THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Office of Research and Graduate Studies

# ANNUAL CANINE RESEARCH REPORT

FOR

2016

Submitted to:
The Ohio General Assembly

May 2017

## 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 elected by the regular faculty of that department. Each member serves a 3 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 hospital 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 CFR grants may fund orphan projects that are important to dog welfare, but are not likely to be funded by other sources.

**PROJECT TITLE: PROSPECTIVE AND RETROSPECTIVE STUDIES ANALYZING THE EPIDEMIOLOGY AND EVOLUTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MRSP) IN A VETERINARY TEACHING HOSPITAL**

*RELEVANT TITLE: THE INCIDENCE AND EVOLUTION OF STAPH RESISTANCE IN A VETERINARY HOSPITAL*

**INVESTIGATOR: A. HOET**

*Final Report*

Methicillin-resistant Staphylococci (MRS) are notorious healthcare-associated and emerging community pathogens that cause significant morbidity and mortality worldwide. Methicillin-resistant *S. pseudintermedius* (MRSP) in particular is considered an emerging canine pathogen responsible for severe skin and ear infections, and methicillin-resistant *S. aureus* (MRSA) is an important cause of healthcare associated infections in humans and animals. MRS are resistant to a broad range of antibiotic classes; therefore, treatment failure is frequently associated with these pathogens. *Nevertheless, little is known about the ecology and epidemiology of MRS in veterinary hospitals. This knowledge is necessary for the development of effective infectious disease prevention and control programs*. The first objective of this research was to perform a prospective epidemiological study for one calendar year to determine the frequency of isolation, the distribution, and the type of MRS, especially MRSP, circulating at the Ohio State University Veterinary Medical Center (OSU-VMC). The second objective was to elucidate the evolution of pathogens such as MRSP over time in regards to their antimicrobial resistance, by performing a comparative analysis of strains found in the hospital during the last 6 years with those isolated during the prospective study.

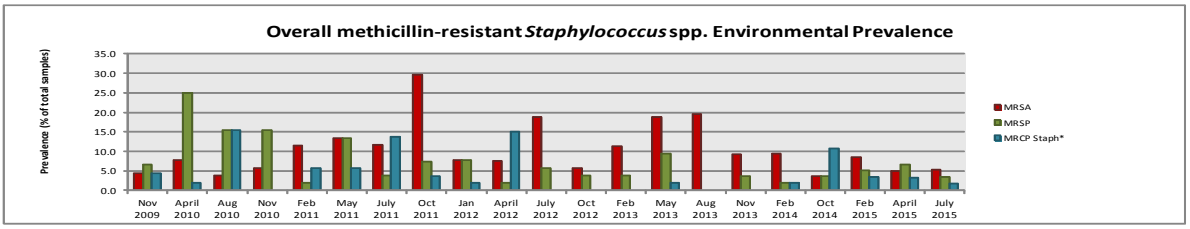
The first objective of this proposal was completed. Samples for the prospective study from incoming dogs, the environment, and personnel were simultaneously collected from October 2014 to July 2015. In the case of canines, 57% (83/145) and 11% (16/145) of dogs were positive for *S. pseudintermedius* and MRSP, respectively. Even though it is not surprising to find *S. pseudintermedius* in dogs, as this bacterium is part of their normal flora, it is concerning that the proportion of MRSP is now in the double digits. On the other hand, the prevalence of *S. aureus* (12%, 17/145) and MRSA (0.7%, 1/145), not common flora in dogs, were found in lower proportions. In any case, these results are comparable to other studies performed in other geographic locations in similar veterinary settings. Interestingly, samples

collected from skin lesions in 26 dogs were never positive for *S. aureus*/MRSA, but 84.6% of them were found to be positive for *S. pseudintermedius*, and 23.1% were positive for MRSP. This again is a very concerning trend, as infected lesions by MRSP are very difficult to treat due to their broad resistance to antimicrobial drugs.

In the hospital environment, approximately 6.0% (14/234) of the surfaces were positive for MRSP, and 5.6% (13/234) were positive for MRSA. Nonetheless, compared to previous years, a general decrease of MRS environmental contamination has been observed (see image below). Currently we are preparing a publication analyzing the different patterns of circulation and survivability of these pathogens throughout the hospital during the yearlong study.

In regards to the veterinary personnel, the first quarterly sampling was performed as a cross-sectional study in which CVM and VMC personnel were sampled. Overall, personnel nasal colonization with *S. aureus* (25.7%) and MRSA (2.0%) was found to be close to the US general population range (28-30% and 1.8-2% respectively). However, when analyzed by hospital and non-hospital personnel, it was found that the former were 1.55 (*p*-value >0.5) times more likely to be colonized with MRSA than non-hospital personnel. When evaluating by occupation within these two groups, interns/residents were 2 times (*p*-value 0.16) more likely to be nasally colonized with *S. aureus* than other hospital personnel. In the case of veterinary students, senior students were 3 times (*p*-value 0.03) more likely to carry *S. aureus* in their nose than 3rd and 2nd year students. Moreover, when comparing hand contamination, senior veterinary students were 3 times (*p*-value 0.02) more likely to have hands positive for *S. aureus* than 3rd year students. Colonization rates from the 2nd, 3rd and 4th samplings, performed only in VMC personnel, ranged from 21.7-42.3% for *S. aureus* and 0-3.8% for MRSA.

One of the most significant findings when analyzing risk factors associated with *S. aureus* colonization was that hospital personnel that did not wash their hands regularly between patients were 18.9 times (p-value 0.0001) more likely to be colonized with S. aureus than those that did wash their hands frequently. This finding highlights the importance of personal hygiene in the control and prevention of *Staphylococci* (and MRS) colonization among




personnel with high occupational exposure. In addition, colonized personnel and

contaminated hands appear to play a role in the contamination of the hospital environment, since 10% of *S. aureus* and 33% of MRSA strains found in personnel are clonal or closely related to those detected in the environment.

We also completed the second objective of this proposal. Phenotypic and genotypic characterization of all MRSP and MRSA isolates has been performed. Linear regression analysis showed that MRSP resistance increased significantly over time, by approximately one new class of antibiotic every 5 years, in environmental isolates pooled across all hospital locations. In contrast, MRSP resistance did not increase significantly over time for canine isolates. These results suggest that environmental MRSP is continuing to acquire novel genetic components that provide them resistance to multiple classes of antimicrobials, becoming even more pathogenic over time. This gain of resistance is a very concerning trend, which requires further attention from the veterinary community. Our study has demonstrated the importance of tracking the evolution of a bacterium that poses serious issues to veterinary medicine, and indirectly, to public health. For animal health, if MRSP continues to acquire resistance, MRSP infections will become ever more challenging to prevent and treat in our animal patients. For public health, if MRSP and MRSA transfer antimicrobial resistant genes between one another (which has been shown to occur), in part because they are both present in multiple common environments, then MRSA might also become more resistant and difficult to treat in human and canine cases. Regardless, our findings highlight the importance of antimicrobial stewardship, cleaning, and hygiene protocols and continuing to perform surveillance of MRS isolates.

Three MPH-VPH students used this data as their Master's theses. One of them, Lane Bookenberger successfully defended her research spring 2017, David Handler will defend his research this coming summer 2017, and Marissa Schreiner who will defend on fall 2017. In addition, an undergraduate student analyzed the antimicrobial susceptibility of all MRSP isolates from the retrospective part of the study and used this information as her undergraduate Honors Thesis. We are currently writing two original manuscripts and one short communication that will be submitted later this year. Finally, this information has already been shared with veterinary professionals in the Midwest Veterinary Conference, sponsored by the Ohio Veterinary Association, as well as through different seminars, core classes, and presentations in our College.

Our results clearly indicate that both MRSP and MRSA are circulating in the personnel, the environment, and incoming animals at the VMC. Therefore, there is a real potential threat of transmission to the animals (nosocomial infections) as well as the personnel working at the facility (zoonotic infections). These results should be considered as further evidence to support improving environmental cleaning and disinfection protocols. They will also help to

plan/establish future interventions to decrease hand contamination and spread of MRS. We believe that the information produced in this study will be used (and has been used) to redesign infectious disease prevention and control procedures. All with the final goal to control and decrease the dissemination and maintenance of important human and animal pathogens, such as MRS, in veterinary hospitals.

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**PROJECT TITLE: SEROREACTIVITY OF OUTER MEMBRANE PROTEINS OF *NEORICKETTSIA HELMINTHOECA***

*RELEVANT TITLE: DETECTION OF NEORICKETTSIA IN DOG BLOOD*

**INVESTIGATOR: M. LIN, Y. RIKIHISA**

*Final Report*

Background and Significance:

***Neorickettsia helminthoeca*** is an **obligatory intracellular bacterium** of digenetic trematodes (internal parasites of molluscs and vertebrates). Dogs acquire Salmon poisoning disease (SPD), an acute and often-fatal illness, when they eat salmonid fish containing encysted trematodes *infected with N. helminthoeca and* the bacterium is transmitted from trematodes to dog monocytes or macrophages. The disease is endemic at the Northwestern pacific coast of the US and Canada, and a part of Brazil, but cases of SPD from non-endemic regions by eating salmon transported from endemic regions have also been reported. In addition, recent reports showed that *Neorickettsia* species in divergent trematodes have been identified throughout the world, including Asia, Africa, Australia, Americas, and even Antarctica, suggesting a global distribution of *Neorickettsia***.**

In untreated dogs, the mortality rate due to SPD can be as high as 90%. Hospitalization is required for dogs with SPD, and the drug of choice is doxycycline. Since there are no vaccines against SPD available, prevention is mainly based on the exclusion of contaminated raw or under-cooked fish from the diet of canids. Current diagnostic techniques to detect SPD include fecal examination for parasite eggs and/or Romanowsky staining of lymph node aspirates from dogs, the polymerase chain reaction assay to detect bacterial DNA in the dog blood specimens or serological tests to detect the presence of antibodies in the blood reactive to *N. helminthoeca*; all require trained lab personnel and lengthy procedures.

*The high morbidity and mortality rates of the disease signified the need for a more rapid and accurate diagnostic test.* Our hypothesis is that some outer membrane proteins of *N. helminthoeca* are highly antigenic in infected dogs, therefore can be used for rapid and specific diagnosis of SPD. Since SPD progression is rapid, and the case fatality rate is quite high, prevention and early diagnosis of SPD are critical. The serological assay based on defined outer membrane protein antigens is fast, specific, objective, and convenient, thus helps generating epidemiological information on *N. helminthoeca* exposure among trematodes, domestic animals, and potentially other animals in nature to raise awareness of SPD. The main goal of this research is to identify potential virulent factors responsible for *N. helminthoeca* pathogenesis, and characterize the major surface protein antigens of *N. helminthoeca* that elicits an immune response in dogs affected with SPD, therefore assist the development of rapid and effective SPD diagnostic and preventive measures.

Final Progress Report:

In this study, we have completed the sequencing and bioinformatic analysis of the *N. helminthoeca* genome, which is composed of a single small circular chromosome of 884,232 base pairs and encodes 774 potential proteins. *N. helminthoeca* is unable to synthesize lipopolysaccharides and most amino acids, but is capable of synthesizing vitamins, cofactors, nucleotides, and bacterioferritin. *N. helminthoeca* is, however, distinct from majority of the family *Anaplasmataceae* to which it belongs, as it encodes nearly all enzymes required for peptidoglycan biosynthesis. Although electron microscopy images suggested that *N. helminthoeca* might not possess a peptidoglycan layer, it is possible that *N. helminthoeca* can still produce precursors or components of peptidoglycan, suggesting its structural hardiness and inflammatory potential.

By comparing with known major immune reactive surface antigens of *Neorickettsia risticii*, the causative agent of Potomac Horse Fever that is closely related to *N. helminthoeca*, we identified five genes encoding putative outer membrane proteins, namely P51, SSA, NSP1, NSP2, and NSP3. Using molecular cloning techniques, genes encoding these proteins were amplified from *N. helminthoeca* genomic DNA and cloned into a bacterial protein expression vector pET33b(+). These recombinant membrane proteins were subsequently expressed and purified in homogeneity from *Escherichia coli* by affinity chromatography.

In order to determine whether these bacterial membrane proteins are recognized by blood from dogs with SPD, the proteins were first analyzed by defined SPD immune sera, which were collected from dogs experimentally infected with *N. helminthoeca* resembling natural infection route, using oral feeding of trematode-infested salmon kidneys infected with *N. helminthoeca*. Results from Western blot analysis showed that all these purified recombinant membrane proteins of *N. helminthoeca* could be detected by SPD immune sera, with NSP2

and SSA among the strongly detected major antigens, whereas NSP1 and NSP3 among the weakest or undetectable antigens. Although *N. helminthoeca* shares few cross-reactive antigens with its closely-related pathogens like *N. risticii* and the agent of canine monocytic ehrlichiosis *Ehrlichia canis*, these cross-reactive antigens are at molecular weights of approximately 64 to 80 kilo-Daltons, which are different from these potential outer membrane proteins (ranging from 23 to 51 kilo-Daltons). Therefore, as a control, horse sera infected with closely related bacterium *N. risticii* did not react with any of these recombinant antigenic proteins of *N. helminthoeca*, and dog sera against *N. helminthoeca* only reacts with proteins at ~64 and 80 kilo-Daltons from *N. risticii*.

The recombinant antigenic membrane proteins of *N. helminthoeca* were further tested with clinical dog sera that were PCR-positive for *N. helminthoeca*. Results showed that membrane proteins P51 and SSA could be detected by two dog sera, whereas NSP1/2/3 was only detected by one of the clinical dog sample with NPS3 among the weakest antigens. In conclusion, these data suggest that these recombinant antigenic proteins of *N. helminthoeca*, especially P51 and SSA, could be used to develop rapid and specific sero-diagnostic techniques for SPD detection.

Patent, Presentation, and Publication:

- **Patent Filed**:
  SERODIAGNOSIS OF SALMON POISONING DISEASE (Application No.: 62/316,254; OSU Reference: 2016-198).
- **Presentation**:
  Minggun Lin, Katherine Bachman, Zhihui Cheng, Sean C. Daugherty, Sushma Nagaraj, Naomi Sengamalay, Sandra Ott, Al Godinez, Luke J. Tallon, Lisa Sadzewicz, Claire Fraser, Julie C. Dunning Hotopp, and Yasuko Rikihisa. 2016. Analysis of Complete Genome Sequence and Major Surface Antigens of *Neorickettsia helminthoeca*, Causative Agent of Salmon Poisoning Disease. The 28th Meeting of the American Society for Rickettsiology, June 11-14, 2016, Big Sky, MT. (*Oral Presentation*)
- **Publication**:
  Mingqun Lin, Katherine Bachman, Zhihui Cheng, Sean C. Daugherty, S. Nagaraj, Naomi Sengamalay, S. Ott, A. Godinez, Luke J. Tallon, Lisa Sadzewicz, S. Parankush, Claire Fraser, Julie C. Dunning Hotopp, and Yasuko Rikihisa. 2017. Analysis of Complete Genome Sequence and Major Surface Antigens of *Neorickettsia helminthoeca*, Causative Agent of Salmon Poisoning Disease. *Microbial Biotechnology* (Manuscript ID: MICROBIO-2016-356-RA, in minor revision).

