



AMERICAN KENNEL CLUB®  
**CANINE HEALTH  
FOUNDATION®**  
PREVENT TREAT & CURE®

# 2022 Research Grants Portfolio

Volume 2

Dear Fellow Dog Lover,

Since 1995, CHF has addressed the important health needs of our closest companions. With the support of our donors, CHF's mission to improve the health and well-being of dogs is being met through funding of peer-reviewed scientific research, educational programs, and outreach. Within these pages are the active research grants being funded through the AKC Canine Health Foundation (CHF).

This electronic issue of the CHF Research Grants Portfolio represents active research projects categorized by research program areas specifically selected to advance the Foundation's mission for healthier dogs. Each grant proposal is reviewed through CHF's rigorous peer review process including the CHF Scientific Review Committee ([akcCHF.org/Scientific-Review-Committee](http://akcCHF.org/Scientific-Review-Committee)) and with subject-matter and clinical experts from across the scientific and veterinary community. Each grant is assessed for scientific merit, impact in the field of study, and significance to dogs and their people. CHF embraces the concept of One Health where both dogs and people can benefit from scientific advancements. The peer-review process ensures the contributions of our donors will be directed to studies that have the greatest potential benefit for dogs.

You will find the study abstracts listed on the following pages represent cutting-edge research as well as applied clinical studies chosen to improve the lives of dogs and contribute to our understanding of complex disease processes, including genetics. The research program areas funded by CHF encompass a broad range of health concerns, including issues that affect all dogs as well as those that affect specific breeds and body systems. Through defined research program areas CHF applies recent advancements in science and technology to address unmet canine health needs and take advantage of areas with the opportunity for immediate impact.

From the CHF Board of Directors and Staff, and from the dogs whose lives are positively impacted by this work, we thank you for your interest, passion, and generosity.

Sincerely,



Andrea Fiumefreddo, MS  
Vice President of Programs & Operations



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# Behavior

02700

One  
Health

## Longitudinal Comparison of Cognitive and Emotional Development in Assistance Dog Puppies

**Principal Investigator:** Brian Hare, PhD; Duke University

**Total Grant Amount:** \$107,880

**Grant Period:** 4/1/2020 - 3/31/2024

**Project Abstract:** A revolution in our understanding of dog cognition has occurred in the past decade, with previous work by the Hare research group linking individual differences in cognition to working dog performance in adults. However, there has yet to be a large-scale longitudinal study tracking the course of cognitive development in any breed of puppies, and limited understanding of how different rearing strategies influence the development of canine cognition. This study will characterize the development of the cognitive traits that this team's previous work has shown predicts a dog's ability to succeed in assistance dog training. Working with the Canine Companions for Independence (CCI), cognitive traits will be characterized using a longitudinal design during the critical period of brain development from 8-20 weeks of age. Next, to test for the influence of different but common service dog rearing strategies on these skills, the investigators will test individual CCI puppies reared in human homes or together with same age peers on a college campus. In studying the cognitive abilities of service dogs, a better understanding of what psychological mechanism(s) successful service dogs rely on or are constrained by when helping humans will be detailed. This information can be used to better predict which puppies will be successful service dogs – improving the success of training while increasing the potential number of service dogs available. These findings will also provide the first set of baseline data on normal cognitive development in dogs as it relates to success in training programs and socialization strategies.

## Blood Disorders

### **Resolving the Major Dyslipidemia Phenotypes and Genetic Risk Factors for Familial Hyperlipidemia in Miniature Schnauzers**

**Principal Investigator:** Eva Furrow, VMD, PhD; University of Minnesota

**Total Grant Amount:** \$98,404

**Grant Period:** 4/1/2020 - 9/30/2022

**Project Abstract:** Familial hyperlipidemia reportedly afflicts more than 75% of Miniature Schnauzers by 10 years of age. This condition is defined as too much circulating lipid (fat) in the bloodstream. Familial hyperlipidemia predisposes dogs to other serious diseases which impact their health and wellbeing. Management of familial hyperlipidemia is hampered by gaps in the understanding of its metabolic and genetic origin. Investigators aim to uncover the metabolic and genetic causes of hyperlipidemia in Miniature Schnauzers. Preliminary data revealed evidence for more than one type of familial hyperlipidemia within the breed. In this study, metabolomics/lipidomics will be used to identify and quantify over 2000 substances in the blood related to metabolism of lipids and other nutrients, classifying them into types of familial hyperlipidemia. Whole genome sequencing will be used to generate a list of hyperlipidemia mutations that may contribute to one or more types of familial hyperlipidemia. Identifying the different types of familial hyperlipidemia and their respective genetic risk factors in Miniature Schnauzers will lead to improved understanding and treatment of the condition, with individualized therapies and genetic tests used to identify at risk dogs that will benefit from early intervention and monitoring.



## Plasminogen-activator Inhibitor-1 (PAI-1) and Impaired Fibrinolysis in Immune-Mediated Hemolytic Anemia

**Principal Investigator:** Tracy Stokol, BVSc, PhD and Steven Friedenberg, DVM, PhD; Cornell University and University of Minnesota

**Total Grant Amount:** \$116,865

**Grant Period:** 1/1/2022 - 12/31/2023

**Project Abstract:** Immune-mediated hemolytic anemia (IMHA) is a common, and often life-threatening, blood disorder in dogs where the dog's immune system attacks its own red blood cells, leading to a severe anemia that is treated with immunosuppressive drugs. However, affected dogs suffer from more than just anemia. They also have over-active clotting systems that lead to abnormal blood clot formation. These blood clots can be fatal if they block off the blood supply and delivery of nutrients and oxygen to vital tissues, causing serious organ damage and failure. Veterinarians now treat dogs with blood thinners to try and prevent these clots from forming, but dogs with IMHA continue to suffer from lethal blood clots, indicating that a more effective therapy is needed.

