each of these interventions in the context of surgical repair for spontaneous cruciate ligament (CCL) rupture in client-owned dogs. We hypothesize 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 will be lower in dogs that received an epidural or FSNB compared to those who receive opioids alone.

Results:
We have currently enrolled 33 of 45 patients for this study. We anticipate finishing enrollment by the end of summer and to have completed data analysis this fall.

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.

Conclusions:
We are still in the data collection phase of this study and thus have no conclusions to report at this time.

Publications/Presentations/Grant Submissions:
Data collection is still in progress thus no presentations or manuscripts have been given/submitted. We hope to submit preliminary data for abstract presentation at the 47th Annual Veterinary Orthopedic Society Conference February 2020.

PROGESS 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 (if applicable)	

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*. Finally, we tested the ability of new PRMT5 inhibitor drugs to kill canine lymphoma cells, testing these drugs both on dog lymphoma cell lines and on cells collected directly from affected dogs.

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 have 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, 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, target methylation, and changes in gene expression.

Results:
We have determined that PRMT5 is expressed in varying amounts in most canine lymphomas, with approximately half 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.

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 will further enhance the preclinical development of PRMT5 inhibitor drugs being developed by OSU investigators. Results of this study should 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 PRMT55 inhibitors for treating cancer in people.

Publications/Presentations/Grant Submissions:

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.

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.

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.

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.

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.

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.

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.

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 2019

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

PROGESS 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)	Megan Brown, DVM, MS, DACVIM (Oncology)
Co-PIs (if applicable)	Brittany Evans, DVM Joelle Fenger, DVM, PhD, DACVIM (Oncology)

Introduction:
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 propose 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:
Fifteeen dogs with diagnosis of lymphoma undergoing a CHOP chemotherapy protocol will be recruited from the Integrated Oncology Service. Each dog that is enrolled must have a definitive diagnosis of lymphoma in addition to complete bloodwork to evaluate general health and ability to receive chemotherapy treatment. In addition, informed written consent must be obtained prior to enrollment. As part of the study, dogs will have baseline blood collection prior to starting the protocol and again before and 3-, 6- and 24-hours after doxorubicin (week 9) administration. A final blood draw will be performed one week after doxorubicin treatment. Owners will also be asked to fill out a quality of life survey at screening, week 9 and week 10. Samples will be processed and cytokine measurement (IL6, MCP1) will be performed. Cytokine concentrations will be compared from baseline, before and after doxorubicin treatment. We expect serum cytokines concentrations in dogs with lymphoma to significantly increase after doxorubicin administration due to induction of cellular senescence.

Results:
Since the trial opened in January of 2018, 16 canine patients have been enrolled. Of these 16 patients, 8 have completed the study and 4 have been removed from study due to progressive disease. Therefore, 4 are currently on study and expected to complete the study by the end of May. We expect to be able to finish enrollment by September of 2019. Data analysis will be performed once enrollment and study procedures have been completed for all 15 patients.

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:
Enrollment is on schedule and expected to finish in fall of 2019. Data analysis and manuscript preparation will occur at the end of 2019/beginning of 2020 with anticipated manuscript submission by April of 2020.

Publications/Presentations/Grant Submissions:
None at this time.

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PROGESS 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)	Karina Creighton
Co-PIs (if applicable)	Daniel Gordon, Adam Rudinsky

Introduction:
The reported 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 vitamin C supplementation in humans has been reported, including reduction in time on vasopressors and improvement in 28-day mortality rates. The results of this pilot study will allow us to determine if vitamin C levels are low in critically ill canine patients, leading to further investigation into whether parenteral supplementation of vitamin C improves clinical outcomes in septic canine patients.

Approach:
Twenty client-owned dogs admitted to ICU with evidence of a systemic inflammatory response and hypotension will be enrolled in this study. Half of the study group will have a documented bacterial infection as the cause of their systemic disease. Baseline data will be collected to calculate a severity of illness score (the canine Acute Patient Physiologic and Laboratory Evaluation score). Serum Vitamin C levels will be measured at admission to ICU, and 24 and 48 hours after admission to ICU. As there are no reference ranges for serum vitamin C in dogs, vitamin C levels will also be measured in 10 healthy control dogs. We hypothesize that patients with a systemic inflammatory response and hypotension will have low circulating levels of vitamin C compared with control dogs, and that patients with sepsis will have lower levels of vitamin C than patients with other causes of shock.