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**PROJECT TITLE: VITAMIN D METABOLICS, PARATHYROID HORMONE AND THE FIBROBLAST GROWTH FACTOR-23 – KLOTHO AXIS IN DOGS WITH VARIOUS STAGES OF CHRONIC KIDNEY DISEASES**

*RELEVANT TITLE: NUTRITIONAL AND HORMONAL FACTORS IN DOGS WITH KIDNEY DISEASE*

**INVESTIGATORS: V. PARKER, R. TORIBIO, D. CHEW**

*Final Report*

Chronic kidney disease (CKD) is a progressive condition commonly diagnosed in people and dogs. CKD frequently induces renal secondary hyperparathyroidism (RHPT), characterized by overproduction of parathyroid hormone (PTH), contributing to disease progression. In people, a large emphasis is placed on correcting hypovitaminosis D which is associated with progression of disease and decreased survival by providing vitamin D (cholecalciferol) or by supplying a vitamin D receptor activator (calcitriol). However, it is important to diagnosis RHPT early in the course of CKD so that treatment will induce minimal toxicities while maximizing benefit. In dogs with CKD, little is known regarding the nature of vitamin D dysregulation, precluding our ability to provide earlier, more effective therapies. The primary goal of this study was to measure vitamin D metabolite, PTH, and FGF-23 concentrations in dogs with CKD and to determine their association with IRIS stages of CKD. We hypothesize that: 1) dogs with CKD will have lower vitamin D metabolite and higher PTH and FGF-23 concentrations than healthy dogs; and 2) these aberrations will be proportional to IRIS stage. A secondary goal of this study was to assess calcium and phosphorus concentrations in dogs with CKD. Lastly, we wanted to determine if there was an association between vitamin D metabolites, namely 25(OH)D, and dietary vitamin D (i.e., cholecalciferol) intake. Data from this study will provide critical new information regarding the relationship of vitamin D metabolites, PTH and FGF-23 in dogs with CKD, laying the foundation for future prospective clinical trials designed to correct the PTH/Vitamin D dysregulation, slow disease progression and improve quality of life in dogs with CKD.

- The study is complete. Results were presented in abstract form at the 2016 ACVIM Research Forum. A manuscript has been published in the Journal of Veterinary Internal Medicine (First published online**:** 10 February 2017; **DOI:** 10.1111/jvim.14653). The abstract of this manuscript is copied below.

**Background:** Hypovitaminosis D is associated with progression of renal disease, development of renal secondary hyperparathyroidism (RHPT), chronic kidney disease-mineral bone disorder (CKD-MBD), and increased mortality in people with CKD. Despite what is known regarding vitamin D dysregulation in humans with CKD, little is known about vitamin D metabolism in dogs with CKD.

**Objectives:** The purpose of our study was to further elucidate vitamin D status in dogs with different stages of CKD and to relate it to factors that affect the development of CKD-MBD, including parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23), calcium, and phosphorus concentrations.

**Methods:** Thirty-seven dogs with naturally occurring CKD were compared to 10 healthy dogs. Serum 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], and 24,25-dihydroxyvitamin D [$24,25(OH)_2D$], and PTH and FGF-23 concentrations were measured. Their association with serum calcium and phosphorus concentrations and IRIS stage was determined.

**Results:** Compared to healthy dogs, all vitamin D metabolite concentrations were significantly lower in dogs with International Renal Interest Society (IRIS) stages 3 and 4 CKD ($r$[creatinine]: −0.49 to −0.60; $P < .05$) but not different in dogs with stages 1 and 2 CKD. All vitamin D metabolites were negatively correlated with PTH, FGF-23, and phosphorus concentrations ($r$: −0.39 to −0.64; $P < .01$).

**Conclusions and Clinical Importance:** CKD in dogs is associated with decreases in all vitamin D metabolites evaluated suggesting that multiple mechanisms, in addition to decreased renal mass, affect their metabolism. This information could have prognostic and therapeutic implications.

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## PROJECT TITLE: RIGHT VENTRICULAR FUNCTION IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE

*RELEVANT TITLE: CARDIAC FUNCTION IN DOGS WITH HEART DISEASE*

**INVESTIGATORS: J. BONAGURA, E. CHAPEL**

*Final Report*

The most common canine heart disease is age-related degeneration of the mitral valve, also called myxomatous mitral valve degeneration. This disorder affects millions of our canine companions, and leads to progressive leaking of the valve (mitral regurgitation), heart enlargement and often to heart failure. Many dogs live with this disease for years without

complications, but others progress rapidly to symptoms that include exercise intolerance, difficult breathing and respiratory distress. Current techniques, mainly focused on blood hormones or the size and function of the left ventricle, do not sufficiently identify dogs at highest risk for heart failure. Interestingly, the function of the right ventricle – a chamber on the opposite side of the heart from the affected cardiac valve – has been shown to predict the risk for heart failure or death in human patients with mitral valve disease. Advanced tissue-based imaging techniques, derived from cardiac ultrasound (echocardiograms), permit measurement of right ventricular function noninvasively. We proposed to evaluate similar heart function measurements in dogs with naturally-occurring mitral valve disease. Our expectation is dogs with more advanced right ventricular dysfunction. If this is the case, analysis of right ventricular function could prove useful for establishing functional risk categories in dogs with mitral regurgitation, prompt more careful home monitoring, and allow for earlier therapeutic interventions.

This study involved dogs with mitral valve disease evaluated at The Ohio State University Veterinary Medical Center. The testing procedures performed are considered standard of care for the disease and included: blood pressure measurement, chest x-rays, and echocardiography with Doppler studies. These are noninvasive examinations and dogs were lightly sedated to reduce stress and to foster high-quality imaging. Right (and left) ventricular size and function were measured using conventional echocardiography as well as advanced methods such as segmental deformation (strain) of the heart muscle. Dogs were categorized into one of three accepted stages of disease severity based on their screening examinations. We enrolled 36 dogs in the study; 12 in each of the three groups. Statistical analysis comparing these three groups of dogs determined that right ventricular function differs across the stages of disease severity. We found that right ventricular function demonstrates a “U-shaped” pattern; function is normal in dogs with mild heart disease, it is increased in dogs with advanced compensated disease, and then returns to normal at the onset of decompensated heart disease (heart failure). Based on our results, we have identified two reliable markers of right ventricular function that have potential utility for prognostication in canine mitral valve disease. Future investigations will include large-scale studies monitoring these markers of right ventricular function in dogs with mitral valve disease to determine whether they can help predict the onset of heart failure and help guide therapy.

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**PROJECT TITLE: EFFECT OF A. *MUCINIPHILA* ADMINISTRATION ON GLP-2 AND INTESTINAL HYPER-PERMEABILITY IN DOGS WITH IBD**

*RELEVANT TITLE: THERAPEUTIC EFFECTS OF A PROBIOTIC ON THE INTESTINES OF DOGS WITH INFLAMMATORY BOWEL DISEASE*

**INVESTIGATORS: P. BOYAKA, M. JUGAN**

*Final Report*

**Reason for research:** Antibiotic-associated diarrhea is a costly issue in human and veterinary medicine, with few treatment options and high relapse rates in severe cases. Mucin-degrading bacteria reside in close proximity to the mucin layer of the gastrointestinal barrier (GIT) and help to maintain the GIT barrier through their metabolic by-products. *Akkermansia muciniphila* is the predominant mucin-degrading bacterium in humans, and populations are decreased in chronic gastrointestinal diseases, such as inflammatory bowel disease. Treatment of rodents with *Akkermansia* augments the GIT barrier in models of inflammatory bowel disease and metabolic syndrome, demonstrated by increased intestinal weight and decreased serum lipopolysaccharide (LPS). Effects are likely mediated through increased GIT L-cell numbers and increased secretion of the enteroendocrine hormone glucagon-like peptide 2 (GLP-2). *Akkermansia muciniphila* may serve as an effective probiotic therapy for decreased GIT barrier function and antibiotic-associated diarrhea in dogs.

**Study conduction:** A double-crossover, placebo controlled study was performed with 8 healthy research dogs to determine the effects of oral *Akkermansia muciniphila* administration on markers of intestinal permeability (LPS) and epithelial damage (cytokeratin-18; CK-18) following antibiotic administration. *Akkermansia muciniphila* was cultured anaerobically on a hog gastric mucin medium, evaluated through 16S rRNA sequencing for strain confirmation, and concentrated through hemocytometer counting. Dogs were randomized to receive either *Akkermansia* ($10^9$ cells/kg; N=4) or vehicle (N=4) for 6 days following a 7-day course of metronidazole. After a 20-day washout, dogs were crossed-over to the alternate treatment. After an additional 20-day washout, the experiment was repeated with amoxicillin-clavulanate. Fecal *Akkermansia* qPCR and plasma concentrations (ELISA) of CK-18, LPS, and glucagon-like peptides (GLP-1, GLP-2) were measured at baseline (T0), post-antibiotic (T1), and post-treatment (vehicle or *Akkermansia*; T2). For each antibiotic, absolute or delta concentrations were compared between time-

points using paired *t* tests. Fecal scores were performed daily and average fecal score for each T0, T1, and T2 time-period were compared using paired *t* tests.

**Results:** *Akkermansia* was detected in feces in 7/8 dogs following supplementation (T2) but not at T0 or T1. Delta (T2-T1) cytokeratin-18 after metronidazole was significantly lower on vehicle (-0.27 ng/ml) versus *Akkermansia* (2.4 ng/ml; p=0.03). Cytokeratin-18 concentrations tended to decrease from T0 to T1 on amoxicillin-clavulanate (p=0.05). Post-prandial GLP-1 concentrations (38.2 pM) were higher than pre-prandial (15.5 pM) concentrations. Fecal score was higher following metronidazole vs amoxicillin-clavulanate (p<0.05).

**Contribution to problem at hand:** Administration of *Akkermansia mucinipihila* to dogs impacted serum CK-18 concentrations and fecal score, demonstrating an impact on the gastrointestinal epithelium. Improvement in fecal score following metronidazole therapy suggests a positive impact of *A. muciniphila* and suggests the need to further evaluate *Akkermansia* as a probiotic therapy in naturally-occurring gastrointestinal disease. Lack of *Akkermansia* detection in fecal samples during baseline periods highlights suggests the need for further research into the canine GIT microbiome, particularly those closely associated with the GIT barrier, for species-specific therapeutic targets.

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## PROJECT TITLE: IMPACT OF THE THERAPEUTIC APPLICATION OF CARBAPENEMS FOR THE TREATMENT OF CANINE URINARY TRACT INFECTIONS

*RELEVANT TITLE: THE IMPACT OF CARBAPENEM ANTIBIOTICS ON TREATMENT OF BLADDER INFECTIONS IN DOGS*

**INVESTIGATORS: T. WITTUM**

*Final Report*

Antimicrobial resistance is a serious concern for human and veterinary medicine. Cephalosporins are a diverse class of beta-lactam antimicrobials used commonly in both veterinary and human medicine. Extended spectrum cephalosporins (ESC) are used for the treatment of skin, wound and complicated urinary tract infections in dogs, such as cefpodoxime and cefovecin.[1-3] This widespread therapeutic use provides selection pressure leading to the emergence and dissemination of resistant bacteria harboring highly mobile genes conferring resistance to all beta-lactam antimicrobials.[4-7] Narrow spectrum

cephalosporins are also frequently used in veterinary medicine, most commonly cephalexin and cefazolin. These narrow spectrum cephalosporins are used routinely for uncomplicated skin and urinary tract infections, and commonly used prophylactically to prevent post-surgical wound infections.[8-10] Thus, resistance that emerges in response to the use of ESCs also negatively impacts the efficacy of the narrow spectrum cephalosporins.

The most common mechanism of bacterial resistance to β-lactam antimicrobials is the production of β-lactamases.[11] Resistance genes conferring ESC resistance can be exchanged among different species of bacteria via horizontal gene transfer.[12] *E. coli* can transfer resistance genes via conjugation not only to other commensal bacteria, but also to pathogenic species including *Salmonella* and pathogenic *E. coli.*[12] Thus when selective pressure is placed on the normal flora within the canine gastrointestinal tract by ESCs, a reservoir of ESC resistance genes can emerge that can later be acquired by pathogens.[13] Antimicrobial resistant infections, including ESC resistant infections in humans have been associated with more severe health outcomes including increased mortality, increased length of hospitalization, and increased medical costs for sepsis patients.[14] Direct contact with pets in the household may be one source for the zoonotic transmission of ESC resistant bacteria since people and their pets can share fecal flora.[15] Additionally, *E. coli* clones have been shown to be shared among human and pet populations of the same household.[16] In the US, clinical diagnostic isolates from dogs and cats have been reported to express extended spectrum β-lactamases (ESBL) and AmpC β-lactamases, both of which confer resistance to ESCs.[17-18] Extended-spectrum cephalosporin resistant Enterobacteriaceae have been identified by the CDC in their 2013 Threat Report as being a serious risk to the public health.[19]

Close contact between owners and their pets may result in the direct zoonotic transmission of canine gastro-intestinal flora to their human owners. Clonal *E. coli* strains resistant to ESCs have been found in humans and their pet dogs living in the same household.[20] Additionally, pet ownership is a risk factor for ESBL rectal colonization.[21] We aimed to determine how antimicrobial therapy in dogs affects the resistance of enteric bacteria to clinically important antimicrobials, extended spectrum cephalosporins (ESC). We obtained fecal swabs from 48 dogs within 24 hours of being prescribed antimicrobials at The Ohio State University Veterinary Medical Center and subsequently 7 days later. Additionally, if the household contained another dog, fecal swabs were obtained from that dog at similar time points. Finally, owners collected a Swiffer® sample of the treated dog's food/water bowl area. Samples were selectively cultured to identify coliform bacteria expressing the $bla_{CMY}$ (AmpC) and $bla_{CTX\text{-}M}$ (ESBL) and fluoroquinolone resistance phenotypes. Genotypes for AmpC and ESBL genes were confirmed employing conventional PCR techniques.

There were 48 participating households with 48 antimicrobial treated dogs and 21 untreated co-habitating dogs. Treated dogs were treated most commonly with Amoxicillin, Clavamox,

Cephalexin, Baytril, Clindamycin, Metronidazole, Doxycycline, Amikacin, and TMS respectively. There were 48 participating households, with an average of 2 adults/household, 20% with children under the age of 18, 42% with multiple dogs, 27% with cats, 10% had humans in the household with livestock exposure, 23% had humans in the household with medical field exposure, 10% with previous human antimicrobial use in the last 30 days, and 21% with international travel within the last 6 months. Of the 48 treated dogs, 69% of them visited a veterinarian in the past 30 days, 44% had antimicrobial use within the last 30 days, and 2 % had livestock exposure. Of the 21 untreated co-habitating dogs, only 4% had previous antimicrobial use in the last 30 days.

Table 1: Prevalence (95% CI) of *bla* $_{CMY}$ and *bla* $_{CTX-M}$ among dogs submitting fecal samples for routine parasitology

| N | *bla* $_{CMY}$(95% CI) | *bla* $_{CTX-M}$(95% CI) |
|---|---|---|
| 212 | 14.2% (9.42% –18.88%) | 4.2%(1.51% – 6.98%) |

Among 51 antimicrobial therapy courses from 48 treated dogs with an initial fecal swab; 28%, 30%, and 38% harbored bacteria expressing the *bla*$_{CMY,}$ *bla*$_{CTX-M}$ genotype, and fluoroquinolone resistance phenotype, respectively. Among treated dogs with a 7 day post antimicrobial fecal swab; 41%, 33%, and 35% harbored bacteria expressing *bla*$_{CMY,}$ *bla*$_{CTX-M}$ genotype, and fluoroquinolone resistance phenotype, respectively. Of the 21 untreated dogs with fecal samples from within 24 hours of co-inhabiting dog's antimicrobial administration; 35%, 24%, and 24% contained bacteria expressing the *bla*$_{CMY,}$ *bla*$_{CTX-M}$ genotype, and fluoroquinolone resistance phenotype, respectively. Among untreated dogs with fecal swabs from 7 days post of co-inhabiting dog's antimicrobial administration, 30% and 22% contained bacteria expressing the *bla*$_{CMY}$ and *bla*$_{CTX-M}$ genotype, respectively, and 26% expressing fluoroquinolone resistance (Table 2). Among the 48 household environmental samples, 16% and 10% contained bacteria expressing the *bla*$_{CMY}$ and *bla*$_{CTX-M}$ genotype, respectively, and 18% expressing fluoroquinolone resistance.