When clots form in the body, they are gradually broken down by enzymes – this normal process is called fibrinolysis. If clots are not broken down properly, they will persist in the blood vessels, causing tissue damage. Researchers suspect that clot breakdown is defective in dogs with IMHA, leading to persistence of blood clots. They believe the decreased fibrinolysis is caused by too much of a blood protein, called PAI-1. PAI-1 protein is the main inhibitor of clot breakdown and if it is too high, clots remain in blood vessels and prevent normal blood flow. Preliminary studies have also shown decreased clot breakdown in blood samples from dogs with IMHA.

In this study, investigators will determine whether dogs with IMHA have high levels of active PAI-1 protein as a major cause of reduced clot breakdown. They will collect blood samples from 40 dogs with IMHA and 40 healthy control dogs and measure PAI-1 protein activity levels and mRNA levels, and perform laboratory tests of clot breakdown. They will also test whether a drug that blocks PAI-1 activity can improve fibrinolysis in these test samples since blood-thinning drugs currently given to dogs with IMHA to prevent clot formation do not affect clot breakdown at all. If investigators find that high PAI-1 levels result in reduced clot breakdown in dogs with IMHA, then PAI-1 inhibitor drugs will open up new possibilities for more effective treatment and improve current IMHA treatment so that abnormal blood clot formation no longer limits dogs' survival.

## Cardiology

### Use of Gene Therapy to Treat Dilated Cardiomyopathy

**Principal Investigator:** Margaret Sleeper, VMD; University of Florida

**Total Grant Amount:** \$146,774

**Grant Period:** 9/1/2016 - 2/28/2023

**Project Abstract:** Dilated cardiomyopathy (DCM) is the second most common cause of heart disease in dogs, and medical management of the secondary signs is the only therapeutic option. The outcome for affected dogs depends on the stage of disease and the breed. Once diagnosed, dogs typically exhibit rapid and uniform progression to congestive heart failure (CHF), with most living less than 6 months. Previous research has shown that heart function is critically dependent upon calcium channel function. These gate-like channels found within the wall of cardiac muscle cells open and close, allowing calcium ions to flow into the cell. Calcium influx then regulates muscle contraction. Heart disease is strongly associated with malfunctioning calcium channels within cardiac cells. Gene transfer strategies to reduce calcium cycling abnormalities improve heart function in animal models as well as in human clinical trials. In this study, Dr. Sleeper will conduct a placebo-controlled, double blinded study to evaluate gene delivery approaches for treatment of Doberman Pinschers affected with DCM and CHF. If results show that the gene delivery slows progression of heart failure in Dobermans with DCM, the results will have significant ramifications for all dogs with heart disease, as calcium handling proteins are abnormally expressed in dogs with heart disease of varying causes.

## The Role of *Bartonella* spp. Exposure and Cardiac Genetic Variation on the Clinical Expression of Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer Dog

**Principal Investigator:** Edward Breitschwerdt, DVM and Kathryn Meurs, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$63,105

**Grant Period:** 2/1/2019 - 7/31/2022

**Project Abstract:** Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in the Boxer dog is an adult onset, familial disease characterized by the presence of ventricular arrhythmias, fainting and sudden death. The investigators have identified a causative mutation in the cardiac Striatin gene that is highly associated with the development of Boxer ARVC, and have demonstrated that some Boxer dogs with the mutation have a more severe form of the disease and will become quite sick while others will remain free of clinical signs. The reason for the variability in clinical signs is unknown but is thought to be associated with concurrent factors for an individual dog which could include a role for chronic infections, as well as genetics, hormonal levels, or other external factors including diet or exercise. The range of disease manifestation of *Bartonella* infection in dogs is broad, but has been shown to infiltrate the heart muscle, and has also been identified in human beings with ARVC. The investigators hypothesize that chronic *Bartonella* spp. infection may lead to the development of a more severe form of Boxer ARVC. Understanding the role of this, and other infectious diseases, in the severity of ARVC may greatly improve the ability to manage this common and sometimes fatal heart disease.



## **Circulating Cortisol Concentrations in Canine Congestive Heart Failure**

**Principal Investigator:** Jessica Ward, DVM; Iowa State University

**Total Grant Amount:** \$35,474

**Grant Period:** 12/1/2019 - 11/30/2022

**Project Abstract:** Congestive heart failure (CHF) is a common disease in dogs. A major contributor to disease progression is the renin-angiotensin-aldosterone system (RAAS), whose end-product aldosterone binds to mineralocorticoid receptors (MRs) and causes negative effects on the heart and blood vessels. RAAS activation is associated with a worse prognosis in humans and dogs with CHF. The stress hormone cortisol can also bind MRs. In healthy individuals, cortisol occupies the MR without activating it, while in disease states, bound cortisol can activate MRs just like aldosterone. In people with CHF, higher blood cortisol levels are associated with a higher risk of death. However, in the subset of patients treated with drugs that block MRs, cortisol is not associated with outcome. These findings suggest that the benefit of MR-blocking drugs may have more to do with blocking cortisol than with blocking aldosterone. The role of cortisol in dogs with CHF remains unknown. The purpose of this study is to determine the prognostic value of blood cortisol in dogs with CHF. Results of this study will help veterinarians better predict outcome for dogs with CHF and may suggest that MR-blocking drugs are indicated in treatment.