Results:
Enrollment for this study is ongoing. 10 control dogs and 10 critically ill dogs have been enrolled. Preliminary data is available for 10 control dogs and 7 critically ill dogs.

Relevance & Impact to Canine Health:
The significance of this pilot study will allow us to determine if vitamin C levels are truly low in these patients, leading to further investigation whether parenteral supplementation of vitamin C improves clinical outcomes in septic patients as seen in humans.

Conclusions:
This study is ongoing.

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

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Computed Tomography Quantification of Airflow Resistance
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.

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:

Statistical analysis will be run in a bulk when all dogs are enrolled to minimize costs related to the computed programs.

Publications/Presentations/Grant Submissions:

Previous publication:

Hostnik 2017 (Vet Radiol Ultrasound. 2017 Sep;58(5):542-551. doi: 10.1111/vru.12531. Epub 2017 Jul 17).

Previous presentation:

ACVR/IVRA 2018 Conference at Ft. Worth, Texas.

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

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

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

Results:
Data collection will be concluded in 2019.

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

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

Conclusions:
Data collection not completed yet.

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

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	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 will 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:
The study was officially started in August 2018. We recruited 3 cases.
Data collection for the retrospective analysis of this study is in progress.

Relevance & Impact to Canine Health:
We seek to identify new evidence-based guidelines in the treatment of MCT, and reduce the complications associated with surgery.

Conclusions:
This study is ongoing.

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

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

Introduction:
Soft tissue sarcoma (STS) is a common cancer in dogs, especially Labrador and Golden Retrievers. In this study we will analyze STS tumors 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 perform RNA sequencing (RNAseq) of a subset of tumors with 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 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. Additionally, the allelic information will reveal whether any haplotypes carry hotspots for structural mutation and, if so, whether any GWAS risk loci are among those. 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 a subset of these 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. Unfortunately, additional samples identified and requested from the Canine Comparative Oncology Genomics Consortium (CCOGC) are not currently available for release from the CCOGC. Recently, we identified additional tumor samples and a set of germline control samples from the Cornell University biobank.

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:
None at this time.

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PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Incidence of acute kidney injury in dogs undergoing contrast-enhanced computed tomography
Principal Investigator (PI)	Karina Creighton
Co-PIs (if applicable)	Catherine Langston, Jessica Hokamp

Introduction:
Iodine-base contrast agents are commonly used in computed tomography (CT scans). These agents are a recognized cause of acute renal injury in human patients, but there is little information regarding the effects of these agents in dogs undergoing CT scans. The purpose of this study is to determine the prevalence of renal injury in dogs administered iodinated contrast, using recognized criteria for diagnosis of acute kidney injury. Additionally, we will evaluate a novel urinary biomarker, NGAL, to determine whether it is a more sensitive and earlier marker of renal injury than traditional diagnostic tools.

Approach:
125 client-owned dogs undergoing contrast-enhanced computed tomography that are expected to be hospitalized for 48 hours after contrast administration will be enrolled in the study. Creatinine will be measured prior to contrast administration and at 24 and 48 hours post-contrast administration. Urinary NGAL will be measured prior to contrast administration and at 6, 12, 24, and 48 hours after contrast administration. We hypothesize that the prevalence of CI-AKI will be around 8% in our study population, as assessed using the veterinary AKI criteria. Additionally, we hypothesize that urinary NGAL will be a more sensitive indicator of renal injury than creatinine, and that changes in urinary NGAL will be observed earlier than changes in serum creatinine.

Results:
Enrollment for this study is ongoing.

Relevance & Impact to Canine Health:
With the increasing use of contrast-enhanced computed tomography in veterinary medicine it is important to determine the prevalence of CI-AKI in dogs, and whether we can use novel biomarkers for more sensitive and timely diagnosis. This will allow for further investigation of treatment strategies that will decrease the risk and/or severity of CI-AKI in at-risk patients.