Table 2: Prevalence of *bla* $_{CMY}$ and *bla* $_{CTX-M}$ genotype and fluoroquinolone resistant phenotype among dogs undergoing antimicrobial treatment and untreated co-habitating dog within the same household

| Treated Dogs | | | Co-habitating Untreated Dogs | | |
|---|---|---|---|---|---|
| Resistance | Pre | Post | Resistance | Pre | Post |
| *bla* $_{CMY}$ | 27.7% | 40.8% | *bla* $_{CMY}$ | 35.3% | 30.4% |
| *bla* $_{CTX}$ | 29.9% | 32.7% | *bla* $_{CTX}$ | 23.5% | 21.7% |
| Cipro | 38.3% | 34.7% | Cipro | 23.5% | 26.1% |

Both treated and untreated dogs had high levels of antimicrobial resistance in their enteric flora compared to the healthy dog population from a previous study (Table 1 & 2). There was no difference between the prevalence of these clinically important resistance genotypes and phenotypes between pre and post antimicrobial therapy in treated or untreated dogs.

However, there was a high level of level of environmental contamination of a heavy traffic human and pet household surface. Finally, there were no significant household (# of adults, # of children, cat or livestock exposure, human medical field exposure, antimicrobial use, and international travel) or dog (previous veterinary visit or antimicrobial use or livestock exposure) characteristics that were associated with a higher level of antimicrobial resistant bacteria recovery in the enteric flora or in the environmental samples. These findings highlight the level of resistant enteric bacteria transmission between dogs in the same household, due to the high level resistant enteric bacteria within untreated co-habitating dogs compared to healthy population of dogs from the same geographic area. Additionally, antimicrobial therapy can increase the level of antimicrobial resistant bacteria in the household environment.

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**PROJECT TITLE: IN VITRO CHANGES TO CANINE PACKED RED BLOOD CELL CONCENTRATES FOLLOWING IRRADIATION AND STORAGE**

*RELEVANT TITLE: EFFECT OF RADIATION AND STORAGE ON RED BLOOD CELL CONCENTRATES FROM DOGS*

**INVESTIGATORS: E. COOPER, S. PRESS**

*Final Report*

Irradiation of blood prior to transfusion is required to prevent transfusion-associated graft-versus-host disease in patients undergoing bone marrow transplant. Additional application for irradiated blood may exist to allow the blood salvaged in surgery that may be contaminated with cancer cells to be re-transfused safely without risking dissemination of malignancy. The effect of irradiation on canine packed red blood cells (pRBCs) is unknown. Our aims were to characterize changes to *in vitro* lymphocyte viability, lymphocyte activation potential, electrolytes, acid-base variables and oxygen-carrying capacity in pRBCs following irradiation and storage.

Ten units of pRBCs were irradiated using a linear accelerator, and flow cytometric analysis of lymphocyte viability and activation was performed at days 0, 7, 10 and 17 of storage. Multiple values were measured to determine if irradiation causes adverse effects to red bloods or makes them unsafe to administer. Additional assessments were made to determine how effectively irradiation decreased the number of white blood cells, and affected their ability to activate and potentially trigger transfusion-associated graft-versus-host disease.

While there were some biochemical changes which occurred as a result of irradiation, these changes were fairly small and not likely of clinical significance. Further, there was not an appreciable impact on the ability for red blood cells to carry oxygen. There were significant differences in lymphocyte viability following irradiation, though not immediately. Additionally, irradiation induced activation of lymphocytes. A decrease in the percentage of viable lymphocytes over time occurred in both irradiated and nonirradiated cells, similar to changes in WCC.

In conclusion, irradiation and storage altered *in vitro* properties of pRBCs compared to nonirradiated blood. Complete loss of viable lymphocytes was not seen immediately following irradiation, and presence of highly activated lymphocytes following irradiation

warrants further investigation. The magnitude of the differences in electrolyte, acid-base and oxygen-carrying capacity was small. The clinical impact of irradiation on these variables may be negligible and is unlikely to preclude safe use of these products.

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**PROJECT TITLE: QUANTIFICATION OF AIRFLOW RESISTANCE BY COMPUTED TOMOGRAPHY IN BRACHYCEPHALIC DOGS BEFORE AND AFTER SURGERY**

*RELEVANT TITLE: USE OF CT SCANS TO MEASURE RESPIRATION IN SHORT NOSED DOGS BEFORE AND AFTER SURGERY*

**INVESTIGATORS: K. HAM, E. HOSTNIK**

*Final Report*

The Bulldog breed is growing in popularity. However, the breed is predisposed to multiple congenital disorders that may have a negative impact to the health of the dog. Brachycephalic obstructive airway syndrome is a collection of respiratory structural anomalies of the respiratory tract. Each anomaly contributes to an overall compromise of airway patency. The abnormalities span from the nares to the distal trachea. The severity of abnormalities for an individual dog is difficult to objectively assess using only physical examination and laryngeal visualization. Bulldogs may have few clinical signs of airway disease; however, the spectrum of affliction is wide and more severely brachycephalic dogs may rapidly develop respiratory distress with the potential of death. Surgery is performed to address conformational obstruction with the goal to decrease the airflow impedance and reduce the upper airway resistance. There is no quantitative tool to objectively assess if a reduction in airflow resistance occurs post-surgery. We developed a method to quantitate airway resistance using computed tomography. We used this method to evaluate airway resistance prior to and following surgery to attempt to quantify the change.

Computed tomography examinations were performed using conscious sedation. Dogs received an intravenous injection of butorphanol and dexmedetomidine 10 to 20 minutes prior to scheduled CT scan. The dogs were positioned in sternal recumbency with the neck extended, the forelimbs slightly abducted, and the hard palate parallel to the CT table with table straps to help secure the dog in place. A 8-multi-detector computed tomography (MDCT) was used. Following the initial computed tomography, the patient was prepared for surgery with endotracheal intubation. Routine ventral wedge alarplasty and partial

staphylectomy was performed. The computed tomography was repeated approximately 21 days after the surgery.

The process used for model construction and meshing for CFD analysis is shown in Figure 1. First, the raw MDCT bone algorithm transverse dataset is imported into ScanIP software (Figure 1A). The anatomy imported into ScanIP includes the entire nasal passage including frontal sinuses from nasal planum to the laryngopharynx. A threshold was then applied to the CT layers by automatic segmentation using -1024 to -450 Hounsfield units (HU) to highlight the airways (blue in Figure 1B). A fill threshold was then applied to isolate the air-filled nasal passage throughout contiguous images, which will be used to generate a 3D solid model (Figure 1C). Still within the ScanIP program, the solid model is sub-divided into a CFD mesh consisting of 6-sided tetrahedral elements which are then exported a MATLAB file into the COMSOL Multiphysics 5.0 software package. During this meshing procedure, we specify the minimum edge length, which is defined as the minimum length of any side of all tetrahedral elements in the model (Figure 3D and Figure 3E). As a result, the density of the mesh (and hence the number of elements) can be controlled.

A finite element algorithm in the COMSOL Multiphysics 5.0 software package was then used to simulate airflow in each 3D model of the nasal passage and to calculate airway resistance. For this analysis, the airways are filled with an incompressible, Newtonian fluid with constant density and viscosity that mimicked the properties of air at environmental conditions. Iterations of the Navier-Stokes equation for incompressible flow are used to solve the momentum balance and continuity equations governing airflow.

Six Bulldogs were enrolled in the study for evaluation of airway flow resistance before and after surgery. The effect of surgery trended towards significance with a decrease in overall airway resistance that is significant at an alpha of 0.08. The trend towards significance suggests that surgical intervention involving routine alarplasty and partial staphylectomy will quantifiably decrease the airway resistance through the upper airway. We feel that if we had a larger number of dogs enrolled then significance would be achieved. We are currently waiting on a collaborator to return additional statistical analysis looking at a breakdown of the local changes in airway resistance (Figure 3).

We have utilized the data acquired in this study to submit an intramural grant for funding to further evaluate surgical intervention on obstructive airway disease. We plan to use laser ablation to erode nasal turbinates in addition to routine surgical methods to evaluate for airway resistance changes.

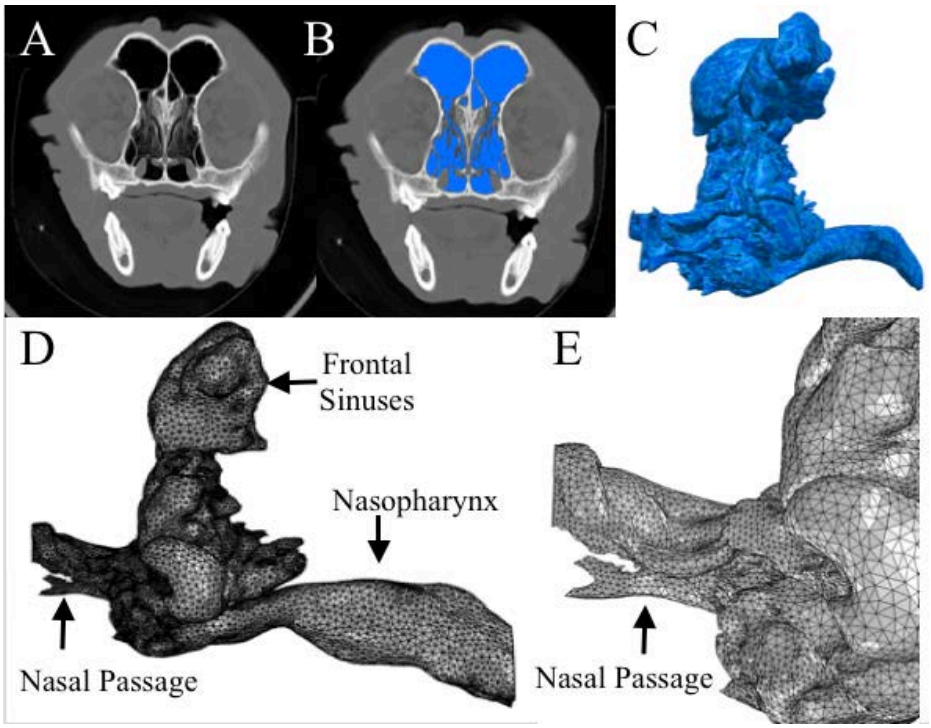


Figure 1. The process for generation of a 3D model. A: Computed tomographic images of the nasal passages were acquired in a transverse image plane using multi-detector computed tomography. A representative image of the caudal nasal passage at the level of the choanae is shown. B: Threshold segmentation in the ScanIP program isolated the airways (blue) using a threshold of -1024 to -450 Hounsfield units (HU). C: A fill threshold algorithm in ScanIP was used to generate a solid model of the nasal passages which was then sub-divided into 6-sided tetrahedral elements and exported to the COMOSL Multiphysics software package. D: 3D model of the nasal passages within the COMSOL Multiphysics package with E: Zoomed in image of the 3D model and tetrahedral mesh elements used in the CFD analysis. Note that each tetrahedral element has 6 sides or edges and the minimum edge length controls the mesh density where a smaller minimum edge length leads to a more dense mesh and more elements.

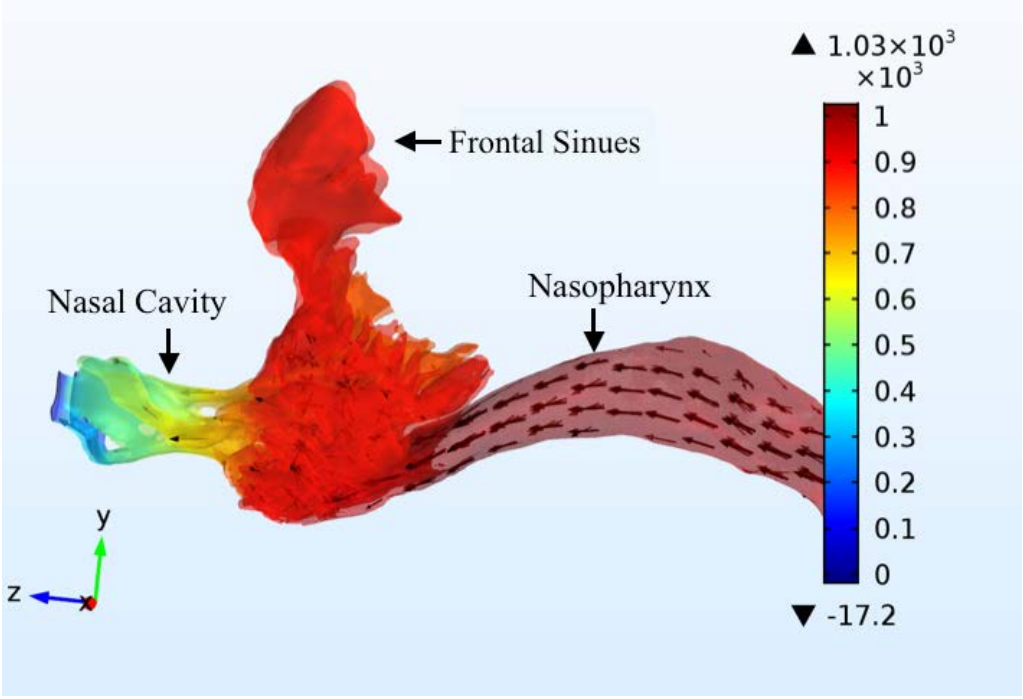


Figure 2. COMSOL Multiphysics 5.0 with MATLAB® model of pressure generated using computational fluid dynamics. The scale on the right is a relative scale displaying pressure with measurements in Pascals. Low-pressure areas are blue and high-pressure areas are dark red. The areas with a transition in color represents an increase in pressure within the model. The black arrows within the model are a logarithmic representation of velocity of air flow in m/s. A larger arrow represents a higher velocity. The arrows are directed to the nares reflecting the pressure drop that correlates to the zero reference for the Navier-Stokes equation. The z-, y-, and x-axes refer to the imaging planes of computed tomography and Cartesian planes.

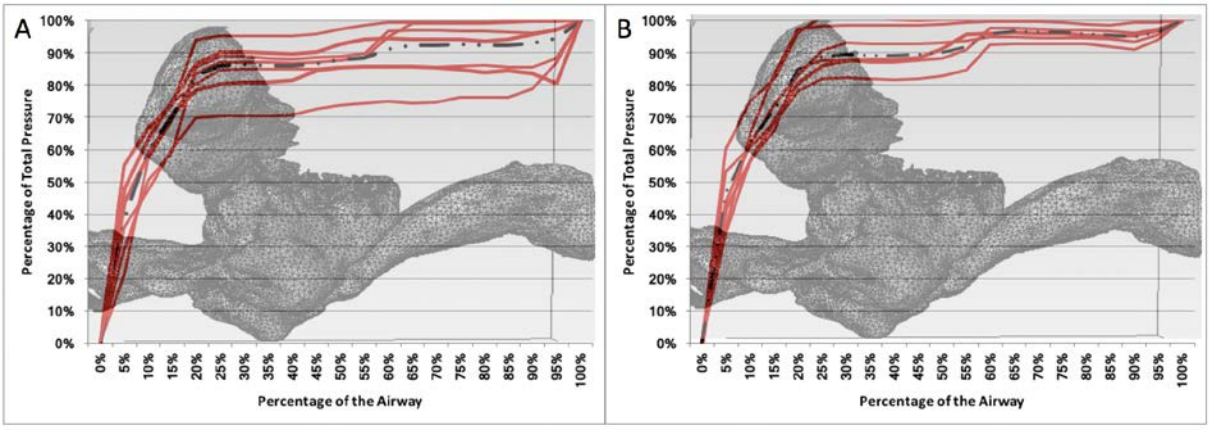


Figure 3. Each line indicates the change in pressure from rostral to caudal through the upper airway. The greater the slope reflects a greater increase of pressure relating to that area of the anatomy. Areas with a low slope, are areas of relatively static airway pressure. The lines are superimposed on a model of the airway to correlate the airway change. The solid red lines indicate individual dogs, while the dashed black line is the average of the six dogs. A: Represents the dogs before surgery. B: Represents the dogs after surgery.