## **Canine Chagas Disease: Characterizing Cardiac Disease and Developing Screening Recommendations for Asymptomatic Dogs Seropositive for *Trypanosoma cruzi***

**Principal Investigator:** Ashley Saunders, DVM; Texas A&M AgriLife Research

**Total Grant Amount:** \$65,691

**Grant Period:** 6/1/2019 - 10/31/2022

**Project Abstract:** Chagas disease (Trypanosomiasis) is caused by a parasite that infects the heart of humans and dogs in the United States causing heart disease and acute death. It is transmitted by kissing bugs, and there is no vaccination or approved treatment. Dogs in the Southern U.S. have a higher risk of parasite infection, and while all dog breeds can be affected, non-sporting, toy and herding breed groups are over-represented. Currently, there is only one commercially available test for Chagas disease in dogs and not all dogs with a positive test will develop clinical signs of disease. Characterization of the heart disease and recommendations for screening naturally infected dogs do not exist and veterinarians and owners are forced to make decisions about the health of their dog based on the currently available test results. This study will evaluate asymptomatic dogs with a positive Chagas test to characterize the presence of heart disease using electrocardiography ultrasound of the heart, and cardiac troponin I, a non-invasive biomarker of heart injury. Additionally, other Chagas tests will be evaluated as potential additional tests to confirm Chagas disease. Results will provide useful information to help owners and veterinarians screening dogs for evidence of heart problems associated with Chagas disease and will expand knowledge of the natural history of this disease in dogs.

## Three-dimensional Echocardiographic Determinants of the Age of Onset of Myxomatous Mitral Valve Disease in Cavalier King Charles Spaniels

**Principal Investigator:** Michele Borgarelli, DVM, PhD; Virginia-Maryland College of Veterinary Medicine

**Total Grant Amount:** \$149,657

**Grant Period:** 5/1/2019 - 10/31/2022

**Project Abstract:** Myxomatous mitral valve disease (MMVD) represents a major health issue in Cavalier King Charles Spaniels (CKCSs). The disease appears at an earlier age, compared to other breeds, and has a genetic, heritable basis. The cause of the disease is unknown, but the role of altered stresses on the mitral valve (MV) has been proposed as one of the triggers for developing the disease. Three-dimensional echocardiography (RT-3DTTE) is a non-invasive technique that allows for characterization of the morphology of the MV. The investigator's preliminary studies using RT-3DTTE found that some CKCSs have a MV of a different shape compared to other breeds. This altered shape of the MV could impose abnormal forces on the valve and predispose the breed for early development of the disease. This study will determine whether the shape of the MV is linked to the age of onset of MMVD in CKCSs, and, if so, the shape of the MV could be used as a screening method, and for directing breeding decisions in order to lower the prevalence of MMVD in CKCSs.

## Investigation into Diet-Associated Dilated Cardiomyopathy in Dogs

**Principal Investigator:** Darcy Adin, DVM; University of Florida

**Co-Investigators:** Lisa Freeman, DVM, PhD and John Rush, DVM, MS, Tufts University; Rebecca Stepien, DVM, MS, University of Wisconsin, Madison; Amara Estrada, DVM and Margaret Sleeper, VMD, University of Florida; Joshua Stern, DVM, PhD, University of California, Davis

**Total Grant Amount:** \$211,521

**Grant Period:** 4/1/2019 - 9/30/2022

**Project Abstract:** Dilated cardiomyopathy (DCM) is a serious disease of the heart muscle whereby the heart becomes enlarged with weak contractions. DCM can result in abnormal heart rhythms, congestive heart failure or sudden death. In dogs, DCM most often occurs in large- and giant-breeds, such as Doberman Pinschers, Boxers, Irish Wolfhounds, and Great Danes; in these dogs, survival time after diagnosis is often only months, even with aggressive medical therapy. Recently, veterinary cardiologists have recognized DCM more frequently in all breeds of dogs including mixed breeds, and even those not usually associated with DCM. There is suspicion that the disease in some dogs is associated with boutique, exotic ingredient, or grain-free (BEG) diets. Some affected dogs on such diets have shown reversal or improvement of their disease after changing their diet, supporting a potential association between consumption of a BEG diet and development of DCM. A specific cause, however, has not been identified, despite extensive nutritional testing of the dog foods and the canine patients. Moreover, the extent of the problem is unknown because only dogs that are symptomatic for DCM have been reported. It is possible that more dogs may be affected but not yet showing signs of heart disease. To investigate the extent of diet-associated heart problems in dogs, this multi-institutional team of veterinary cardiologists and nutritionists will prospectively screen a large population of apparently healthy dogs for DCM and compare important cardiac disease measures, including ultrasound of the heart, blood biomarker and taurine concentrations, and the frequency of DCM in dogs eating BEG versus non-BEG diets.

## Identification of a Second Genetic Risk Allele(s) Associated with the Development of Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer Dog

**Principal Investigator:** Kathryn Meurs, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$115,474

**Grant Period:** 5/1/2020 - 4/30/2022

**Project Abstract:** Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited heart disease diagnosed most commonly in the Boxer dog. Investigators previously identified the first known causative mutation for ARVC in the dog in the striatin gene. However, this mutation does not explain all affected canine cases. As in the human form of ARVC, there appears to be more than one genetic cause of this disease in the Boxer dog. To identify a second causative variant, DNA samples from Boxer dogs with confirmed Boxer ARVC that are negative for the striatin mutation will be whole genome sequenced to find a second genetic variant that is causative for the development of ARVC in the Boxer dog. The investigators previously used a similar approach to successfully identify a second causative mutation for dilated cardiomyopathy in the Doberman Pinscher. Ultimately, this information will improve understanding of the pathophysiology of ARVC, help improve treatment modalities, and provide information to develop a strategy to gradually reduce the prevalence of the additional variant and this disease.