Conclusions:
This study is ongoing.

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

PROGESS 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 Fenger, DVM, PhD, DACVIM (Oncology)
Co-PIs/Co-Is	

Introduction:
Mast cell tumors (MCTs) are a common malignant skin tumor in dogs. They possess a range of biologic behaviors with some dogs developing benign tumors that are cured by surgery, to extremely aggressive MCTs that are refractory to surgery, radiation and chemotherapy. The identification of activating mutations in the receptor tyrosine kinase Kit in patients with advanced MCTs has provided insight into the genetic changes underlying this disease; however, responses are generally not durable beyond 12 months and treatment is often unsuccessful in the 50-70% of dogs that do not possess Kit mutations. The exact mechanisms through which other genetic factors influence multiple aspects of malignant MCTs have yet to be fully defined.

Our laboratory has been studying the genetic factors that contribute to the aggressive behavior of canine MCTs. The WWOX tumor suppressor gene is frequently deleted in human cancers and plays a key role in regulating DNA damage repair and maintaining genomic stability. We found that WWOX is decreased in canine mast cells (MCs) and primary MCTs compared to normal MCs, suggesting that loss of WWOX contributes to the aggressive behavior of MCTs. The goal of this study is to dissect how WWOX affects normal and malignant MC biology. ***We hypothesize that WWOX loss impairs DNA damage repair and promotes genomic instability in normal and malignant MCs. We predict that in malignant MCs possessing low basal levels of WWOX, WWOX overexpression will restore DNA damage repair functions and enhance the sensitivity of MCs to DNA damaging agents.***

Approach:
The overarching goal of this study is to gain a more complete understanding of the functional role of WWOX in mediating DNA damage repair and maintaining genomic stability normal and malignant mast cells. To accomplish this, we will use the following experimental approaches:

AIM 1: Assess expression of WWOX in canine mast cell tumor cell lines and tumor samples. We will determine the gene transcript and protein expression levels of WWOX in canine MC lines and primary MCTs using a combination of real-time PCR, immunohistochemistry, and Western blotting.

AIM 2: Determine the role of WWOX in repairing DNA damage in normal and malignant MCs lines. Canine MC lines expressing low basal levels of WWOX will be transduced with lentiviral gene expression constructs in order to dissect the consequences of WWOX overexpression on MC behavior. We will evaluate the impact of restoring WWOX on DNA damage repair pathway activation following treatment with DNA damaging agents or ionizing radiation in canine MC lines.

AIM 3: Assess the biological and molecular consequences of WWOX loss in normal MCs using *Cpa3-Cre; WWOXfl/fl* transgenic mice. We have generated a novel transgenic mouse model (***CPA3-Cre; WWOXfl/fl***) allowing for conditional deletion of WWOX largely restricted to mast cells and basophils. We will investigate the effects of *WWOX* deletion on the DNA damage response and repair in normal bone marrow-derived mast cells (BMMCs) generated from single (***WWOXfl/fl***) or double (***CPA3-Cre; WWOXfl/fl***) transgenic mice.

Results:
Our preliminary data demonstrates that WWOX gene expression is frequently decreased in primary canine MCTs compared to normal canine bone marrow-cultured mast cells (BMCMCs). Concordant with these findings, we have shown that WWOX protein is expressed at low levels in primary canine MCTs. We are in the process of generating a tissue microarray containing N=40 primary MCTs and paired normal skin tissues from dogs undergoing MCT surgery at the OSU-VMC. Using this TMA, we will correlate WWOX staining intensity with markers of DNA damage (BP53, γH2AX) and cellular proliferation (Ki67) and other clinicopathologic variables (histologic tumor grade, overall survival). We have generated canine MC lines that express high levels of WWOX in order to better understand the influence of WWOX in regulating DNA damage repair and maintaining genomic stability. Preliminary data generated from these canine MC lines demonstrates that while WWOX overexpression results in decreased cell viability, it does not appear to effect the invasive capacity of MCs. Studies are ongoing to better understand the effect of WWOX restoration on sensitivity to DNA damaging agents (ionizing radiation), DNA repair pathway activation, and genomic stability in canine malignant MC lines and normal BMCMCs generated from single (***WWOXfl/fl***) or double (***CPA3-Cre; WWOXfl/fl***) transgenic mice.