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**PROJECT TITLE: COMPARISON OF BOLUS ADMINISTRATION OF HYPERTONIC SALINE COLLOID AND HYPERTONIC SALINE-COLLOID COMBINATION ON ISOFLURANE-INDUCED HYPOTENSION IN DOGS**

*RELEVANT TITLE: COMPARISON OF A SINGLE DOSE HIGH SALT COLLOID VERSUS A HIGH SALT COLLOID COMBINATION ON ANESTHESIA-INDUCED LOW BLOOD PRESSURE*

**INVESTIGATORS: T. AARNES**

*Final Report*

Gas anesthesia in dogs is associated with unwanted side effects, e.g. low blood pressure, that can lead to post-anesthetic complications. Traditionally, administration of fluids containing electrolytes at physiologic concentrations (crystalloids) during anesthesia was thought to improve blood pressure under anesthesia. However, studies have documented that large volumes of crystalloids results in fluid overload, without lasting improvement in blood pressure. In contrast, fluids composed of starch molecules in solution (colloids) and fluids with high concentrations of electrolytes (hypertonic saline) require administration of smaller quantities and lead to increased blood pressure. A combination of colloids and hypertonic saline has not been studied in anesthetized dogs. The purpose of this study is to determine the effects of a combination of a colloid and hypertonic saline on blood constituents, blood pressure, tissue blood flow and urine production during gas anesthesia. This will provide valuable information for making anesthesia safer for dogs.

The research was undertaken to determine the effect of different types of fluids for the treatment of isoflurane-induced hypotension in dogs. The results of the study indicate that all three fluids types (hypertonic saline, hetastarch, and hypertonic-hetastarch) increased blood pressure and cardiac output during hypotension caused by inhaled anesthetics, but that hetastarch increased blood pressure for a longer period of time than the other two fluids. The duration of increase in cardiac output was not dependent on the type of fluid administered. The microvascular circulation data is being analyzed and will be submitted as a separate manuscript. An abstract reporting the hemodynamic results is in preparation and will be submitted in April 2017 for presentation at the International Veterinary Emergency and Critical Care Symposium. The manuscript detailing the complete hemodynamic results of the project is in preparation.

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**PROJECT TITLE: EXPRESSION AND FUNCTION OF MCT1 AND MCT4 IN CANINE OSTEOSARCOMA AND MELANOMA**

*RELEVANT TITLE: CHARACTERIZING THE EXPRESSON AND FUNCTION OF THE TRANSPORTERS MCT1 AND MCT2 IN DOGS BONE CANCER AND MELANOMA*

**INVESTIGATORS: C. LONDON**

Dysregulation of cellular bioenergetics is one of the distinctive mechanisms used by tumor cells to adapt to a variety of environmental conditions. For example, cancer cells undergo aerobic glycolysis, utilizing glucose to produce lactate, even in the presence of oxygen. In order to continue proliferating, tumor cells maintain their intracellular pH by transporting lactate in and out of the cell. This is accomplished by the solute transporters MCT1 and MCT4. Both are expressed in human osteosarcoma and melanoma, and have been identified as critical for growth and metastasis. Lastly, inhibition of MCT1 and MCT4 in murine xenograft models results in delayed tumor growth and metastasis. The overriding goal of this proposal was to elucidate the contribution of MCT1 and MCT4 expression to the metabolic phenotype of canine tumors. We hypothesized that canine osteosarcoma and melanoma cell lines would express MCT1 and MCT4, and that downregulation of MCT1/4 expression would inhibit cell proliferation and survival. In addition, we predicted that these effects would be synergistic with both doxorubicin and metformin.

We initiated our studies in canine osteosarcoma first as we had undertaken prior studies indicating that cellular metabolism was dysregulated in this tumor type. Osteosarcoma cell lines and fresh frozen primary osteosarcoma tissue obtained from the Biospecimen Repository at the OSU Veterinary Medical Center were evaluated for expression of MCT1 and MCT4 using a combination of real-time PCR and Western blotting. Two novel first in class small molecule inhibitors targeting MCT1 and MCT4 were used to investigate the effects of MCT inhibition on cell proliferation, apoptosis and oxygen consumption in canine osteosarcoma cell lines. A standard cytotoxic chemotherapeutic (doxorubicin) and metformin (a biguanide that inhibits mitochondrial complex I) were evaluated in conjunction with MCT1/4 inhibition for synergistic activity *in vitro.* To further interrogate the role of MCT1, a lentiviral shRNA targeting MCT1 was generated and used to transduce canine osteosarcoma cell lines; proliferation, invasion and oxygen consumption were subsequently evaluated.

Expression of MCT1 and MCT4 were confirmed in all canine and human osteosarcoma cell lines and primary tumor samples. Two small molecule inhibitors NGY-066 (MCT1 inhibitor)

and NGY-008 (MCT4 inhibitor) were evaluated in canine osteosarcoma cell lines and no effects on cell survival were observed. However, the combined use of NGY-066 (MCT1 inhibitor) with doxorubicin or metformin markedly decreased the $IC_{50}$ of either drug when used alone *in vitro*.

To evaluate the target specificity of NGY-066/NGY-008 and investigate the role of MCT1 in osteosarcoma, we generated a lentiviral shRNA targeting MCT1. The loss of MCT1 expression was not lethal to the cell lines, however cells had a decreased invasive capacity when MCT1 was not expressed. In addition, when cultured under growth conditions, cells demonstrated upregulation of oxidative phosphorylation, measured via increased oxygen consumption after administration of an uncoupling agent (FCCP). Notably, this effect was lost when cells were cultured in the absence of glucose. Lastly, proliferation of osteosarcoma cell lines decreased significantly when exposed to doxorubicin in the absence of MCT1 expression, however this effect was not observed in the presence of metformin.

Our data provides evidence in support of continued evaluation of novel strategies targeting key proteins involved in regulating cancer cell metabolism. Evaluation of the effects of selective inhibition of these proteins through gene editing is ongoing. To help improve our understanding of the unique role cell metabolism plays in cancer treatment strategies, we will continue to investigate mechanisms by which dysregulated proteins can be effectively targeted to modulate cellular metabolism. Moreover, now that we have generated the tools and reagents necessary to genetically manipulate MCT expression in osteosarcoma cell lines, we will extend our studies to canine melanoma cell lines.

**Lay Summary:**

Cancer cells have high energy needs, and therefore need to adapt to many different environments for survival. Cancer cells can take advantage of the available nutrients and oxygen in their environment to help maintain their high metabolic rate. One way in which they accomplish this is by using glucose (sugar) for energy. This process is not efficient and generates lactic acid, which is toxic to cells in high amounts. To help prevent this toxicity, cancer cells increase expression of two proteins, MCT1 and MCT4, allowing lactate to move in and out of the cell. Lactate cannot be removed from human cancer cells when MCT1 and MCT4 are blocked, ultimately causing cell death. Very little is known about the function of MCT1 and MCT4 in canine cancer. The purpose of this proposal was to determine expression patterns of MCT1 and MCT4 in canine osteosarcoma and melanoma, define the anti-cancer effects of inhibiting MCT1 and MCT4, and determine if the use of novel MCT1/MCT4 inhibitors has synergistic activity when combined with chemotherapy.

Expression of MCT1 and MCT4 were evaluated in canine osteosarcoma cell lines and

primary osteosarcoma tumor samples obtained from the Biospecimen Repository at the Veterinary Medical Center. Novel MCT1 and MCT4 inhibitors were used to determine the impact of MCT1/4 inhibition on canine osteosarcoma cell growth and death. These inhibitors were also combined with a chemotherapy drug (doxorubicin) and a drug used to treat high blood sugar in people (metformin) to determine if there is a synergistic effect when the drugs are used together. To further investigate the role of MCT1 in canine osteosarcoma, gene editing techniques were used to decrease expression of MCT1 in the osteosarcoma cell lines.

Expression of MCT1 and MCT4 were confirmed in canine osteosarcoma cell lines and primary tumor samples. The use of a novel inhibitor targeting MCT1 and MCT4 (NGY-066) in canine osteosarcoma cells indicated that cells were more sensitive to treatment when alternative sources of energy (lactate, glucose) were absent. In addition, osteosarcoma cell proliferation was substantially decreased when cells were treated with an anti-cancer drug (doxorubicin or metformin) in combination with the MCT1 inhibitor (NGY-066). To confirm that these results were due to MCT inhibition, gene editing techniques were used to inhibit MCT1 expression in the osteosarcoma cell lines. The cells that did not express MCT1 had increased sensitivity to doxorubicin, however this effect was lost when cells were treated with metformin. Lastly, when osteosarcoma cells did not express MCT1, their ability to invade through a membrane was decreased and they relied more heavily on alternative energy sources (respiration).

In summary, we have demonstrated that both MCT1 and MCT4 are expressed in canine osteosarcoma cell lines and primary tumor samples. Our data also provide evidence to support continued evaluation of the use of drugs that target key proteins involved in cancer cell metabolism. Additional studies of the effects of selective inhibition of these proteins through gene editing is ongoing. To help improve our understanding of the unique role cell metabolism plays in cancer treatment strategies, we will investigate ways in which cancer cells exploit other proteins as their metabolic needs change. Lastly, now that we have generated the tools and reagents necessary to genetically manipulate MCT expression in osteosarcoma cell lines, we will extend our studies to canine melanoma.

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Interim Reports

**PROJECT TITLE: THE HEALTH OF DOGS ON LIVESTOCK FARMS: HUSBANDRY, INFECTION CONTROL AND PATHOGEN COLONIZATION**

*RELEVANT TITLE: PATHOGEN CONTROL IN WORKING FARM DOGS*

**INVESTIGATORS: J. STULL**

*Interim Report*

Reason the Research was Undertaken

Dogs often live on farms where there are livestock (e.g., pigs, cattle, chickens). Livestock animals can carry bacteria that they may transmit to dogs causing disease. Many of these diseases can also be spread further from dogs to people. Appropriate dog practices are important to limit the movement of disease-causing organisms from livestock and promote dog health. Yet, little is known of the current practices of dogs on livestock farms or the frequency of livestock-derived organisms in these dogs. The aims of this project are to determine dog practices and preventive healthcare measures occurring on livestock farms in Ohio and determine how these practices influence the frequency of specific disease-causing organisms from livestock (*Salmonella* and multi-drug resistant organisms) in these dogs.

Methods

A paper-based 10-minute survey was developed and distributed through the mail to a random sample of 2,000 Ohio livestock owners identified through a farming magazine distribution list. The survey gathered information on respondents' current livestock ownership, household demographics, presence and purpose of dog(s) on the farm, husbandry and preventative care practices for dogs, dog-livestock-person interaction, dog health, and level of attachment to dogs on the farm. Respondents to the survey who currently had both livestock and dogs were sent a follow-up kit and requested to collect and return fecal samples from up to two of their dogs. Additional recruitment for individuals with both livestock and dogs occurred by sending surveys and fecal collection kits to 62 interested OH 4-H club members. Dog fecal samples were tested for *Salmonella* and multi-drug resistant organisms (ie., ESBL-producing organisms $bla_{CMY}$, $bla_{CTX\text{-}M}$, $bla_{KPC}$, $bla_{NDM\text{-}1}$), using biochemical and molecular techniques. Reminders and follow-up mailings were used to maximize response for both the primary survey and follow-up dog fecal request.

As part of a pilot study and to serve as a comparison group for dogs on livestock farms, dogs

at dog shows were recruited. During the summer of 2016, exhibitors at five AKC conformation shows within Ohio were asked to participate in a two-part study. The first part consisted of a 15 minute survey on current dog husbandry practices at home and at shows. It also served to assess participants' current knowledge of preventative veterinary health measures. The second part of study consisted of a one page dog-specific survey and providing fresh fecal samples from up to two of their dogs currently present at the show. Fecal samples were screened for antimicrobial resistant bacteria and presence of *Salmonella* as described above. The fecal sample was linked to both surveys in order to identify potential risk factors at the dog and environmental levels. During the shows a centrally located table was staffed by a study representative to enroll interested show exhibitors. Enrollment was voluntary.

Results and Discussion

Surveys were returned by 47% of individuals, with 446 meeting the study inclusion criteria as an Ohio livestock farm operator, and 297 (67%) of these also dog owners. Fecal samples from 100 dogs were received and processed.

*Respondent demographics*

Of the 297 respondents with both livestock and dogs, 52% indicated one or more individuals at higher risk to infectious disease were present in their household. This included individuals < 5 yrs (7%), ≥ 65 yrs (32%), or immunocompromised (32%). These results are similar to findings in the general population and indicate an important potential for disease transmission to people on livestock farms.[1]

*Dog demographics*

On average, 1.8 dogs were reported by dog-owning livestock farmers (range 1-7). Many respondents (49%) reported their dogs were outdoor only and were not fenced or in a yard when outdoors (53%). These proportions are much higher than the general population (~5%) and indicate a risk for frequent exposure to and movement of infectious diseases on the farms.[1]

*Dog-livestock contact*

Direct contact between dogs and livestock (or the livestock environment) allow for movement of infectious diseases between the animals. Survey respondents reported frequent contact between dogs and livestock; dogs had direct access to livestock (70%), access to livestock stalls/pens (62%), access to sick/isolation pens (40%), and contact with new livestock (46%). The occurrence of these high-risk dog-livestock practices was not associated with the presence of household members at higher risk to infectious disease (Chi-square test; $P$ = 0.3).

*Dog husbandry practices*

Some items fed to dogs, such as raw/undercooked meat- or milk-based products are frequently contaminated with disease-causing organisms (e.g., *Salmonella*), that can cause illness in dogs and be transmitted to both people and livestock. These products were sometimes fed to dogs on livestock farms; home killed meat (6%), raw eggs (6%), raw meat (7%), raw milk (5%), raw animal product treats (11%). One or more of these high-risk products was fed to 24% of dogs. There was no association between the reported feeding of these high-risk items and the presence household members at higher risk to infectious disease (Chi-square test; $P = 0.4$).

*Dog-person contact*

Respondents reported close contact with many of the dogs. Approximately 50% of dogs were allowed to sleep in-doors, often in a family member's bed (13%) or other living area (25%). Licking of household members' faces by dogs was frequently reported – daily/several times per week (17%).

*Respondent disease concerns*

The majority (94%) of respondents reported no to minimal concern that they or their family members could acquire a disease from their dog. The reported lack of concern appears to be higher than reported for the general dog-owning population (86% reported minimally or not concerned).[1] Similarly, respondents were overall unconcerned their dog could acquire a disease from their livestock (90%).

*Attachment to dog*

Respondents reported a high level of attachment to dogs, with 62% stating they were "very attached" and 34% "somewhat attached." This level of attachment is slightly lower to that reported by the general dog-owning population (92% very attached).[2]

*Veterinary care*

Overall, the level of veterinary care received by farm dogs was high and consistent with that reported by the general dog-owning population.[2] Most respondents stated all owned dogs were current on rabies vaccine and other vaccines in the past 3 years (85%), received flea/tick preventative (86%), and were on an intestinal parasite preventative program (71%). Only 59% claimed their dogs were examined by a veterinarian annually, substantially lower than 85% previously reported in another study by the general dog-owning population.[2] Access to veterinary care was not a concern or barrier for most (78%) respondents.

*Dog fecal carriage of pathogens*

Dog samples were tested for *Salmonella* spp. and ESβL-producing organisms. Survey data were analyzed to identify predictors for dog carriage of pathogens. Seven percent of dogs were shedding *Salmonella* spp., and 4% and 39% *Escherichia coli* carrying $bla_{CTX\text{-}M}$ and $bla_{CMY\text{-}2}$, respectively. A majority of dogs that tested positive for *Salmonella* and $bla_{CTX\text{-}M}$ carriage were found to have exposures to multiple variables within the farm environment/livestock and human domains. Several factors were identified as significant predictors for dog carriage of $bla_{CMY\text{-}2}$ [access to livestock feed (OR=3.8), "has full run of the house for sleeping" (OR=3.5), access to livestock feed (OR=3.8), fed meals in kitchen (OR=2.9)].