*Funding for the research is provided through the collaborative efforts and generosity of the American Boxer Charitable Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## **Towards Precision Medicine for Canine Cardiac Disease: A Genomic and Machine-Learning Approach to Prediction of Risk and Outcomes in Canine Myxomatous Mitral Valve Disease**

**Principal Investigator:** Lucy Davison, VetMB, PhD; Royal Veterinary College, University of London

**Total Grant Amount:** \$107,922

**Grant Period:** 10/1/2021 - 9/30/2023

**Project Abstract:** Myxomatous mitral valve disease (MMVD) is the most common cause of heart disease in adult dogs, affecting millions of dogs worldwide. Small- and medium-sized dogs, especially Cavalier King Charles Spaniels, Dachshunds, Poodles, and Yorkshire Terriers, are predisposed to MMVD, suggesting that the disease has a genetic basis. MMVD typically progresses slowly, over several years, eventually resulting in heart failure in approximately half of affected dogs. Treatment in most cases is directed at reducing the impact and clinical signs of heart failure in an attempt to extend lifespan. There is need for better and more specific MMVD treatments, ideally to prevent the disease progressing to heart failure. Understanding why some dogs develop heart failure with MMVD and others do not, would make it easier to identify dogs who would benefit from closer monitoring or preventative treatment. Using a technology called 'whole genome sequencing' to study approximately 200 dogs, investigators have already identified 500 genetic variants that are more common in dogs or breeds with MMVD and/or heart failure. The research team will now study these variants in a larger number of dogs (240 dogs with MMVD and 240 dogs without MMVD) using archived clinical samples. Investigators plan to process this combined clinical and genetic information with machine learning to determine whether they can predict an individual dog's risk of MMVD and heart failure. By the end of the study, investigators aim to use genetic information to identify dogs who would benefit from closer monitoring and early treatment of MMVD with appropriate medication, before they develop heart failure. Studying genetics will also help to understand more about MMVD itself, assisting with the design of better and more specific treatments to slow the progression of this common disease.

## Tolerability and Clinical Efficacy of Acetazolamide for Treatment of Hypochloremia in Canine Congestive Heart Failure

**Principal Investigator:** Darcy Adin, DVM; University of Florida

**Total Grant Amount:** \$99,913

**Grant Period:** 4/1/2022 - 9/30/2023

**Project Abstract:** Congestive heart failure (CHF) is a very common heart condition in older, small breed dogs. Dogs with CHF almost always die of their disease or complications associated with medications used to treat CHF and therefore this is a significant health concern for dogs. Although medications can increase the quality and quantity of life, additional treatment approaches are needed to improve the outcome for affected dogs. Blood chloride levels are often low (hypochloremia) in CHF because of diuretic treatment and this is a marker for advanced CHF in people and in dogs. Hypochloremia also appears to contribute to disease progression and therefore restoration of blood chloride levels is considered a therapeutic target in people. This study seeks to determine if acetazolamide, a chloride-retaining diuretic that is not currently used to treat CHF, can restore blood chloride levels in dogs whose values are low. If this study shows that acetazolamide increases blood chloride levels in dogs with CHF that are treated with standard of care medications, then investigators will plan a long-term study to assess the effect of this intervention on survival outcomes. This study has significant potential to improve the lives of dogs living with CHF.

# Dermatology & Allergic Disease

02694

One  
Health

## **Investigation on the Molecular Crosstalk between Canine Atopic Skin and Microbes: Unraveling Potential Pathomechanisms for Chronic Recurrent Skin Infections**

**Principal Investigator:** Domenico Santoro, DVM, MS, DrSc, PhD; University of Florida

**Total Grant Amount:** \$79,369

**Grant Period:** 5/1/2020 - 10/31/2022

**Project Abstract:** Environmental allergy is extremely common in dogs. Alterations of the skin barrier may lead to an altered inflammatory signal in allergic skin cells that perpetuates the inflammatory response and alters the interaction between skin cells and the external environment. Microorganisms have been shown to be more adherent on allergic compared with healthy skin. This increased adhesion on allergic skin cells has been associated with an increased susceptibility of allergic dogs to skin infections. Furthermore, an altered expression of natural defenses has been shown in allergic compared to healthy canine skin. How the interaction between microorganism and canine skin occurs and what this interaction activates in the host (canine skin) and the microorganisms is incompletely understood. Understanding which genes are activated in the host and the microbes in the early stage of adhesion is fundamental to better design treatments for skin infection in allergic dogs. The investigators will evaluate what occurs in the skin cells of allergic dogs and microbes (bacteria and yeasts) in the first hour of contact to increase our understanding of disease mechanisms in atopic dogs.



## Investigating the Potential of Phage Therapy to Tackle *Staphylococcus pseudintermedius* Infections in Dogs

**Principal Investigator:** Gavin Paterson, PhD; R(D)SVS and Roslin Institute, University of Edinburgh

**Total Grant Amount:** \$99,830

**Grant Period:** 1/1/2021 - 4/30/2022

**Project Abstract:** The spread of antimicrobial resistance is a major threat to modern medicine, for both humans and animals. In the case of dogs, *Staphylococcus pseudintermedius* is an important cause of infections, especially pyoderma. Antimicrobial resistance in *S. pseudintermedius* is making infections more difficult to treat which is affecting dog welfare and might pose a threat to humans too. There is a need to explore alternative treatments to antibiotics with one approach being to use phage therapy. This therapy uses naturally-occurring viruses, called bacteriophages (phages) which infect and kill bacteria to treat bacterial infections. Phage therapy has a long history of safe and effective use in humans and has the advantage that it can target drug resistance bacteria with few side effects. This project has a team of veterinarians and scientists working together to isolate and characterize phages that kill *S. pseudintermedius* which may contribute to the development of new, exciting treatments to benefit dog health and wellbeing.