Relevance & Impact to Canine Health:
Mast cell tumors (MCTs) are the most common malignant skin tumor in dogs and account for up to 20% of all skin cancers. MCTs possess a wide range of biological behaviors with benign tumors being cured with surgery alone, to biologically aggressive, metastatic mast cell tumors that spread to distant sites such as lymph nodes, spleen and liver. While activating mutations in KIT are found in approximately 20% of aggressive MCTs, the other molecular alterations that drive the development and spread of these tumors remains largely unknown. Our laboratory has been investigating factors the influence the biology of malignant of canine MCTs. The tumor suppressor gene WWOX is deleted/lost in a number of malignancies and WWOX has been implicated in playing a critical role in regulating DNA damage repair processes and maintaining genomic stability in cell lines. The impact of WWOX in canine MCT biology is unknown and dissecting the downstream pathways altered by WWOX has the potential to uncover novel targets for therapeutic intervention in MCTs. As such, the overarching goal of this proposal is to use an integrated *in vitro* and *in vivo* studies to dissect how WWOX affects normal and malignant MC biology at the molecular and cellular levels 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.

Conclusions:
We have demonstrated that canine primary MCTs frequently express low basal levels of WWOX transcript and protein compared to normal canine MCs. We have generated canine MC lines expressing low/high levels of WWOX in order to study the effects of WWOX restoration on MC biology. Enforced WWOX expression appears to decrease MC viability but does not appear to affect the invasive phenotype of cultured MCs. We have generated a novel transgenic mouse model allowing for deletion of WWOX specifically in MCs and basophils; this model will allow us to study the role of WWOX in DNA damage repair and genomic instability in normal MCs. A more thorough understanding of how dysregulation of WWOX signaling mediates the aggressive behavior of canine MCTs 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 malignant mast cell disease.

Publications/Presentations/Grant Submissions:
None at this time.

PROGESS 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:
Results are pending as the samples will be batch analyzed at the end of the study after all dogs are enrolled. Since opening the study, 1/3 of the total required enrollment has been successfully completed.

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:
The conclusions to this study are pending until the complete data set is acquired, samples are analyzed, and evaluated with statistical analysis.

Publications/Presentations/Grant Submissions:

Presentation Target: ACVIM June 2020 Meeting – Baltimore Maryland
Publication Target: PLoS One – Expected submission July/August 2020
Grant Target: To be determined following obtaining complete results from this study.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Analgesic effects and tolerability of tapentadol in combination with NSAIDS in dogs with osteosarcoma
Principal Investigator (PI)	Megan Brown, DVM, MS, DACVIM (Oncology)
Co-PIs/Co-Is	Vincent Wavreille, DVM, MRCVS, DAVCS Nina Kieves, DVM, DACVS, DACVSMR, CCRT Turi Aarnes, DVM, MS, DACVAA

Introduction:
Canine osteosarcoma is a bone tumor that causes pain and interferes with quality of life. Amputation of the affected limb is the best form of pain control, but not always pursued due to coexisting diseases or owner wishes. In such cases, adequate pain control is paramount.

Approach:
Tapentadol is a novel opoid pain medication that has been shown to be well tolerated and provided pain relief in healthy dogs, but has not been studied in dogs with naturally occurring pain. Therefore, the goal of this study is to evaluate the pain relief effects of tapentadol in dogs with osteosarcoma. Dogs with suspected appendicular osteosarcoma will be enrolled in the study. At the time of screening, owners will complete a baseline pain assessment. On Day 1, dogs will undergo a standardized pain assessment by a veterinarian and pressure sensitive walkway evaluation. Patients will be discharged on tapentadol and an NSAID. Owners will also be given a daily drug log to document mediation administration and adverse events associated with tapentadol treatment. Patients will have a repeat veterinarian and owner pain assessments and pressure sensitive walkway evaluation on Day 5. We expect that tapentadol, in conjunction with NSAIDs, will provide analgesia and be well tolerated in dogs with appendicular osteosarcoma.

Results:
This study was recently funded and just getting started.