From dog shows, 38 individuals agreed to participate, returning completed general surveys. In total, 29 dog fecal samples were submitted by those completing the general survey. Twenty-one percent of the samples (n=6) exhibited a resistance profile for $bla_{CMY}$; none had a resistance profile consistent with $bla_{CTX}$. *Salmonella* was cultured from 7% (n=2) of the samples. Both dogs harboring *Salmonella* were the only dogs fed raw meat/egg diets. The high frequency of antimicrobial resistance and *Salmonella* observed among fecal samples from AKC show dogs was consistent with findings from livestock farms; these appear to be higher than dogs in the general popualtion.[3]

Contribution of this Study

Based on currently received and evaluated survey and dog fecal data, several themes have emerged from this study. Dogs are frequently a part of Ohio livestock farms (~70%), with very close contact between dogs and livestock. A number of frequently reported practices likely put dogs at increased risk to livestock diseases, such as close contact with livestock (well and sick). Dogs may further move livestock diseases throughout a farm or to other farms through these practices. Gaps in dog preventive veterinary care may further increase these risks. Additionally, there are potential human health concerns given the frequent occurrence of higher-risk people living on livestock farms, suboptimal dog preventive care and husbandry, close dog-person contact, and limited personal hygiene measures reported by respondents. Disease risks are documented by the presence of *Salmonella* and multi-drug resistant organisms in dogs' feces. Dogs at AKC confirmation shows appear to have similarly high proportions of fecal carriage of antimicrobial resistance and *Salmonella*. Overall, surveyed livestock farmers reported a limited concern for disease transmission to and from dogs, potentially complicating the ability to promote change in practices.

Presentations

These findings have been presented as invited oral presentations at the International Society for Companion Animal Infectious Diseases bi-annual conference (October 2014; Ontario,

Canada) and Conference of Research Workers in Animal Disease (December 2015; Chicago, IL), as well as poster presentations at the American College of Veterinary Internal Medicine annual conference (June 2015) and International Conference on Emerging Infectious Diseases (August 2015; Atlanta, GA). A manuscript is in final stages of peer-review with the journal *Zoonoses and Public Health* – it is expected to be accepted shortly.

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**PROJECT TITLE: DECODING THE GENOMIC ARCHITECTURE OF CANINE LUNG CANCER**

*RELEVANT TITLE: GENOME ANALYSIS OF CANINE LUNG CANCER*

**INVESTIGATORS: G. LORCH**

*Interim Report*

**Publication resulting from this award**:

Clemente-Vicario F, Alvarez CE, Rowell JL, Roy S, London CA, Kisseberth WC, **Lorch G**, "Human genetic relevance and potent antitumor activity of heat shock protein 90 inhibition in canine lung adenocarcinoma cell lines". *PLOS ONE*. Vol. 10, no. 11: e0142007. 2015.

**Purpose**

Response rates to current therapies for treatment of canine lung cancer are poor. The metastatic incidence is approximately 71% at the time of diagnosis, resulting in a dismal median survival time of only 90 days in dogs with stage T3 tumors. While low-grade solitary primary tumors are potentially cured by complete surgical resection, unresectable or metastatic tumors require additional therapy. The goal of this research is to define canine lung cancer mutations. This information will increase understanding of lung adenocarcinoma development and allow us to better predict which currently available small molecule inhibitors may have biologic activity as

well as develop novel treatments to target common mutations or pathways for this cancer in dogs.

**Methods**

To begin to establish the genes affected by genomic alterations or copy number alterations (CAN) present in lung cancer, we custom designed a 966,903 feature comparative genomic hybridization (CGH) array tiling the canine genome. The array is comprised of isothermal 60-nucleotide probes targeting all regions of unique or low copy repeats (i.e., with otherwise-unique sequence) based on the CanFam2 assembly (including the unmapped contigs annotated as chrUn). Average spacing of probes is 1.9kb for unique sequence and 1.2kb for low copy repeats. The CGH platform and probe design method is Agilent SurePrint G3 (it includes 7,113 additional Agilent control probes). DNA quality control, array hybridization and scanning were performed by Asuragen1 (Austin, TX) under Agilent certified conditions. The two tumor cell line samples were compared against a healthy male Labrador retriever as the reference. The reference specimen was obtained under informed owner consent and the following Ohio State University IACUC approved protocol (2010A0015-R1, Canine Specimen Collection and Banking) which covered the procedure used to obtain the sample and their subsequent use for research application. Agilent uses a linear normalization process (including dye based normalization using copy-neutral normalization probes) for their LogR values. This data was imported into Golden Helix SNP and Variation Suite, and converted from LogR10 to LogR2. Sample quality metrics were performed, including percentile based Winsorizing, derivative log ratio spread, and wave detection/correction. For segmentation, we used the univariate Optimal Copy Number Analysis Module (CNAM in Golden Helix), which uses a change-point identification algorithm. While this powerful algorithm accurately identifies changes in sequential data, it is computationally intensive. We selected 10 max segments per 10,000 bases, 20-marker minimum for a copy number call, and a max pairwise permuted p-value of 0.005 (with 2000 permutations per pair). CNV calls were based on a logR2 ratio threshold of -0.40/0.40 for losses and gains, respectively. Because this segmentation algorithm is more sensitive to deletion copy number changes, we used a more stringent threshold for deletions for further analysis (-0.45 for deletions and 0.4 for gains). Mean symmetric smoothing was applied to all figures.

In 2016, we have used funds from this award to further our understanding of the genomic landscape of canine pulmonary adenocarcinoma through next generation sequencing and analysis from 5 frozen tumor and matched normal lung tissue. DNA and RNA were isolated from the biospecimens and whole exome next generation sequencing (NGS) of tumor DNA and matched normal in addition to NGS data through existing pipelines based on CanFam 3.1 in order to identify high quality genomic alterations in these cancers. Dr. Lorch has also developed eight cell lines derived from canine pulmonary adenocarcinoma tumors. Four of the cell lines have had exome NGS performed, the other four in the sequence pipeline.

**Results**

Comparative genomic hybridization was conducted on BACA and CLAC to establish the genes affected by genomic alterations or Copy Number Alterations. Stringent thresholds (0.4 and 0.45 Log2 ratios) and high minimum-number of probes per CNA segment (20 probes) were applied. We identified many large alterations that affect known cancer driver genes in these cell lines. For example, chr13, which contains the MYC gene that is commonly amplified in human lung adenocarcinoma, has 2-copy gains in both cell lines. Both cell lines have 2-copy loss of the most commonly deleted genome segment in human lung adenocarcinoma—which contains the genes CDKN2A/B/B-AS1 (one focal, the other larger CNA). BACA has large CNA deletions of the lung adenocarcinoma tumor suppressor PIK3R1 (<1% of human cases) and the pan cancer tumor suppressor CASP3. CLAC has a large CNA gain including NRAS, a known lung adenocarcinoma driver in 0.4% of human cases. CLAC also has gain of the lung adenocarcinoma oncogene CCND1 associated with 4% of human cases. Because the large CNAs contain very high numbers of presumptive bystander genes, it is not straight forward to evaluate all as potential oncogenic drivers. However, it is possible to study focal alterations—here arbitrarily defined as <3Mb—to implicate known cancer genes and pathways. Using the results of Cancer Gene annotation system for Cancer Genomics (CaGe) analysis with conservative criteria to minimize false positives, we found that the total number of genes affected by focal alterations was 263. Of those, 129 genes were called as either cancer drivers (89 genes) or pathway genes. We determined that at least 28 and 13 of those genes, respectively, are gained or lost in the predicted direction to be oncogenic (i.e., gain of oncogenes and loss of tumor suppressors). Two of those genes–CDKN2A and LRP1B –are reported to be significantly mutated in lung adenocarcinoma (but the latter, called only in the earlier study, may have been due to the large size of the gene). Gene Set Enrichment Analysis (GSEA) of all focal CAN genes yielded the top match by significance as genes altered in a complex therapeutic model (GSEA gene set name Zhang_antiviral_response_to_ribavirin_dn) applied to the human lung adenocarcinoma A549 cell line; another top hit was genes down-regulated by stable expression of SEMA3B in the human lung adenocarcinoma cell line H1299 (p-values/false detection rate q-values of 4.5E-06/3.27E-02 and 2.99E-05/4.65E-02). While those mechanistic studies with human cell lines may not be directly relevant to the canine BACA and CLAC cell lines, this finding and the implicated driver genes mentioned indicate that the dog lines are relevant to human lung adenocarcinoma.

The NGS analyses have thus far revealed a low somatic coding mutation (SNV) burden in 5 tumors (mean of 45) with one outlier bearing 131 SNVs. Notably a recurrent ERBB2 mutation has been identified in 3/5 cases with an additional ERBB2 mutation discovered in a fourth case. We have also validated the somatic nature of these mutations vis Sanger sequencing. Additional putative copy number alteration have been identified in CDKN2A/B, MTAP, ARID1B, ERBB2, FANCD2 and PTEN.

**Conclusions and Relevance**

Somatic mutations are the predominant mechanism that gives rise to cancer. The average cancer cell has approximately four sequence mutations of oncogenes (mean 1) and tumor suppressors (3), 11 very large CNAs involving whole chromosomes (2 gain, 2 loss) or chromosome arms (3 gain, 5 loss), and 23 focal CNAs (11 gains, 12 losses). Here we have determined the CNAs for BACA and CLAC using high resolution array CGH. The most striking findings were 2-copy gains of chr13, which contains MYC, and 2-copy loss of a small part of chr11, for which the overlapping segment between the two cell lines includes only CDKN2A/B/B-AS1. These are among the most common CNAs seen in human lung adenocarcinoma, with CDKN2A being involved in 43% of cases. Notably CDKN2A encodes ARF, which directly interacts with overexpressed MYC protein to block its transformation and proliferation activities. Other large CNA genes gained or lost in the correct direction to drive cancer are PIK3R1 and CASP3 in BACA, and NRAS and CCND1 in CLAC. We found many cancer driver and pathway genes affected in the cell lines by focal CNAs, including TSC2, NF2, BCL6, CHEK2, CDK6, KAT2B, PKD1 and TP63. Among the mechanistic insights, the CNAs suggest relevance for HSP90 inhibition that would be expected to have therapeutic effects through the PI3K and MAPK pathways (e.g., PIK3R1, TSC2, BCL6, NF2, PKD1, and NRAS). Other pathways that are likely to be affected according to our findings are cell cycle progression (e.g., CDKN2A and CCND1) and apoptosis (CASP3). The array CGH data thus support both lung adenocarcinoma and pan cancer relevance. Additionally, the GSEA analysis of these two canine cell lines strongly implicates human lung adenocarcinoma among all other cancer data. These facts formally demonstrate that BACA and CLAC can serve as comparative oncogenomic models for development of drug treatments to mammalian lung adenocarcinomas in which a set of common driver mechanisms are present.

Our sequencing results have now allowed us to design the first canine cancer clinical trial under the umbrella of a personalized medicine approach (PMed) to cancer therapy. We have also developed the first of its type biomarker assay to identify the dogs with lung cancer that would benefit from the use of a small molecule inhibitor drug *vs*. traditional standard of cancer chemotherapy.

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**PROJECT TITLE: *CLOSTRIDIUM DIFFICILE* IN DOGS: RISK FACTORS FOR COLONIZATION, INFECTION AND OWNER TRANSMISSION**

*RELEVANT TITLE: C. DIFF IN DOGS: RISK FACTORS FOR INFECTION AND TRANSMISSION TO OWNERS*

**INVESTIGATORS: J. STULL**

*Interim Report*

Reason the Research was Undertaken

*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. Using a survey of dog-owners with and without *C. difficile* infections and testing of fecal samples from them and their dogs, this project will 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.

Methods

Dog-owners tested for clinical *C. difficile* infection at The Ohio State Wexner Medical Center were recruited. Dog owners with *C. difficile* (case; n=160) and without *C. difficile* (control; n=160) will be enrolled. Fecal samples from owners (1 sample) and up to two dogs (2 samples each) per owner were requested and cultured for *C. difficile*. A questionnaire was used to capture important owner, household, and dog information (time point 1). A follow-up survey and requested dog fecal samples were used to assess changes in fecal *C. difficile* carriage and dog-owner practices one month after initial survey (time point 2). Molecular analysis will be used to characterize *C. difficile* samples (e.g., carrying disease-causing toxins, strains implicated in important human disease). Analysis will focus on the prevalence of *C. difficile* in human and dog feces, distribution of unique subtypes in each population and indistinguishable dog-owner pair subtypes. Results will be analyzed to determine risk factors for dog *C. difficile* carriage, dog infection, and suggested dog-owner transmission.

Results and Discussion

To date, we have enrolled 114 human patients in this study. Thirty human patients (26%) returned a completed survey and canine fecal samples at baseline (time point 1). From these 30 households, 41 dogs were enrolled with 2 samples collected per dog (total of 82 canine fecal samples). Respondents from 15 of the 30 (50.0%) households comprising 20

dogs responded to the follow-up survey and sent in follow-up samples 1 month after initial sampling (total of 39 canine fecal samples). *C. difficile* was detected (tpi+) in 25% of human samples. *C. difficile* was detected in dogs at time point 1 and 2. More dogs tested positive for *C. difficile* at time point 1 (22%) versus time point 2 (10%); no dogs tested positive at both time points. It is possible that dogs had short-term colonization or were contaminated with *C. difficile* as shedding did not persist from time point 1 to time point 2.

Table 1: Results of microbiological testing on human and canine fecal samples

| | Number of Individuals | tpi+ | tcdA+/tcdB+ | tcdA+/tcdB- | tcdA-/tcdB+ |
|---|---|---|---|---|---|
| **Human** | 114 | 29 (25.4%) | 16 (55.2%) | 5 (17.2%) | 1 (3.4%) |
| **Canine** (time point 1) | 41 | 9 (22.0%) | 1 (11.1%) | 0 (0.0%) | 0 (0.0%) |
| **Canine** (time point 2) | 20 | 2 (10.0%) | 1 (50.0%) | 0 (0.0%) | 0 (0.0%) |

Surveys were completed for 41 dogs. No statistically significant associations were identified between dog risk factors (as reported by survey respondents) and presence of *C. difficile* in a dog's feces (Fisher's Exact test; all $P > 0.05$; Table 2). While exposure to antimicrobials and the development of diarrhea have been strongly associated with *C. difficile* infection in humans, the current results suggest that this is not the case in dogs. This may be limited to a small sample size, and further data will be useful to examine these associations. Patients are still actively being recruited for this study in attempt to increase sample size.

Table 2: Association between selected risk factors and dog colonization with *C. difficile* at time point 1 (n=41).

| | | *C. difficile* | | |
|---|---|---|---|---|
| **Risk Factor** | | **Negative** | **Positive** | **p-value**[1] |
| Antimicrobial exposure | Yes | 1(3.1) | 1 (11.1) | 0.395 |
| | No | 31(96.9) | 8(88.9) | |
| Dog park visits | | | | 0.645 |
| | Yes | 3(9.4) | 1 (11.1) | |
| | No | 29(90.6) | 8(88.9) | |
| Livestock exposure | | | | |
| | Yes | 3(9.4) | 2(22.2) | 0.299 |
| | No | 29(90.6) | 7(77.8) | |
| Widlife exposure | | | | |
| | Yes | 8(25.0) | 2(22.2) | 0.620 |
| | No | 24(75.0) | 7(77.8) | |
| Raw food exosure | | | | |
| | Yes | 4(12.5) | 1 (11.1) | 0.700 |
| | No | 28(87.5) | 8(88.9) | |
| Diarrhea | | | | |
| | Yes | 3(9.4) | 0(0.0) | 0.465 |
| | No | 29(90.6) | 9(100.0) | |
| Veterinary visit | | | | |
| | Yes | 8(25.0) | 2(22.2) | 0.620 |
| | No | 24(75.0) | 7(77.8) | |

Toxigenic *C. difficile* (toxin A, toxin B, or both) was detected in 22 human samples and 1 of the dogs each at time points 1 and 2 (Table 1). These results suggest toxigenic *C. difficile* is rare in dogs, even when living with a person recently diagnosed with and shedding *C. difficile*. Due to Institutional Review Board (IRB) requirements, the research team is still currently blinded to the identity of human-dog pairs and human *C. difficile* results. At the conclusion of enrollment, the study team will compare results of human-dog pairs to investigate likely human-dog *C. difficile* transmission. Future research will include further characterizing isolates for binary toxin, ribotyping, & whole genome sequencing of human-dog pairs to determine if transmission between these groups was likely.