## **Duration of Antibiotic Therapy for Canine Superficial Pyoderma: Is the One-Week Post Resolution of Clinical Signs a Valid Rule-of-Thumb?**

**Principal Investigator:** Clarissa Souza, DVM, MS, PhD; University of Illinois

**Total Grant Amount:** \$25,354

**Grant Period:** 11/1/2020 - 10/31/2022

**Project Abstract:** Canine superficial bacterial skin infection in dogs is the main presentation leading to antibiotic use in small animal practice. Commonly prescribed duration of treatment for bacterial infections are not evidence-based and it has been anecdotally recommended that all cases of bacterial skin infections take antibiotics for seven days beyond clinical resolution of lesions. Bacterial skin infections often recur. This study will determine if superficial bacterial skin infection recurs sooner in dogs not treated beyond clinical resolution of the lesions. The secondary goals are (i) to determine the average time for a bacterial skin infection to resolve, (ii) to investigate if the clinical severity of infection correlates with the bacterial resistance pattern, and lastly (iii) to compare the susceptibility pattern of the bacteria isolated at the study entrance and at infection recurrence. The study results will establish appropriate recommendations for the treatment of canine superficial bacterial skin infection.

## Transcriptome Profiling of Canine Familial Dermatomyositis Skin Lesions and Treatment with a JAK Inhibitor to Identify Novel Pathways Involved in Pathogenesis

**Principal Investigator:** Frane Banovic, DVM, PhD; University of Georgia

**Total Grant Amount:** \$57,658

**Grant Period:** 4/1/2021 - 3/31/2023

**Project Abstract:** Dermatomyositis (DMS) is a chronic inflammatory and autoimmune disorder affecting primarily skin and muscle in both humans and dogs. Familial canine DMS is mainly described in predisposed breeds, such as Collies and Shetland Sheepdogs, and is characterized by severe inflammatory lesions leading to skin scarring with disfiguration, increased morbidity and decreased quality of life. Full clinical remission of DMS skin lesions can be difficult to achieve, reflecting a poor understanding of the pathogenesis of skin lesions in this disease. The transcriptome (gene expression analysis) investigation of human DMS tissues has revolutionized the understanding of the molecular fingerprint of DMS, further defining pathogenic immune pathways and identifying disease-specific biomarkers. Recently, a novel mechanism-based treatment using Janus kinase (JAK) inhibitors has led to a significant clinical and molecular improvement in refractory cutaneous DMS in humans. Oclacitinib is a safe and well-tolerated JAK inhibitor that has been used for the treatment of allergic dermatitis in dogs; however, the therapeutic effect of oclacitinib on canine immune-mediated diseases, such as DMS, has not been investigated. The main objective of this study is to perform a large-scale molecular signature analysis of canine familial DMS. Investigators hypothesize that integrating deep sequencing-based transcriptome profiling with systems biology analysis will identify novel pathogenic pathways and inflammatory biomarkers as canine DMS disease drivers, with potential for the development of novel targeted therapeutics. Furthermore, they will evaluate the effect of oclacitinib, a novel veterinary JAK inhibitor, on the modulation of the cutaneous DMS clinical signs in dogs. These results may suggest that JAK inhibition has the potential to reverse canine DMS pathomechanisms, opening the door to a new era of targeted treatment for this debilitating inflammatory skin disease.

*Funding for the research is provided through the collaborative efforts and generosity of the Collie Health Foundation and American Shetland Sheepdog Association. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

# Endocrinology

02428

## Identifying the Disease-Defining Autoantibodies in Canine Addison's Disease

**Principal Investigator:** Steven Friedenber, DVM, PhD; University of Minnesota

**Total Grant Amount:** \$181,864

**Grant Period:** 3/1/2018 - 8/31/2022

**Project Abstract:** Addison's disease is a common and life-threatening disorder in dogs in which the body's immune system destroys the outer layer of the adrenal glands. The adrenal glands produce hormones that are critical for energy metabolism, immune system function, intestinal health, and kidney function. Symptoms of Addison's disease can mimic other conditions, and as a result, many dogs remain undiagnosed for years. About one-third of dogs with Addison's disease are diagnosed only after suffering an acute adrenal crisis, which can cause a wide range of complications that require emergency stabilization and hospitalization. Today, there is no way to predict which dogs will develop Addison's disease before they become sick. If such a test were available, veterinarians would be able to evaluate high-risk dogs before they show signs, helping to prevent disease-related complications and potentially enabling earlier treatment. In this study, the investigator will use a novel approach combining gene and protein sequencing to identify the antibodies that target the adrenal glands in Standard Poodles, Portuguese Water Dogs, and English Cocker Spaniels with Addison's disease. These antibodies are produced by the immune system before the onset of clinical signs. The ability to identify these antibodies would therefore provide a test for early diagnosis. This research will contribute to progress in developing an important clinical test for Addison's disease that can help improve the lives of the many dogs at high risk of developing this life-threatening condition.

**Breed Specific Reference Ranges for Canine Thyroid Testing****Principal Investigator:** Brian Petroff, DVM, PhD; Michigan State University**Total Grant Amount:** \$139,975**Grant Period:** 6/1/2019 - 5/31/2023

**Project Abstract:** Thyroid disease is common in dogs with the incidence of hypothyroidism approaching 30% in some breeds. Evaluation of thyroid function currently involves comparison of thyroid hormone concentrations in an individual patient with reference ranges generated from past testing of dogs of many breeds. However, while such all-breed reference ranges are generally accurate, they may not be optimal for some individual breeds, necessitating the generation of breed specific thyroid reference ranges. In thyroid testing specific for four breeds: Irish Setters, Rhodesian Ridgebacks, Borzois and Whippets, dogs will be examined and tested sequentially to insure a healthy cohort for generation of a breed specific reference range for a panel of thyroid function assays. Once completed this study will generate breed specific thyroid testing reference ranges for three dog breeds. This work offers immediate and tangible improvements in canine health by refining thyroid testing interpretation in purebred dogs.