Relevance & Impact to Canine Health:
Pain medication for dogs include non-steroidal anti-inflammatory drugs (NSAIDS). However, NSAIDs can cause side effects and may not be sufficient for moderate to severe pain. Tramadol, an opoid-like medication is also used, although growing evidence demonstrates that Tramadol does not produce reliable pain controls in dogs. Therefore, new pain medications are needed. If successful, this study would support further investigation of tapentadol as an analgesic in small animals, including management of hard to treat conditions, such as chronic and cancer-related pain.

Conclusions:
This study was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Morphologic, morphometric and functional characterization of degenerative lumboscacral stenosis in Labrador Retrievers
Principal Investigator (PI)	Ronaldo da Costa, DVM, MS, PhD
Co-PIs/Co-Is	Stephen Jones, MVB, MS Carolyn Nye, DVM

Introduction:
Degenerative lumbosacral stenosis (DLSS), also known as cauda equine syndrome, is a common disease affecting the caudal spine of dogs, primarily large breed dogs. DLSS frequently affects Labrador retrievers causing compression of nerves in caudal lumbar region (cauda equine), nerve roots and vessels leading to pain and neurological deficits.

Approach:
DLSS is typically diagnosed using advanced imaging, such as computed tomography (CT) or magnetic resonance imaging (MRI), however the correlation between clinical and imaging findings is complex, and new methods may lead to advances and refinement in the diagnosis of the disease. We propose to characterize DLSS in Labradors using a combination of a novel kinematic MRI technique in combination with electrodiagnostic tests. In order to fully comprehend the disease, we need to perform through advanced imaging and functional tests of the lumbar spine of healthy and affected Labrador retrievers.

Results:
This study was recently funded and is just getting started.

Relevance & Impact to Canine Health:
Canine DLSS bears similarities to human degenerative lumbar spinal disease, the most common cause of back pain in people, as both affect the cauda equine resulting in 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. While many studies have been done to assess canine lumbosacral disease, a prospective, objective study looking at the anatomical and functional abnormalities in dogs clinically affected with DLSS comparing it to clinically normal dogs using kinematic MRI and electrodiagnostics is lacking. This study will be the foundation for future therapeutic and genetic studies of DLSS in dogs.

Conclusions:
This study was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Assessment of regional intestinal perfusion by infrared thermography during foreign body surgery
Principal Investigator (PI)	Julien Guillaumin, DVM
Co-PIs/Co-Is	Soscha Tencate, DVM Edward Cooper, VMD Karina Creighton, BVSc Page Yaxley, DVM Mary McLoughlin, DVM, MS

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 as leaking 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:
Digital and infrared thermographic images will be obtained of the FBO-affected intestinal loop, as well as adjacent oral and aboral intestinal loops. Images will be acquired before and after completion of the surgical technique, either enterotomy or resection-anastomosis (R&A). Systemic perfusion status will be assessed by blood pressure, lactate measurement and sublingual microvascular imaging. Illness severity will be stratified with $APPLE_{full}$ score. R&A intestinal segments will be submitted for histopathological analysis.

Results:
This study was recently funded and is just getting started.

Relevance & Impact to Canine Health:
Intraoperative assessment of intestinal perfusion, is important to avoid intestinal dehiscence. Intestinal dehiscence can lead to septic peritonitis, multi-organ dysfunction syndrome and septic shock. If shown to be reliable, this technique could provide objective, real-time information about local perfusion and tissue viability. This information could then be used in surgical decision making, reducing the number of surgical complications.

Conclusions:
This study was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

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, DVM, PhD
Co-PIs/Co-Is	Deborah Knapp, DVM, MS Morgan Evans William Kisseberth, DVM, PhD

Introduction:
Translational cell carcinoma (TCC) is the most common bladder cancer in dogs and is associated with herbicide and pesticide exposure. 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 herbicides and pesticides.

Approach:
The primary goal of this study is to characterize the urinary and stool microbiota in dogs with and without TCC. The second goal is to quantify and compare microbial genes from urine that are involved in herbicide and pesticide metabolism to determine if these genes are enriched in dogs with TCC. We will free-catch urine and stool samples from healthy dogs and dogs that present with TCC. Samples will undergo DNA extraction and 16S rRNA sequencing. A subset of urine samples will be selected for metagenomics sequencing and microbial gene analysis.