Contribution of this Study

The results of this study will allow us to determine the relevance of *C. difficile* in the dog population, better understand the movement of this bacterium between people and dogs, and identify possible areas for disease control and prevention. Given the critically important nature of this bacterium in human disease and suggested concerns in dogs, this project is likely to have far-reaching benefits to the veterinary and public health communities.

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**PROJECT TITLE: A NOVEL ANALGESIC DRUG, TAPENTADOL, FOR THE TREATMENT OF PAIN IN DOGS WITH CLINICAL OSTEOARTHRITIS**

*RELEVANT TITLE: TAPENTADOL, A NEW DRUG FOR TREATING ARTHRITIS PAIN IN DOGS*

**INVESTIGATORS: T. AARNES**

*Interim Report*

Tapentadol is a novel analgesic that treats pain through two different mechanisms.
The dual activities of tapentadol have been purported to result in increased analgesia (relief of pain) with an opioid sparing effect and decreased side effects compared with other drugs commonly used to treat pain in people. This study will determine if tapentadol causes analgesia (relief of pain) when administered orally to dogs, and concentration of the drug in a dog's blood at which they experience analgesia. These results will provide veterinarians with the information required to use tapentadol to treat pain in dogs and determine if a previously investigated dose provides appropriate analgesia. This knowledge will be beneficial for increasing veterinarians' abilities to effectively administer pain management and improve the quality of life of dogs with chronic pain conditions.

The research was undertaken to evaluate how the drub tapentadol is absorbed, distributed throughout the body, and its duration of effect in dogs and determine if tapentadol treats pain in dogs with osteoarthritis. The first portion of the study is complete and we determined the best dose of the drug based on how long it lasts in the body of dogs. The second portion, determination of analgesic effect, is ongoing.

An abstract reporting the pharmacokinetic results is in preparation and will be submitted in April 2017 for presentation at the American College of Veterinary Surgeons symposium. The manuscript detailing the pharmacokinetics has been submitted and is under consideration for publication. Patient recruitment for the second portion of the study is ongoing and we expect to complete data collection within one year.

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**PROJECT TITLE: CHARACTERIZING THE ROLE OF W WOX DEFICIENCY IN CANINE OSTEOSARCOMA**

*RELEVANT TITLE: CHARACTERIZING THE ROLE OF THE TUMOR SUPPRESSOR GEN Wwox IN BONE CANCER*

**INVESTIGATORS: J. FENGER**

*Interim Report*

**Rationale for conducting research**

Osteosarcoma (OS) is the most common form of malignant bone cancer in children and dogs. Despite aggressive treatment including surgery and chemotherapy, 30-40% of children and >90% of dogs with OS develop chemotherapy resistant metastatic disease. Significantly, little improvement in survival times have been achieved in either children or dogs in over 30 years. A variety of clinical trials have been completed in an attempt to improve outcome for children and dogs with OS, yet to date, none have proven successful. It is likely that until the molecular alterations underlying OS are identified and characterized, the development of more effective therapies that will substantially improve outcomes for children and dogs with OS will remain a significant challenge.

The WW domain-containing oxidoreductase (*WWOX*) is a tumor suppressor gene that is deleted in many human cancers and cancer-derived cell lines. *WWOX* plays a central role in regulating normal bone homeostasis and *WWOX*-deficient mice spontaneously develop OS, suggesting that loss of *WWOX* predisposes to the development of osteosarcoma. We have generated preliminary data demonstrating that Wwox protein expression is decreased in canine OS tumors and OS cell lines compared to normal canine osteoblasts. In cell culture models, we found that *WWOX* deletion impairs DNA damage repair and checkpoint activation, suggesting that loss of *WWOX* may drive genomic instability and provide an advantage for clonal expansion of neoplastic cells. The purpose of this proposal is to evaluate the functional role of *WWOX* deficiency in normal canine osteoblast biology and its contribution to the initiation of canine OS. *We hypothesize that Wwox deficiency impairs cellular DNA damage responses and cell-cycle checkpoint activation, thereby contributing to genomic instability and initiation of canine osteosarcoma.* As such, the specific aims of this proposal are:

1) Characterize the functional consequences of *WWOX* deficiency in canine osteosarcoma and normal canine osteoblasts.

2) Determine the effects of *WWOX* deficiency on sensitivity to DNA damage, cell-cycle checkpoint activation, and DNA damage repair in normal and malignant canine osteoblasts.

3) Assess the effects of osteoblast-specific ablation of the *WWOX* gene *in vivo* by generating *Col1a1*-Cre/*Wwox*$^{-/-}$ transgenic mice.

We anticipate that this work will generate significant new data regarding the function of the tumor suppressor *WWOX* in canine osteosarcoma biology and potentially identify novel targets for therapeutic intervention.

**How was the study conducted:**

To more fully characterize Wwox protein expression in primary canine OS tumors, we have generated a tissue microarray (TMA) consisting of 22 primary OS tumors and 8 paired primary OS tumors with matched lung metastatic lesions. Briefly, representative areas of tumor tissue were identified on H&E stained sections and cores were spotted in triplicate onto predetermined sites on the TMA recipient block. The expression of Wwox in OS tumors and paired lung metastasis will be assessed and scored using immunohistochemical staining. In conjunction with our TMA findings, we have acquired fresh canine OS samples available through the OSU Veterinary Clinical Research Support Shared Resource Biospecimen Repository. We have performed real time PCR and Western blotting to evaluate Wwox transcript and protein expression in primary canine OS tumors and in available canine OS cell lines.

To assess the consequences of Wwox deficiency on normal and malignant canine osteoblast cell functions *in vitro*, we have generated Wwox shRNA constructs and cloned these into the pGreenPuro™ shRNA Cloning and Expression Lentivector (Systems Biosciences). We have transduced normal canine osteoblast cells (Cell Applications, Inc.) and in canine OS cell lines expressing high basal levels of Wwow with pGreenPuro-scramble or pGreenPuro-shWwox lentivectors. Stably transduced cells were sorted based on GFP positivity and knockdown efficiency was confirmed with Western blotting. *WWOX*-deficient cells were evaluated for changes in cellular morphology, bone differentiation marker expression (alkaline phosphatase) and mineralization activity (von Kossa) by immunocytochemistry. Real time PCR was performed to detect expression of markers of osteoblast commitment (*Runx2*, *Alp, Bsp*, *ColI*, *Oc*). Lastly, cells expressing *WWOX* shRNA or scramble vector were assessed for differences in proliferative capacity (CyQUANT® Cell Proliferation Assay Kit), anchorage-independent cell growth (soft-agar growth assay), cell migration (wound-healing assay), and Matrigel invasion in the presence or absence of hepatocyte growth factor (HGF).

To determine the role of *WWOX* in DNA damage response and DNA repair, Wwox-deficient

and control canine osteoblasts will be treated with aphidicolin to chemically induce DNA replication stress. Differences in the expression of DNA damage proteins (γH2AX) and repair markers (53BP1) in response to aphidicolin treatment in Wwox-deficient and control osteoblasts will be evaluated and quantified using immunofluorescence. To determine DNA repair pathways affected by Wwox, western blotting will be performed to detect expression of DNA repair markers associated with homologous recombination (HR) repair mechanisms (Rad51, CtIP) or non-homologous end joining (NHEJ) repair (53BP1, RIF1). To asses if WWOX loss provides a survival advantage by allowing cells with accumulating DNA damage to undergo repair and avoid apoptosis, Wwox-deficient and control osteoblasts will be subject to aphidicolin treatment and we will evaluate Caspase-3/7 activity (SensoLyte® Homogeneous AMC Caspase- 3/7 Assay kit, Anaspec Inc), cell apoptosis (Annexin-V-PE/7-AAD kit, BD Biosciences), and cell viability (WST-1 assay, Roche). To evaluate checkpoint activation, phosphorylation and expression of the DNA damage sensors/checkpoint activators, pATM, pATR, and their downstream effectors pChk1 and pChk2 will be evaluated by western blot. Propidium iodide staining for DNA content will be used to evaluate cells in different phases of the cell cycle, with accumulation of cells in $G_2$ indicative of $S/G_2$ checkpoint activation.

Targeted ablation of the *WWOX* gene in mice results in the spontaneous development of OS in approximately 30% of mice; however, knockout mice also suffer from growth retardation, severe metabolic defects, and have a high incidence of other tumors. Therefore, specific ablation of *WWOX* in mouse bone without the compound effects of total *WWOX* deletion is necessary to specifically address the role of Wwox in osteoblast biology *in vivo*. To this end, we have crossed transgenic mice (Tg) that carry a conditional allele of *WWOX* (*Wwox*$^{fl/fl}$-Tg mice) with mice expressing Cre recombinase under the osteoblast-specific Collagen1α1 promoter (*Col1a1*-Cre-Tg mice). We have shown that in our double transgenic *Col1a1*-Cre; *Wwox*$^{fl/fl}$-Tg mice, deletion of the “floxed”-*Wwox* allele is restricted to osteoblasts and is not present in other non-bone tissues (liver, spleen, lung) using Western blotting, demonstrating that our model functions in a tissue-specific fashion. We have backcrossed our F1 *Col1a1*-Cre; *Wwox*$^{fl/fl}$-Tg mice to C57/B6 mice for 8 generations (F8). To characterize the consequences of *WWOX* gene deletion in osteoblasts *in vivo*, single and double Tg mice were sacrificed at 12 weeks of age (n=3 per genotype) and submitted to the Comparative Pathology and Mouse Phenotyping Resource (CPMPR) for full gross necropsy to characterize any phenotypic abnormalities, particularly with regard to musculoskeletal growth rate and development of OS lesions. Immunohistochemical (IHC) analysis will be performed by the CPMPR to characterize the effects of Wwox in osteoblasts on bone quality, skeletal development (alizarin red/Alcian blue), number and activity of osteoclasts (tartrate-resistant acid phosphatase), tumor histology and phenotype, and general effects on the musculoskeletal tissues in mice. Lastly, to assess the phenotypic consequences of WWOX

deletion on osteoblasts in vitro, we have generated osteoblast cultures from the calvaria of single or double Tg mice. Cultures were evaluated at day 7, 10, and 14 for differences in cellular proliferation, expression of bone differentiation markers (alkaline phosphatase) and mineralization activity (von Kossa) as previously described. The expression of bone commitment markers was determined using real time PCR and the effects of WWOX deletion osteoblast functions (migration/invasion, sensitivity to DNA damage, checkpoint activation, DNA repair) was evaluated using assays described above in to confirm their role in Wwox-induced phenotypic changes.

**Results and progress of the study**

To determine whether *WWOX* loss is a common event in common event in canine OS, we performed Western blotting to evaluate Wwox protein expression in normal canine osteoblasts, canine OS cell lines, and primary canine OS tumors and found that normal canine osteoblasts express high levels of Wwox protein compared to most OS cell lines and tumors. We further demonstrated by real time PCR that Wwox transcript is reduced in primary canine OS tumor specimens and OS cell lines and that Wwox transcript levels are similar to that expressed in human OS cell lines. These data suggest that dysregulation of *WWOX* may be fundamental to the disease process in both human and canine OS. To more fully characterize Wwox protein expression in primary canine OS tumors, we have generated a tissue microarray (TMA) consisting of 22 primary OS tumors and 8 paired primary OS tumors with matched lung metastatic lesions. We are currently optimizing the Wwox antibody for immunohistochemical detection of the Wwox protein in canine tissues. Following optimization of the immunohistochemistry protocol, the expression of Wwox in OS tumors and paired lung metastasis will be assessed and scored using immunohistochemical staining. We anticipate completion of these data in the next 4 months.

To assess the consequences of Wwox deficiency on normal and malignant canine osteoblast cell functions *in vitro*, we have generated Wwox shRNA constructs and cloned these into the pGreenPuro™ shRNA Cloning and Expression Lentivector. Normal canine osteoblast cells and canine OS cell lines that express high basal levels of Wwox were transduced with either scramble or WWOX shRNA lentiviral vectors and stably transduced cells were sorted based on GFP positivity. We confirmed knockdown efficiency in cell lines using real time PCR and Western blotting. The expression of bone commitment markers, bone morphogenetic protein-2 (BMP-2), alkaline phosphatase (ALP), runt related transcription factor-2 (RUNX2), and osteocalcin (OC) was determined using real time PCR and we found enhanced expression of RUNX2 (a known binding partner of Wwox) following *WWOX* knockdown in osteoblasts. Lastly, cells expressing *WWOX* shRNA or scramble vector were assessed for differences in proliferative capacity and we found that cell proliferation was enhanced following loss of Wwox in osteoblasts and osteosarcoma cells. We are currently completing

experiments evaluating the functional consequences of Wwox deletion on anchorage-independent cell growth, cell migration and Matrigel invasion. Now that we have generated stably transduced cell lines expressing Wwox shRNAs, we will utilize these cell lines to perform the experiments outlined in Aim 2 investigating the role of WWOX in DNA damage response and DNA repair. We anticipate completion of these cellular assays in 4-5 months.

Wwox plays a central role in regulating bone homeostasis and Wwox-deficient mice spontaneously develop OS, suggesting that loss of WWOX predisposes to the development of this cancer. However, globally deficient WWOX mice do not live beyond a few weeks post birth as they suffer from growth retardation, severe metabolic defects including metabolic acidosis and kidney failure, and have a high incidence of other tumors (lung tumors, lymphoma, squamous cell carcinoma). To more critically evaluate the effects of *WWOX* deficiency in a tissue specific manner, we have generated a conditional transgenic mouse model of *WWOX* deletion in osteoblasts (*Col1a1*-Cre*; Wwox*$^{fl/fl}$-Tg mice). Therefore, the consequences of WWOX deletion can be evaluated directly in osteoblasts in vivo and in vitro without the confounding influence of altered Wwox expression in other tissues. We have shown that the *Col1a1*-Cre*; Wwox*$^{fl/fl}$-Tg mice function in a tissue-specific manner based on the expression of Cre recombinase by crossing *Col1a1-Cre*-Tg mice with *Wwox*$^{fl/fl}$-Tg mice and demonstrating by Western blotting that primary osteoblast cultures derived from *Col1a1*-Cre*; Wwox*$^{fl/fl}$-Tg mice express significantly decreased levels of Wwox protein compared to single transgenic mice (*Col1a1-Cre*-Tg or *Wwox*$^{fl/fl}$-Tg). Furthermore, non-bone tissues (liver, spleen, lung) from single and double transgenic mice express equal amounts of Wwox protein providing additional data to support the idea that our model of osteoblast-specific Wwox deletion functions in a tissue-specific manner. To characterize the consequences of *WWOX* gene deletion in osteoblasts *in vivo*, single and double Tg mice were sacrificed at 12 weeks of age and submitted to the Comparative Pathology and Mouse Phenotyping Resource (CPMPR) for full gross necropsy to characterize any phenotypic abnormalities, particularly with regard to musculoskeletal growth rate and development of OS lesions. We anticipate that phenotypic analysis of our transgenic mouse model will be completed in the next 6 months.