## **Pattern of Thyroid Function Tests during Recovery from Acute Nonthyroidal Illness**

**Principal Investigator:** Timothy Bolton, DVM; Virginia-Maryland Regional College of Veterinary Medicine

**Total Grant Amount:** \$13,792

**Grant Period:** 1/1/2020 - 6/30/2022

**Project Abstract:** Hypothyroidism is the most common endocrine disease in dogs. A diagnosis of hypothyroidism relies on finding both appropriate clinical signs and low thyroid hormone levels. Unfortunately, other illnesses can suppress thyroid hormone levels and result in a misdiagnosis. This phenomenon of low thyroid hormone levels caused by a disease not involving the thyroid gland is known as nonthyroidal illness or euthyroid sick syndrome. It is important to distinguish between nonthyroidal illness and hypothyroidism as the treatment for each is different. Historically, the recommendation for a dog with nonthyroidal illness has been to resolve the underlying disease, followed by a recheck of thyroid hormone levels thereafter. However, the duration of time after resolution of the nonthyroidal illness necessary to perform accurate thyroid hormone level testing is unknown. This study will provide information about thyroid hormone levels during the course of nonthyroidal illness, and also establish the approximate duration of time for recovery of thyroid hormone levels to normal following illness resolution. These results will correlate clinically with more concrete recommendations for thyroid hormone level testing following resolution of nonthyroidal illness.

## Potential Reduction of Systemic and Pancreatic Inflammation using Fenofibrate in Diabetic Dogs

**Principal Investigator:** Allison O'Kell, DVM, MS; University of Florida

**Total Grant Amount:** \$15,000

**Grant Period:** 2/1/2021 - 1/31/2023

**Project Abstract:** Diabetes mellitus (DM) is a common disease in dogs and a cause of significant patient morbidity as well as emotional and financial stress to pet owners. In this study, investigators will examine the effect of fenofibrate on alleviating some of the complications of DM. Fenofibrate is a drug known to decrease blood lipid levels that has also shown promise in laboratory settings to decrease other consequences of DM such as gut barrier dysfunction, systemic inflammation and pancreatitis. Investigators hypothesize that fenofibrate treatment in spontaneous canine DM will reduce systemic inflammation and decrease serum pancreatic lipase activity (a marker of pancreatic inflammation), and positively alter lipoprotein profiles without affecting blood sugar (glycemic) control. The results of this study will be used to design a larger long-term longitudinal clinical trial which will evaluate risk-reduction of pancreatitis and other DM complications by fenofibrate in dogs.

## **Canine Pituitary Adenoma Organoids (CaPitO) as in vitro Model for Canine Cushing's Disease**

**Principal Investigator:** Karin Sanders, DVM, PhD; Utrecht University

**Total Grant Amount:** \$106,939

**Grant Period:** 4/1/2021 - 9/30/2022

**Project Abstract:** Pituitary-dependent hypercortisolism (PDH) caused by a pituitary tumor (adenoma) is one of the most common endocrine disorders in dogs. Medical treatment options that focus on the pituitary tumor are currently lacking. In this project, investigators aim to validate three-dimensional Canine Pituitary adenoma Organoids (CaPitOs) as a model for canine PDH, and use this to find new pituitary-targeted treatment options. Organoids are grown from stem cells and resemble the primary organ or tumor they derive from and therefore can be viewed as avatars of the tumor that can be cultured in the laboratory. Investigators will perform next generation sequencing techniques to identify new treatment targets and then inhibit these targets with drug screens in the validated CaPitOs. Drugs identified in this study may eventually improve the survival and quality of life of dogs with PDH, improve the treatment of PDH in humans, as well as offer a sound but less-invasive alternative to pituitary surgery.



## Understanding the Genetic Basis of Addison's Disease in Portuguese Water Dogs

**Principal Investigator:** Steven Friedenber, DVM, PhD; University of Minnesota

**Total Grant Amount:** \$207,381

**Grant Period:** 5/1/2021 - 4/30/2024

**Project Abstract:** Addison's disease (AD) is a common, life-threatening disorder in dogs characterized by the immune-mediated destruction of portions of the adrenal gland. This damage prevents the adrenal gland from synthesizing hormones that are necessary for normal cell metabolism, kidney function, and maintenance of the immune system. Dogs with AD are also highly predisposed to succumbing to a life-threatening adrenal crisis. AD is most common in Portuguese Water Dogs (PWDs), which have a 29-fold greater risk of developing the disease compared to other dog breeds, indicating a strong genetic component. To date, no genetic variants have been associated with AD in PWDs. This lack of knowledge has prevented the development of a genetic test that would allow for prediction of a dog's disease risk and the development of informed breeding practices related to AD. In this study, investigators will use state-of-the-art scientific tools to understand the genetic basis of AD in PWDs. The data generated here will provide the foundation for the development of a genetic test for AD in PWDs, enabling early diagnosis and treatment, as well as maintenance of genetic diversity within the breed while helping to decrease disease incidence.

*Funding for the research is provided through the collaborative efforts and generosity of the Portuguese Water Dog Foundation, Inc. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