Results:
This study was recently funded and is just getting started.

Relevance & Impact to Canine Health:
The microbiome is a good target for developing diagnostics or treatments and preventions as it can be readily manipulated. This study has the potential to lead to alternative diagnostics for TCC – as biopsies can be challenging and invasive. It will also provide early insights into microbial genes enriched in TCC – potentially including those related to herbicide/pesticide metabolism.

Conclusions:
This study was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PROGESS REPORT (lay report) to the Ohio General Assembly	
Title	Pilot study on the effects of intra-articular allogenic stem cell therapy for the treatment of osteoarthritis
Principal Investigator (PI)	Nina Kieves, DVM
Co-PIs/Co-Is	Jennifer Barrett, DVM, PhD Eric Hostnik, DVM, MS

Introduction:
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, evening 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. This arthritis develops in a predictable manner, making it an excellent model to study arthritis treatment.

Approach:
This 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. Our laboratory has previously validated, screened and optimized three-dimensional cultured (3D) canine adipose-derived stem cells for their anti-inflammatory properties as an allogenic treatment of osteoarthritis.

Results:
This study was recently funded and is just getting started.

Relevance & Impact to Canine Health:
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 was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

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 Wittum, MS, PhD
Co-PIs/Co-Is	Dubraska Diaz-Campos, DVM, PhD Greg Ballash, DVM, MPH Dixie Mollenkopf, MS, PhD

Introduction:
Urinary tract infections (UTI), most commonly caused by *Escherichia coli*, are one of the most common diseases affecting dogs that require antibiotics. The use of antibiotics can influence the types of virulence and antibiotic resistant gene in *E. coli* predisposing these dogs to persistent and chronic UTIs. We propose to study the association of antibiotic therapy with virulence and antibiotic resistance of *E. coli* isolates causing UTIs.

Approach:
Using whole genome sequencing, we aim to identify the antimicrobial resistance and virulence genes for each isolate. Previous antimicrobial therapy will be assessed for each of the canine patients by investigating the clinical history and medical record of each patient. In doing so, we expect to determine if there is an association between antimicrobial therapy and antimicrobial resistance and /or virulence in canine patients with UPEC UTIs.

Results:
This study was recently funded and is just getting started.

Relevance & Impact to Canine Health:
This data will assist clinicians to make more educated decisions about antibiotic use for canine UTIs.

Conclusions:
This study was recently funded and is just getting started.

Publications/Presentations/Grant Submissions:
This study was recently funded and is just getting started.

FUNDING OF PROJECTS	
TITLE	**BUDGET**
Clostridium difficile in dogs: risk factors for colonization, infection and owner transmission	$22,727
Pharmacokinetics, pharmacodynamics, and sedative effects of orally, intramuscularly, and intravenously administered dexmedetomidine in dogs	$22,727
Akkermansia muciniphila effect on intestinal microbiome in healthy dogs post antibiotic treatment	$ 8,182
Pharmacokinetics of intravenous and intranasal naloxone hydrochloride in dogs	$22,050
Safety and efficacy of platelet-mimicking nanoparticles to reduce bleeding time in dogs	$22,549
Microsensitivity of freeze thaw cycled canine plasma	$ 2,740
Liponucleotide therapeutics for canine acute respiratory distress syndrome	$22,571
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
Perfusion index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs	$11,588
Serum cytokine concentrations in dogs with multicentric lymphoma before and after doxorubicin treatment	$22,100
Pilot Study: Serum vitamin C levels in dogs with non-septic and septic critical illness	$ 8,182
Computed tomography quantification of airflow resistance before and after nasal turbinate laser ablation	$22,727
Pulse oximetry pleth variability index as a predicter of fluid responsiveness in dogs	$22,728
Influence of neo-adjuvant steroid administration on histologic margins in canine cutaneous mast cell tumors	$22,698
Germ line and somatic genetics of canine soft tissue sarcoma	$22,493
Incidence of acute kidney injury in dogs undergoing contrast-enhanced computed tomography	$22,727
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
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

APPENDICES

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

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Application Deadline Date

Canine/Equine
Spring ☐
Fall ☐

This is a:
☐ New Proposal
☐ Resubmission

Intramural Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR CFR USE ONLY.