To assess the phenotypic consequences of *WWOX* deletion on osteoblasts *in vitro*, we generated osteoblast cultures from the calvaria of single or double Tg mice. We validated that Wwox protein was reduced in *Col1a1*-Cre*; Wwox*$^{fl/fl}$ osteoblast cultures and we confirmed the purity of our osteoblast cultures by performing immunocytostaining to determine alkaline phosphatase staining as a marker of osteoblast differentiation. The expression of bone commitment markers, bone morphogenetic protein-2 (BMP-2), runt related transcription factor-2 (RUNX2), and osteocalcin (OC) was determined using real time PCR and we found that RUNX2 expression was enhanced following *WWOX* deletion in

osteoblasts. These findings are consistent with data in human studies demonstrating that attenuation of *WWOX* in osteosarcoma is associated with aberrant RUNX2 expression. Cellular proliferation on day 7, 10, and 14 in osteoblast cultures generated from *Wwox*$^{fl/fl}$ or *Col1a1*-Cre*; Wwox*$^{fl/fl}$-Tg and we found that loss of *WWOX* increased cell proliferation in osteoblasts *in vitro*. Studies are currently underway investigating the effects of *WWOX* deletion on osteoblast migration/invasion, sensitivity to DNA damage, checkpoint activation, and DNA repair. We anticipate completion of the experiments outlined in Aim 3 in 3-4 months.

We have made significant progress on the experiments proposed in Aims 1 and 3 and anticipate completion of these studies in the next 6 months. We will be performing experiments outlined in Aim 2 next month and anticipate their completion within 4-5 months.

**Contribution of this study to solving the problem at hand (lay-language summary):**
Osteosarcoma (OS) is the most common malignant bone cancer in children and dogs; however, the incidence of canine OS is approximately ten times higher than that observed in humans. Despite aggressive treatment including surgery and chemotherapy, 30-40% of children and >90% of dogs with OS develop fatal, chemotherapy resistant metastatic disease. Significantly, little improvement in survival times have been achieved in either children or dogs in over 30 years. As such, new therapies are desperately needed if substantial improvements in outcome are to be achieved.

The WW-domain oxidoreductase (*WWOX*) is a tumor suppressor gene that is commonly lost in human OS tumors. Mice deficient in Wwox spontaneously develop OS tumors, suggesting that Wwox deletion predisposes to the development of OS and plays a central role in the pathogenesis of this tumor. The overarching goal of this study is to characterize the role of the tumor suppressor gene WWOX in normal and malignant osteoblast biology and determine the functional consequences of *WWOX* deficiency in promoting the aggressive biology of canine OS. We found that Wwox protein expression is decreased in primary canine OS tumors and canine OS cell lines compared to normal osteoblasts. Furthermore, we demonstrated that Wwox gene transcript levels are reduced in primary canine OS tumor specimens and OS cell lines and that Wwox expression is similar to that observed in human OS cell lines, suggesting that dysregulation of *WWOX* may be fundamental to the disease process in both species. To assess the consequences of Wwox deficiency on normal and malignant canine osteoblast cells, we have generated canine OS cell lines deficient in Wwox and studied the effects of loss of Wwox on the expression of bone differentiation markers and cell proliferation. In these experiments, we found that *WWOX* deletion promotes cell proliferation and up-regulated expression of RUNX2, a transcription factor crucial to osteoblast differentiation and known to be over-expressed in OS.

To better study the role of the tumor suppressor gene *WWOX* in normal osteoblast and osteosarcoma biology, we have developed a transgenic mouse model of osteoblast-specific deletion of WWOX (*Col1a1*-Cre*; Wwox*$^{fl/fl}$-Tg mice) and confirmed the functionality of this model including tissue-specific loss of Wwox in osteoblast cultures generated from *Col1a1*-Cre*; Wwox*$^{fl/fl}$ mice. Similar to our findings in cultured canine OS cell lines, we found that loss of Wwox expression in osteoblasts generated from *Col1a1*-Cre*; Wwox*$^{fl/fl}$ transgenic mice promotes cell proliferation and up-regulated expression of RUNX2.

Together, our findings demonstrate that loss of the tumor suppressor gene *WWOX* commonly occurs in spontaneous canine OS. Furthermore, these data suggest that attenuation of *WWOX* in canine OS cells and normal mouse osteoblasts enhances cell proliferation, in part through up-regulation of the transcription factor RUNX2. We anticipate that the findings of this study will provide a molecular framework for understanding the functional role of Wwox deficiency in normal osteoblast biology and its contribution to the initiation of OS.

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**PROJECT TITLE: LOW-LEVEL LASER THERAPY AS AN ADJUNCTIVE TREATMENT FOR CANINE ACRAL LICK DERMATITIS: A RANDOMIZED, DOUBLE-BLINDED SHAM-CONTROLLED STUDY**

*RELEVANT TITLE: LOW LEVEL LASER THERAPY AS AN ADDITIONAL TREATMENT FOR DERMATITIS IN DOGS*

**INVESTIGATORS: L. COLE, A. SCHNEDEKER, G. LORCH, S. DIAZ, P. RAJALA-SCHULTZ**

*Interim Report*

Canine acral lick dermatitis (ALD) is a disease that manifests as excessive, compulsive licking at a focal area on the limb resulting in a proliferative, ulcerative, and hairless lesion that has secondary deep infection. ALD can have multiple causes such as anxiety, allergic disease, hypothyroidism, orthopedic abnormalities, neurologic and psychogenic. Conventional therapy for ALD includes systemic antibiotics for the deep bacterial infection and a systemic behavior-modifying medication (i.e., trazodone, fluoxetine, clomipramine). Non-conventional treatment options include surgery, radiation, and electrostimulation and

have variable efficacy and numerous side effects.

Low-level laser therapy (LLLT) is an alternative therapy used to treat a multitude of conditions that require stimulation of healing and relief of inflammation, itching (pruritus) and pain. Blue light phototherapy is bactericidal and has been shown to kill methicillin-resistant *Staphylococcus aureus* in the laboratory. As ALD lesions are inflammatory, painful or pruritic, and commonly secondarily infected, the use of both LLLT and blue light phototherapy may be a useful treatment option. The objective of this study is to determine whether the use of LLLT in addition to conventional therapy will result in a significant decrease of the licking behavior of ALD lesions than conventional therapy alone. Our hypothesis is that the addition of LLLT to conventional therapy for canine ALD will result in a >50% reduction in licking behavior than without LLLT.

In this study, 20 dogs will be enrolled and the following procedures will be performed: physical exam, sedation, radiographs, biopsy for histopathology and culture (bacterial and fungal) and study treatments (control or treatment). Dogs are randomized into control (laser off=sham) and treatment group (laser on). All dogs receive antibiotics (based on culture results) and oral anti-anxiety medication (trazodone). The dogs return for treatments (sham or laser) three times weekly for 2 weeks, then twice weekly for 2 weeks, for a total of 10 visits. The owner monitors licking behavior using a licking visual analog scale (LVAS), an objective scoring system.

Study enrollment began September 2015. To date, 10 dogs have completed the study. Preliminary results show that LLLT in addition to oral antibiotics and oral trazodone results in an approximately 20% greater decrease in licking when compared to oral antibiotics and oral trazodone alone. Enrollment for the remaining 10 dogs continues and recruitment efforts have increased by contacting local referring veterinarians and posting on social media sites. Once all dogs are enrolled in the study, the results of the LVAS will be compared between the treatment and control groups to evaluate the efficacy of LLLT in decreasing licking behaviors.

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**PROJECT TITLE: DIAGNOSING AND MANAGING NEUROPATHIC PAIN IN DOGS WITH SPINAL CORD INJURY**

*RELEVANT TITLE: DIAGNOSING AND MANAGING NERVE PAIN IN DOGS WITH SPINAL CORD INJURY*

**INVESTIGATORS: S. MOORE**

*Interim Report*

Neuropathic pain affects 90% of people living with spinal cord injury (SCI), yet it is an under-appreciated problem in canine patients with the same disease. This is likely because assessment of neuropathic pain in people relies heavily on self-reporting, something our veterinary patients are not able to do. In essence, our patients may be suffering in silence. Objective methods to quantify neuropathic pain have been used in rodent models of SCI and in the human clinical setting. Our laboratory has recently adapted one such technique (von Frey anesthesiometry-VFA) for use in dogs, but this technique requires assessment of inter-observer agreement. The time frame for development of neuropathic pain in dogs with SCI is also currently unknown, as is the frequency of its occurrence. Our study has three aims. Aim 1 will use VFA to document the time course for development of and frequency of occurrence of neuropathic pain in dogs with SCI caused by intervertebral disc extrusion (IVDE). Aim 2 will assess the ability of the commercially available neuromodulatory drug gabapentin to modulate sensory threshold (ST) in dogs using a blinded, placebo controlled, cross-over study design. Aim 3 will evaluate the inter-observer agreement of VFA measurements between three trained clinicians in a group of normal dogs.

At this time we have enrolled 16 of 30 SCI-affected dogs (Aim 1) and 7 of 20 normal dogs (Aim 2) in our study. This is in keeping with our overall time line for the project, and we expect the study to finish enrollment by fall of 2017. Canine research funds were obtained to fund the first half of this project (15 SCI-affected dogs and 10 normal dogs) and the project is still on-going with most of the funds from OSU expended and data collected having been used as preliminary data to apply and successfully obtain external funding for the second half of patient enrollment from the Gray Lady Foundation (project # 60049797) to allow us to evaluate an expanded patient enrollment in the same project and for completion of Aim 3. This funding has served as the basis for the thesis work of Dr. Austin Kerns, currently a graduate student and Neurology resident in VCS with an expected graduation date of spring 2018. We anticipate that 2-3 publications will result from this work.

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**PROJECT TITLE: MICROSENSITIVITY OF FREEZE THAW CYCLED CANINE PLASMA**

*RELEVANT TITLE: MICROSENSITIVITY OF DOG PLASMA THAT HAS UNDERGONE MULTIPLE FREEZE-THAW CYCLES*

**INVESTIGATORS: P. YAXLEY, K. GERKEN, E. COOPER**

*Interim Report*

Fresh frozen plasma and frozen plasma are used to replace coagulation factors and protein in veterinary patients. Sometimes, plasma units are discarded because they are thawed but then cannot be used due to patient status change, death, or complications with the actual plasma unit. It has been previously studied that the coagulation factors 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 refreshing is a substantiated complication that would prevent the future use of these units.

A preliminary study has been performed to determine if canine plasma can serve as a medium for bacterial growth at all and has shown that it is possible. However, 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.

The next step of this study will be to compare growth of bacteria in samples of plasma at room temperature and throughout a freeze-thaw-cycle processing. A small sample of each plasma unit will also be inoculated with bacteria in order to determine the conditions were still viable for bacterial growth. It is unlikely that bacterial growth will occur and it is likely to conclude that the freeze-thaw-cycle process is not inhibitory for future use of plasma.

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New Projects Just Funded in 2016

**PROJECT TITLE: COMPARISON OF PREOPERATIVE ANALGESIC PROTOCOLS AND EVALUATION OF CHRONIC NEUROPATHIC PAIN STATE IN DOGS UNDERGOING TPLO**

*RELEVANT TITLE: COMPARISON OF PRE-SURGERY ANESTHESIA PROTOCOLS AND EVALUATION OF NERVE PAIN IN DOGS AFTER KNEE LIGAMENT STABILIZATION SURGERY*

**INVESTIGATORS: N. KIEVES, T. AARNES, J. HOWARD, S. MOORE, J. PENG**

Neuropathic pain is a complex, chronic pain state that can develop secondary to osteoarthritis in people, leading to significant loss of function with substantial healthcare costs. Spontaneous cranial cruciate ligament (CCL) rupture is the most common cause of pelvic limb lameness in the dog, and causes secondary osteoarthritis in the stifle. It is commonly corrected with surgery. Neuroanalgesic procedures including epidurals and direct nerve blocks are common prior to surgical correction of cruciate disease. This investigation will evaluate the efficacy of three common neuroanalgesic procedures on short term perioperative pain management and evaluate chronic sensory threshold as a measure of neuropathic pain in the dog. If dogs are shown to have chronic neuropathic pain with osteoarthritis, spontaneous CCL rupture in the dog may serve as a naturally occurring animal model for studying neuropathic pain in both dogs and humans.

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**PROJECT TITLE: VALIDATION OF PRMT5 AS A CANDIDATE THERAPEUTIC TARGET IN CANINE LYMPHOMA**

*RELEVANT TITLE: VALIDATION OF THE CANCER ASSOCIATED GENE PRMT5 AS A TARGET FOR TREATMENT*

**INVESTIGATORS: W. KISSEBERTH, K. RENALDO, R. BAIOCCHI, K. SHILO**

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 an anticancer therapy. In this set of experiments, we will determine the prevalence and relevance of PRMT5 expression in a large set of lymphoma biopsy samples collected from affected dogs. We will then characterize the molecular and cellular consequences of changes in PRMT5 expression in cancer cells *in vitro*. Finally, we will test the ability of new PRMT5 inhibitor drugs to kill canine lymphoma cells, testing these drugs both on dog lymphoma cell lines and on cell lines directly from affected dogs.

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**PROJECT TITLE: SAFETY AND EFFICACY OF PLATELET-MIMICKING NANOPARTICLES TO REDUCE BLEEDING TIME IN DOGS**

*RELEVANT TITLE: SAFTEY AND EFFICACY OF NANOPARTICLES TO HELP REDUCE HEMORRHAGE IN DOGS*

**INVESTIGATORS: J. GUILLAUMIN**

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. This 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. We will also look for a reduction of bleeding time in normal dogs, which we have shown before in mice. These findings in dogs cannot only lead to a synthetic platelet applicable veterinary medicine, but may subsequently revolutionize platelet transfusions in humans.

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**PROJECT TITLE: PERFUSION INDEX AS A NON-INVASIVE TOOL TO DETERMINE EPIDURAL ANESTHESIA EFFECTIVENESS IN DOGS**

*RELEVANT TITLE: PERFUSION INDEX AS A NON-INVASIVE TOOL TO DETERMINE EFFECTIVENESS OF ANESTHESIA IN THE SPINE*

**INVESTIGATORS: C. RICCO PEREIRA**

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 is to evaluate PI as a non-invasive method to determine epidural anesthesia onset and effectiveness in dogs. Dogs will be anesthetized and epidural injections using two different drug combinations will be performed. Perfusion index will be recorded before and thereafter. During surgery, the dog's reaction to the surgical stimulation will be recorded and additional analgesia will be provided if needed. We expect the PI will increase after epidural anesthesia due to changes in vascular tone. the results of this study will allow us to better predict if an epidural anesthesia is effective before surgery starts. Future applications include studying other regional anesthesia techniques and drug combinations, and conditions that result vascular tone changes (pain, shock, intravascular volume replacement).

**PROJECT TITLE: TILMANOCEPT AS A CANINE CANCER THERANOSTIC**

*RELEVANT TITLE: TILMANOCEPT AS A BIOLOGICAL CANCER DIAGNOSTIC THERAPY FOR DOGS WITH CANCER*

**INVESTIGATORS: T. ROSOL**

Tumor associated macrophages (TAMs) are an important immune cell in the tumor microenvironment and have been shown to contribute to tumor growth and metastasis. In humans, TAMs have been identified in multiple tumor types and increased TAM density is frequently associated with a poor prognosis. Recent work suggests that TAMs are also present in canine cancers and may represent a novel therapeutic target. In partnership with Navidea, we have a unique opportunity to study TAMS in canine cancer. We propose an in vitro and vivo proof-of-concept study that would use a novel mannose-receptor binding radiopharmaceutical agent, Tilmanocept, to identify TAMs in canine tumor biopsies (via immunohistochemistry) as well as dogs with spontaneously occurring soft tissue sarcomas

(via nuclear scintigraphy). Study data will be further used to further develop Tilmanocept as a backbone for novel canine cancer imaging and therapeutic agents.