# Epilepsy Initiative

02561

## Is Gut Dysbiosis Associated with Canine Idiopathic Epilepsy?

**Principal Investigator:** Karen Muñana, DVM, MS; North Carolina State University

**Total Grant Amount:** \$104,453

**Grant Period:** 2/1/2019 - 1/31/2023

**Project Abstract:** Idiopathic epilepsy is the most common chronic nervous system disorder of dogs. Its cause is poorly understood but is believed to involve genetic and environmental factors. Treatment with anti-seizure drugs remains the standard of care. However, approximately one-third of dogs fail to achieve satisfactory seizure control, highlighting the need to investigate factors that may influence disease course. An association between epilepsy and inflammatory gastrointestinal disease is well documented in humans, and several other nervous system disorders have been linked to alterations in gut microbial populations, with considerable attention focused on the bacteria *Helicobacter* and *Lactobacilli*. The aim of this study is to determine whether dogs with idiopathic epilepsy have shifts in the gastrointestinal environment that may influence disease course. The researchers hypothesize that dogs with idiopathic epilepsy have alterations in the gut microbial population characterized by the presence of *Helicobacter*, a decrease in *Lactobacillus*, and resulting inflammation that are associated with epilepsy development and outcome. The investigators will collect and study paired fecal samples from untreated and phenobarbital treated epileptic dogs and including an unaffected dog from the same household. The occurrence of *Helicobacter* and *Lactobacillus* species will be analyzed using molecular genetic techniques and specific biomarkers of inflammation and evaluated for associations with disease onset and outcome. In exploring the association between the gut microbial population and canine epilepsy, this study has the potential to improve our understanding of epilepsy, and ultimately guide the development of more effective therapies for this disorder.

## Do Dogs Get Temporal Lobe Epilepsy? Clinical Signs, Magnetic Resonance Imaging and Pathological Findings in Epileptic Dogs

**Principal Investigator:** Starr Cameron, BVetMed; University of Wisconsin, Madison

**Total Grant Amount:** \$14,555

**Grant Period:** 7/1/2020 - 6/30/2022

**Project Abstract:** Thirty percent of dogs with idiopathic epilepsy have poor seizure control and are considered by their caregivers to have a poor quality of life despite appropriate medical therapy. Temporal lobe epilepsy (TLE) is the most common type of epilepsy in humans, and has been well described in other species, including cats. Many epileptic dogs have a seizure presentation that is very similar to that described in humans with TLE including: excessive salivation, staring off, dilated pupils and facial twitching. Even with these similarities present, the actual anatomical changes within the brain have not been confirmed in dogs. Approximately forty percent of humans with TLE have poor seizure control, and additional treatment options, including surgery and laser ablation, result in the majority of patients becoming seizure-free long-term. The goal of this study is to further evaluate dogs for TLE, with the overall objective of better understanding the causes of canine epilepsy. Magnetic resonance imaging (MRI) findings and detailed anatomical changes from epileptic dogs with a history of TLE-like seizures will be studied. The hippocampus, which is the part of the brain implicated in TLE, will be extensively evaluated based on criteria established for TLE in humans and multiple other species. This project has the potential to further our understanding of epilepsy in dogs, broadening treatment options and ultimately leading to improved seizure control. Additionally, the results from this study will provide the foundation to explore other treatment options routinely recommended in humans with TLE for dogs with drug resistant seizures.

## **A Dose Finding Study of Cannabidiol in Dogs with Idiopathic Epilepsy**

**Principal Investigator:** Stephanie McGrath, DVM, MS; Colorado State University

**Total Grant Amount:** \$107,995

**Grant Period:** 5/1/2021 - 4/30/2024

**Project Abstract:** Affecting approximately 5% of the canine population, idiopathic epilepsy is a widespread disease that is often frustrating and, at times, debilitating to both dogs and their owners. About one-third of dogs afflicted by epilepsy are refractory to the standard drugs available to treat the disease so finding a replacement or adjunctive medication is imperative. Recently, the anticonvulsive properties of cannabidiol (CBD) have been demonstrated in human and canine patients. The primary objective of this study is to find an effective dose of CBD for idiopathic epilepsy in client-owned dogs with uncontrolled seizures ( $\geq 2$  seizures per month). Aim 1 of this study is to determine the dose of oral CBD that will reduce average monthly seizure activity in client-owned refractory idiopathic epileptic dogs by 50% or more when added to standard anticonvulsive therapy. Aim 2 of this study is to evaluate the safety and tolerability of CBD in dogs with idiopathic epilepsy. Investigators hypothesize that, at the appropriate dose, CBD will be effective in lowering the average monthly seizure frequency by 50% in at least 50% of uncontrolled epileptic dogs and that CBD, even at high doses, will be well tolerated. An effective agent with limited side effects has the potential to improve the quality of life of epileptic dogs, and ultimately afflicted humans, as dogs serve as an ideal surrogate for human epileptic conditions.

## Assessment of Frequency of Seizures and Antiseizure Drug (ASD) Efficacy by Electroencephalography (EEG) for Dogs with Epilepsy

**Principal Investigator:** Fiona James, DVM, MS; University of Guelph

**Total Grant Amount:** \$83,318

**Grant Period:** 9/1/2021 - 8/31/2025

**Project Abstract:** Epilepsy is the most common brain disease encountered in dogs. Epilepsy can be caused by several underlying problems responsible for the recurrent seizures seen with epilepsy. Accurate seizure control has an impact on the quality of life and survival time in epileptic dogs as well as on their caretaker's quality of life. Several other conditions such as movement disorders can be mistaken for epilepsy and there are several types of seizures that can go undiagnosed, thus missing an opportunity to improve the dog's quality of life and neurological deficits. The accuracy of epilepsy diagnosis and how veterinarians choose the most appropriate treatment for epileptic dogs potentially delays finding the best therapy because it is currently unknown which antiseizure drug (ASD) is more appropriate for which type of seizures.

As in human medicine, electroencephalography (EEG) evaluates brain function and is the only way to confirm seizure activity and further classify different types of seizures. Another important use of EEG in people is to evaluate, objectively and in a non-invasive manner, the efficacy of an ASD. However, unlike human medicine, the diagnosis of epileptic seizures in veterinary medicine is solely based on subjective information (e.g. owner description or visualization of an episode) which have been shown to be highly unreliable. As such, EEG should be used to obtain objective data in order to diagnose and treat epileptic dogs accurately.