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

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

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

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

3. PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)

3b. DEGREE(S)/BOARD CERTIFICATION

3c. POSITION TITLE

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

3e. DEPARTMENT

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

3g. E-MAIL ADDRESS:

4. HUMAN SUBJECTS RESEARCH
☐ No ☐ Yes

4b. Human Subjects Assurance No.

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

6. VERTEBRATE ANIMALS ☐ No ☐ Yes

4a. Research Exempt
☐ No ☐ Yes

If "Yes," Exemption No.

6a. IACUC Approval and Date

6b. ILACUC Number

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

10. Checklist:

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

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

Principal Investigator (Last, First, Middle):

Abstract and Key Personnel

Intramural Grant Application

College of Veterinary Medicine

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

DO NOT EXCEED THE SPACE PROVIDED (300 words).

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

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

Name	Department	Time Commitment to Project	Signature

Principal Investigator (Last, First, Middle):

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

FROM	THROUGH

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

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

EQUIPMENT *(Itemize and provide justification below)*

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

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

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

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

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

Principal Investigator (Last, First, Middle):

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

INTRAMURAL GRANT APPLICATION
COLLEGE OF VETERINARY MEDICINE

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

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

Principal Investigator (Last, First, Middle):

RESOURCES

INTRAMURAL GRANT APPLICATION
COLLEGE OF VETERINARY MEDICINE

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

Laboratory:

Clinical:

Animal:

Computer:

Office:

Other:

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

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

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

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

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

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

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

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

F. Time Line for Experimental Plan:

G. Literature Cited

III. INVESTIGATOR INFORMATION

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

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

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

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

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

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

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

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

COUNTY PAYMENTS			NUMBER OF TAGS SOLD				
County	Invoice	Amt Paid $	1 - YR	3 - YR	PERMNT	Dangerous Dogs	KENNEL REG
Adams County Auditor	1	$770.70	7,384	76	6	0	35
Allen County Auditor	1	$1,620.80	15,618	139	16	0	13
Ashland County Auditor	1	$903.30	8,364	146	16	0	71
Ashtabula County Auditor	1	$1,052.90	9,370	243	41	0	20
Athens County Auditor	1	$877.30	8,651	33	0	0	23
Auglaize County Auditor	1	$908.60	8,389	189	13	0	0
Belmont County Auditor	1	$984.30	8,576	310	31	0	36
Brown County Auditor	1	$836.70	7,894	116	11	0	15
Butler County Auditor	2						
Carroll County Auditor	1	$775.80					
Champaign County Auditor	1	$807.30	7,681	51	7	0	169
Clark County Auditor	1	$2,153.00					
Clermont County Auditor	1	$1,867.60	16,967	376	56	0	21
Clinton County Auditor	1	$835.80					
Columbiana County Auditor	1	$2,111.60	20,999	5	8	0	22
Coshocton County Auditor	1	$986.50	9,057	16	4	0	720
Crawford County Auditor	1	$859.10					
Cuyahoga County Auditor	1	$6,900.40					
Darke County Auditor	2						
Defiance County Auditor	1	$761.90	7,078	113	12	0	82
Delaware County Auditor	1	$2,059.60					
Erie County Auditor	1	$1,384.80	13,777	2	0	0	65
Fairfield County Auditor	1	$2,460.30	22,515	482	62	0	22
Fayette County Auditor	1	$425.30	3,638	50	46	0	5
Franklin County Auditor	1	$10,634.90					
Fulton County Auditor	1	$812.20	7,610	119	9	0	65
Gallia County Auditor	1	$237.70	1,990	16	1	0	329
Geauga County Auditor	1	$1,250.10	11,348	18	13	0	180
Greene County Auditor	1	$2,621.60	22,483	890	102	0	43
Guernsey County Auditor	1	$612.84	5,925	78	14	0	40