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## FUNDING OF PROJECTS

*Final Reports*

**"Prospective and retrospective studies analyzing the epidemiology and evolution of Methicillin-Resistant *Staphylococcus Pseudintermedius* (MRSP) in a veterinary teaching hospital"** **$28,506**
*Relevant Title: "The incidence and evolution of staph resistance in a veterinary hospital"*

**"Vitamin D metabolics, parathyroid hormone and the fibroblast growth factor-23 – klotho axis in dogs with various stages of chronic kidney diseases"** **$28,350**
*Relevant Title: "Nutritional and hormonal factors in dogs with kidney disease"*

**"Seroreactivity of outer membrane proteins of *Neorickettsia helminthoeca"*** **$31,304**
*Relevant Title: "Detection of neorickettsia in dog blood"*

**Right ventricular function in dogs with myxomatous mitral valve Disease"** **$ 8,191**
*Relevant Title: "Cardiac function in dogs with heart disease"*

**"Effect of *A. muciniphila* administration on GLP-2 and intestinal hyper-permeability in dogs with IBD"** **$ 8,108**
*Relevant Title: "Therapeutic effects of a probiotic on the intestines of dogs with inflammatory bowel disease"*

**"Impact of the therapeutic application of carbapenems for the treatment of canine urinary tract infections"** **$15,637**
*Relevant Title: "The impact of carbapenem antibiotics on treatment of bladder infections in dogs"*

**"In vitro changes to canine packed red blood cell concentrates following irradiation and storage"** **$ 8,180**
*Relevant Title: "Effect of radiation and storage on red blood cell concentrations from dogs"*

**"Quantification of airflow resistance by computed tomography in** **$ 7,368**

**brachycephalic dogs before and after surgery"**
*Relevant Title: "Use of CT scans to measure respiration in short nosed dogs before and after surgery"*

**"Comparison of bolus administration of hypertonic saline colloid and hypertonic saline-colloid combination on isoflurane-induced hypotension in dogs"** **$19,843**
*Relevant Title: "Comparison of a single dose high salt colloid versus a high salt colloid combination on anesthesia-induced low blood pressure"*

**"Expression and function of MCT1 and MCT4 in canine osteosarcoma and melanoma"** **$22,691**
*Relevant Title: "Characterizing the expression and function of the transporters MCT1 and MCT2 in dogs bone cancer and melanoma"*

*Interim Reports*

**"The health of dogs on livestock farms: husbandry, infection control and pathogen colonization"** **$28,610**
*Relevant Title: "Pathogen control in working farm dogs"*

"**Decoding the genomic architecture of canine lunch cancer"** **$22,723**
*Relevant Title: "Genome analysis of canine lung cancer"*

**"*Clostridium difficile* in dogs: risk factors for colonization, infection and owner transmission"** **$22,727**
*Relevant Title: "C. diff in dogs: risk factors for infection and transmission to owners"*

**"A novel analgesic drug, Tapentadol, for the treatment of pain in dogs with clinical osteoarthritis"** **$20,281**
*Relevant Title: "Tapentadol, a new drug for treating arthritis pain in dogs"*

**"Characterizing the role of W wox deficiency in canine osteosarcoma"** **$22,500**
*Relevant Title: "Characterizing the role of the tumor suppressor gene Wwox in dog bone cancer"*

**"Low-level laser therapy as an adjunctive treatment for canine Acral lick dermatitis: a randomized, double-blinded sham-controlled study"** **$ 7,752**
*Relevant Title: "Low level laser therapy as an additional treatment for dermatitis in dogs"*

**"Diagnosing and managing neuropathic pain in dogs with spinal cord injury"** **$12,545**
*Relevant Title: "Diagnosing and managing nerve pain in dogs with spinal cord injury"*

**Microsensitivity of freeze thaw cycled canine plasma"** **$ 2,740**
*Relevant Title: "Microsensitivity of dog plasma that has undergone multiple freeze-thaw cycles"*

New Projects Funded in 2016

**"Comparison of preoperative analgesic protocols and evaluation of chronic neuropathic pain state in dogs undergoing TPLO"** **$22,645**
*Relevant Title: "Comparison of pre-surgery anesthesia protocols and evaluation of nerve pain in dogs after knee ligament stabilization surgery"*

**"Validation of PRMT5 as a candidate therapeutic target in canine lymphoma"** **$21,484**
*Relevant Title: "Validation of the cancer associate gene PRMT5 as a target for treatment"*

**"Safety and efficacy of platelet-mimicking nanoparticles to reduce bleeding time in dogs"** **$22,549**
*Relevant Title: "Safety and efficacy of nanoparticles to help reduce hemorrhage in dogs"*

**"Perfusion index as a non-invasive tool to determine epidural anesthesia effectiveness in dogs"** **$11,588**
*Relevant Title: "Perfusion index as a non-invasive tool to determine effectiveness of anesthesia in the spine"*

**"Tilmanocept as a canine cancer theranostic"** **$22,500**
*Relevant Title: "Tilmanocept as a biological cancer diagnostic therapy for dogs with cancer"*

# APPENDICES

## i. COUNTY PAYMENTS

## ii. INTRAMURAL GRANT APPLICATION TEMPLATE

# 2017 County Dog Tag & Kennel Registration

| | | | Sold | | | | |
|---|---|---|---|---|---|---|---|
| County | Invoice | Amt Paid $ | 1 - YR | 3 - YR | PERMNT | Dangerous Dogs | KENNEL REG |
| Adams County Auditor | 1 | $946.10 | 9,031 | 83 | 9 | 0 | 91 |
| Allen County Auditor | 1 | $1,676.80 | 16,153 | 140 | 8 | 0 | 115 |
| Ashland County Auditor | 1 | $902.10 | 8,639 | 93 | 3 | 0 | 73 |
| Ashtabula County Auditor | 1 | $1,039.00 | 9,697 | 147 | 24 | 0 | 12 |
| Athens County Auditor | 1 | $941.90 | 9,108 | 20 | 2 | 0 | 231 |
| Auglaize County Auditor | 1 | $856.20 | 8,054 | 126 | 13 | 0 | included in other #s |
| Belmont County Auditor | 1 | $1,026.40 | 9,340 | 213 | 23 | 0 | 55 |
| Brown County Auditor | 1 | $1,208.00 | 11,230 | 106 | 11 | 0 | 422 |
| Butler County Auditor** | | | | | | | |
| Carroll County Auditor | 1 | $761.40 | 7,312 | 22 | 2 | 0 | 216 |
| Champaign County Auditor | 1 | $780.60 | 7,654 | 35 | 3 | 0 | 17 |
| Clark County Auditor | 1 | $2,237.00 | 21,634 | 203 | 8 | 0 | 47 |
| Clermont County Auditor | 1 | $1,831.40 | | | | | |
| Clinton County Auditor | 1 | $914.60 | | | | | |
| Columbiana County Auditor | | | | | | | |
| Coshocton County Auditor | 1 | $929.40 | 8,496 | 14 | 7 | 0 | 686 |
| Crawford County Auditor | 1 | $825.70 | 7,894 | 77 | 9 | 0 | 42 |
| Cuyahoga County Auditor** | 1 | $6,977.30 | | | | | |
| Darke County Auditor | 1 | $1,176.20 | 11,514 | 43 | 11 | 0 | 9 |
| Defiance County Auditor | 1 | $755.60 | 7,168 | 73 | 10 | 0 | 69 |
| Delaware County Auditor | 1 | $2,000.50 | | | | | |
| Erie County Auditor | 1 | $1,378.00 | 13,700 | 0 | 0 | 0 | 80 |
| Fairfield County Auditor** | 1 | $2,416.50 | | | | | |
| Fayette County Auditor | 1 | $420.30 | 4,048 | 45 | 1 | 0 | 10 |
| Franklin County Auditor | 1 | $10,239.50 | 85,312 | 4,066 | 486 | 0 | 25 |
| Fulton County Auditor | 1 | $755.90 | 7,142 | 88 | 4 | 0 | 113 |
| Gallia County Auditor | 1 | $247.80 | 2,317 | 13 | 3 | 0 | 92 |
| Geauga County Auditor | 1 | $1,227.90 | | | | | |
| Greene County Auditor | 1 | $2,736.00 | 24,995 | 567 | 61 | 0 | 54 |
| Guernsey County Auditor | 1 | $648.20 | 6,395 | 9 | 1 | 0 | 50 |
| Hamilton County Auditor | | | | | | | |
| Hancock County Auditor | 1 | $1,211.50 | 12,102 | 0 | 0 | 0 | 13 |
| Hardin County Auditor | 1 | $703.60 | 6,973 | 6 | 3 | 0 | 15 |
| Harrison County Auditor | 1 | $289.20 | | | | | |
| Henry County Auditor | 1 | $631.60 | 6,061 | 54 | 7 | 0 | 23 |
| Highland County Auditor | 1 | $443.60 | | | | | |
| Hocking County Auditor | 1 | $499.20 | 4,877 | 20 | 1 | 0 | 45 |
| Holmes County Auditor | | | | | | | |
| Huron County Auditor | 1 | $1,116.10 | 10,868 | 64 | 8 | 0 | 21 |
| Jackson County Auditor | 1 | $688.20 | 6,579 | 27 | 5 | 0 | 172 |
| Jefferson County Auditor | 1 | $503.60 | | | | | |
| Knox County Auditor | 1 | $986.90 | 9,394 | 103 | 76 | 0 | 9 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lake County Auditor | 1 | $2,884.20 | 27,154 | 377 | 45 | 0 | 107 |
| Lawrence County Auditor | 1 | $942.00 | 9,173 | 34 | 14 | 0 | 5 |
| Licking County Auditor | 1 | $3,078.40 | 30,725 | 4 | 2 | 0 | 27 |
| Logan County Auditor | 1 | $606.20 | 5,935 | 32 | 2 | 0 | 11 |
| Lorain County Auditor | 1 | $2,718.00 | 25,833 | 334 | 16 | 0 | 185 |
| Lucas County Auditor | | | | | | | |
| Madison County Auditor | 1 | $644.20 | 6,133 | 62 | 12 | 0 | 3 |
| Mahoning County Auditor | 1 | $3,080.00 | 29,180 | 300 | 32 | 0 | 400 |
| Marion County Auditor | 1 | $878.70 | 8,324 | 87 | 10 | 0 | 102 |
| Medina County Auditor | 1 | $2,502.30 | 21,513 | 743 | 118 | 0 | 101 |
| Meigs County Auditor | 1 | $219.80 | 2,120 | 7 | 3 | 0 | 27 |
| Mercer County Auditor | 1 | $447.30 | 4,328 | 16 | 2 | 0 | 77 |
| Miami County Auditor | 1 | $1,901.40 | 17,430 | 330 | 57 | 0 | 24 |
| Monroe County Auditor | 1 | $376.20 | 3,762 | 0 | 1 | 0 | 275 |
| Montgomery County Auditor | 1 | $6,511.60 | 64,757 | 61 | 12 | 0 | 56 |
| Morgan County Auditor | 1 | $284.30 | 2,523 | 51 | 14 | 0 | 27 |
| Morrow County Auditor | 1 | $567.90 | 5,360 | 82 | 6 | 0 | 13 |
| Muskingum County Auditor | 1 | $1,374.00 | 13,458 | 41 | 11 | 0 | 209 |
| Noble County Auditor | 1 | $183.10 | 1,637 | 22 | 4 | 0 | 88 |
| Ottawa County Auditor | 1 | $819.20 | 7,659 | 124 | 13 | 0 | 31 |
| Paulding County Auditor | 1 | $350.20 | | | | | |
| Perry County Auditor | 1 | $709.40 | | | | | |
| Pickaway County Auditor** | | | | | | | |
| Pike County Auditor** | 1 | $257.50 | | | | | |
| Portage County Auditor | 1 | $3,097.00 | | | | | |
| Preble County Auditor | 1 | $342.80 | | | | | |
| Putnam County Auditor | 1 | $659.80 | 6,045 | 7 | 0 | 0 | 532 |
| Richland County Auditor | 1 | $2,135.50 | 18,775 | 0 | 1 | 0 | 2,570 |
| Ross County Auditor | 1 | $1,545.30 | 15,304 | 4 | 0 | 0 | 137 |
| Sandusky County Auditor | 1 | $1,232.00 | 11,920 | 69 | 6 | 0 | 126 |
| Scioto County Auditor | 1 | $379.80 | 2,217 | 27 | 6 | 0 | 1,440 |
| Seneca County Auditor | 1 | $1,342.20 | 10,261 | 107 | 8 | 0 | 276 |
| Shelby County Auditor | 1 | $871.80 | 8,377 | 71 | 3 | 0 | 98 |
| Stark County Auditor | 1 | $4,875.20 | 45,217 | 821 | 104 | 0 | 32 |
| Summit County Auditor | 1 | $4,173.50 | | | | | |
| Trumbull County Auditor | 1 | $1,860.10 | 17,314 | 267 | 43 | 0 | 56 |
| Tuscarawas County Auditor | 1 | $1,505.50 | 14,598 | 78 | 16 | 0 | 63 |
| Union County Auditor | 1 | $779.70 | | | | | |
| Van Wert County Auditor | 1 | $538.40 | 3,981 | 1 | 0 | 2 | 14 |
| Vinton County Auditor | 1 | $209.80 | 1,509 | 3 | 0 | 0 | 580 |
| Warren County Auditor | 1 | $2,805.30 | 25,153 | 718 | 71 | 0 | 36 |
| Washington County Auditor** | 1 | $1,143.60 | 11,209 | 49 | 4 | 0 | 40 |
| Wayne County Auditor | 1 | $2,116.30 | 20,435 | 112 | 16 | 0 | 232 |
| Williams County Auditor | 1 | $522.10 | | | | | |
| Wood County Auditor | 1 | $2,159.20 | 19,423 | 475 | 64 | 0 | 104 |
| Wyandot County Auditor | 1 | $433.10 | 4,001 | 77 | 4 | 0 | 59 |
| | | | | | | | |
| | **Total:** | **$120,993.70** | **876,430** | **12,050** | **1,519** | **2** | **10,970** |
| **NOTES: | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Invoice 1 sent out 1/30/17 | | | | | | | |
| Invoice 2 sent out 3/15/17 | | | | | | | |
| Invoice 3 sent out 4/27/17 | | | | | | | |
| Payment still not received-third invoice mailed 4/27/17 | | | | | | | |
| Breakdown of tags sold not provided | | | | | | | |
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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. VERTEBRATE ANIMALS ☐ No ☐ Yes

4a. Research Exempt
☐ No ☐ Yes

If "Yes," Exemption No.

5a. Hospital Executive Board Approval and Date

5b. ILACUC Approval Number and Date

| 6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)* | | 7. COSTS REQUESTED FOR FIRST YEAR | 8. COSTS REQUESTED FOR TOTAL PERIOD OF SUPPORT |
|---|---|---|---|
| From | Through | 7a. Direct Costs ($) | 8a. Direct Costs ($) |

9. Checklist:

- ☐ Page 1 (*Form - Cover Page*)
- ☐ Page 2 *(Form – Technical & Lay Abstracts and Personnel)*
- ☐ Page 3 (*Form - First year Budget*)
- ☐ Page 4 (*Form – Total Budget and Justification*)
- ☐ Page 5 (*Form - Resources*)
- ☐ Begin Page 6 – Response to Reviewer Criticism (Form Pages-2 page limit)
- ☐ Research Plan (*Sections A through F – 8 page limit)*
- ☐ Previous Intramural Funding and Investigator Information (*Sections H and I*)
- ☐ Letter(s) of Cooperation
- ☐ Curriculum Vitae *(use 4 page NIH biosketch for 2 pg CV + 2 pg Other Support)*
- ☐ Check if appendices are included
- ☐ Packet contains Original and 3 copies turned into the College Research Office
- ☐ ILACUC or Hospital Executive Committee approval/client consent form
- ☐ Submitted electronic version to Morscher.1@osu.edu

| | | |
|---|---|---|
| 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 with out compelling justification (see Budget page and justification)

| Name | Department | Time Commitment to Project | Signature |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

Principal Investigator (Last, First, Middle):

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

| FROM | THROUGH |
|---|---|
| | |

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

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

EQUIPMENT *(Itemize and provide justification below)*

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

VMC SUPPLIES & SERVICES *(Itemize costs to be charged to the Veterinary Teaching Hospital)*

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*).



# 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 4 page NIH Biosketch form [2 pg CV, 2 pg Other Support]*) NIH Website: https://grants.nih.gov/grants/funding/phs398/biosketch.pdf example: https://grants.nih.gov/grants/funding/phs398/biosketchsample.pdf

**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.*