Hamilton County Auditor	2						
Hancock County Auditor	1	$1,282.30					
Hardin County Auditor	1	$704.90	7,008	7	0	0	20
Harrison County Auditor	1	$357.40					
Henry County Auditor	1	$6,273.30	6,062	31	10	0	18
Highland County Auditor	1	$518.40	4,693	109	12	0	44
Hocking County Auditor	1	$450.10	4,363	21	3	0	45
Holmes County Auditor	1	$1,034.30	9,793	4	2	0	518
Huron County Auditor	1	$1,107.40	10,679	84	10	0	43
Jackson County Auditor	1	$682.60	6,479	53	6	0	128
Jefferson County Auditor	1	$502.10	4,296	146	28	0	7
Knox County Auditor	1	$945.60	8,867	113	16	10	90
Lake County Auditor	1	$2,924.90	26,853	633	47	0	27
Lawrence County Auditor	1	$886.00	8,739	36	1	0	4
Licking County Auditor	1	$3,184.10	31,570	7	0	0	25
Logan County Auditor	1	$605.70	5,740	29	18	0	50
Lorain County Auditor	1	$2,799.50	25,341	548	83	0	180
Lucas County Auditor	1	$5,545.80	52,332	804	39	0	24
Madison County Auditor	1	$665.80	5,790	206	23	0	20
Mahoning County Auditor	1	$2,970.90	27,669	415	39	0	405
Marion County Auditor	1	$902.40	8,228	158	25	0	72
Medina County Auditor	1	$2,509.60	20,781	970	132	0	85
Meigs County Auditor	1	$211.40	2,007	21	2	0	24
Mercer County Auditor	2						
Miami County Auditor	1	$1,878.80					
Monroe County Auditor	1	$364.40	3,563	3	2	0	260 tags sold for 52 accounts
Montgomery County Auditor	1	$6,198.70					
Morgan County Auditor	1	$305.80	2,690	63	13	0	49
Morrow County Auditor	1	$591.50	5,329	118	15	0	82
Muskingum County Auditor	1	$1,225.50	11,848	42	11	0	171
Noble County Auditor	1	$187.00	1,655	29	4	0	88
Ottawa County Auditor	1	$838.70	7,795	136	18	0	4
Paulding County Auditor	1	$351.00	3,239	53	8	0	32
Perry County Auditor	1	$677.80					
Pickaway County Auditor	2	$582.10	5,356	15	4	0	380

Pike County Auditor	2						
Portage County Auditor	1	$3,166.80					
Preble County Auditor	1	$369.30					
Putnam County Auditor	1	$673.90	6,192	9	2	0	500
Richland County Auditor	1	$1,969.90	19,060	0	0	0	639
Ross County Auditor	1	$1,460.20	14,470	1	1	0	119
Sandusky County Auditor	1	$1,256.90	11,671	185	20	0	143
Scioto County Auditor	1	$701.20	3,036	72	6	0	74
Seneca County Auditor	1	$1,077.10	9,702	177	27	0	268
Shelby County Auditor	1	$873.20	8,153	143	7	0	80
Stark County Auditor	2						
Summit County Auditor	1	$4,140.20					
Trumbull County Auditor	1	$1,973.30					
Tuscarawas County Auditor	1	$1,613.40	15,214	143	43	0	61
Union County Auditor	1	$902.60	7,418	374	48	0	6
Van Wert County Auditor	1	$522.90	5,187	21	5	0	16
Vinton County Auditor	1	$176.90	1,324	0	0	0	445
Warren County Auditor	1	$2,879.50	24,184	1,135	118	9	26
Washington County Auditor	1	$1,238.60					
Wayne County Auditor	1	$1,814.10	16,921	141	42	0	377
Williams County Auditor	1	$532.00					
Wood County Auditor	1	$2,255.00	19,553	578	117	0	93
Wyandot County Auditor	1	$434.80	4,071	55	6	0	52
	Total:	**$128,810.54**	**710,235**	**11,356**	**1,481**	**19**	**7,510**
****NOTES:**							
Invoice 1 sent on 2/1/2019							
invoice 2 sent on 4/10/2019							
Payment not yet received as of 4/29/2019							
Breakdown of types of tags sold requested, but not provided							