The goals of this study are to confirm the seizure under-reporting phenomenon in canine epilepsy and estimate ASD efficacy for different seizure types in dogs. Investigators will record EEG from dogs with epilepsy and evaluate the number and type of seizures seen on EEG in comparison to what their caregivers see. Their EEG data will further be examined with respect to what ASDs they are receiving to understand the effects of these drugs on their seizures. These findings have the potential to revolutionize the veterinary approach to canine epilepsy through the provision of an objective measure of treatment success and, hopefully, a way to predict management outcomes for dogs.

## **Validating Genetic Variants Underlying Canine Idiopathic Epilepsy and Exploring Their Functional Roles in the Belgian Sheepdog and Tervuren**

**Principal Investigator:** Anita Oberbauer, PhD; University of California, Davis

**Total Grant Amount:** \$108,000

**Grant Period:** 5/1/2021 - 10/31/2022

**Project Abstract:** Canine epilepsy is a debilitating condition and, unfortunately, an all too common neurological disease with impact on a dog's health and well-being, and a significant emotional toll on owners. Epilepsy is known to be inherited, and therefore characterizing the causal genetic alterations is important to assist in breeding decisions as well as reveal pathways that could be useful targets for therapeutic intervention to mitigate the seizures themselves. Although fairly widespread among all dogs, the prevalence of idiopathic epilepsy (IE) is considered elevated and a recognized health concern in the Belgian Sheepdog (BS) and Belgian Tervuren (BT) relative to other dog breeds, making them good candidate breeds for genetic studies on IE. In addition, it was through research on these breeds that an IE risk variant in the ADAM23 gene was initially discovered and then found to be a common risk variant across many breeds although the ADAM23 variant fails to completely explain disease expression in any of the breeds indicating the need for further exploration of causal variants contributing to disease expression in dogs. Investigators recently identified a novel genomic region on canine chromosome 14 (CFA14) associated with increased risk for IE in BS and BT dogs. When combined with the ADAM23 region, the risk for IE was further increased. The present research, while focusing on the BS and BT breeds, aims to validate the involvement of the risk region on CFA14 in these and other breeds, investigate the functional changes associated with the variants while discovering additional genetic variants underlying IE susceptibility, and provide insight into the regulatory components of this disorder pertinent to many breeds.

## Investigating Neuronal Network Connectivity in Dogs with Idiopathic Epilepsy using Functional Magnetic Resonance Imaging

**Principal Investigator:** Karen Muñana, DVM, MS; North Carolina State University

**Total Grant Amount:** \$75,840

**Grant Period:** 5/1/2021 - 4/30/2024

**Project Abstract:** Idiopathic epilepsy is the most common chronic neurological disorder of dogs, for which the cause remains poorly understood, and the standard of care is limited to symptomatic treatment with anti-seizure drugs. Outcomes are frequently unsuccessful, underscoring the critical need to better understand the underlying physiology responsible for seizures in dogs with idiopathic epilepsy, if more effective management is to be achieved. Functional magnetic resonance imaging (fMRI) is a noninvasive technique to evaluate brain activity that measures small changes in blood flow associated with increased energy demand. Resting state fMRI (rs-fMRI) detects spontaneous fluctuations in the brain's blood flow that are analyzed for synchrony, to identify anatomically distinct but functionally connected regions of the brain, called resting state networks (RSNs). Alterations in RSNs have been documented in humans with epilepsy, and specific changes associated with disease progression, severity, and treatment response. Hence, rs-fMRI has emerged as a powerful tool for investigating the underlying cause of epilepsy in humans and holds similar promise in the study of epilepsy in dogs. The aim of this study is to evaluate RSNs in dogs with idiopathic epilepsy using rs-fMRI. Investigators hypothesize that dogs with idiopathic epilepsy have alterations in the functional connectivity of the brain compared to neurologically normal dogs. Ten epileptic dogs that are not receiving any anti-seizure medications and ten neurologically normal dogs will undergo fMRI and image analysis to identify and compare RSNs. Results from this study will provide novel insight into the brain function of epileptic dogs, to further our understanding of epilepsy and potentially lead to more effective management strategies.

# Gastrointestinal Disease

02523-MOU

## Identifying the Genetic Basis of Protein Losing Enteropathy in Yorkshire Terriers

**Principal Investigator:** Kenneth Simpson, BVM&S, PhD; Cornell University

**Total Grant Amount:** \$46,440

**Grant Period:** 3/1/2018 - 6/30/2022

**Project Abstract:** Chronic intestinal disease associated with the loss of protein into the gut, termed protein losing enteropathy (PLE), is a severe, life threatening condition that affects many dog breeds, including the Yorkshire Terrier, Soft-Coated Wheaten Terrier, Basenji, Norwegian Lundehund, and Chinese Shar-Pei. The syndrome of PLE is most common in Yorkshire Terriers (4.2-10 fold relative risk), and affected dogs frequently suffer from severe weight loss, accumulation of fluid within tissues and body cavities, diarrhea, low levels of circulating proteins, increased risk for abnormal clotting, and derangements in vitamin and mineral homeostasis. The microscopic appearance of the small intestine of Yorkshire Terriers with PLE (YT-PLE) is distinct from PLE in other breeds, suggesting it is caused by a breed-specific genetic abnormality. Despite aggressive treatment, remission is variably achieved and relapse is common. Long-term survival is infrequent with recent studies indicating treatment failure in approximately 50% of Yorkshire Terriers with PLE. The high morbidity and mortality of YT-PLE indicates the desire to eradicate this disease through breeding practices. The investigators are seeking to identify genetic regions and genes associated with YT-PLE to enable prevention of this disease, provide insights into the development of PLE across species, and facilitate the discovery of more specific and effective therapies. Preliminary studies in their laboratory have linked several genetic regions to YT-PLE but additional genotyping of DNA samples from YT with and without PLE is required to enable definitive identification of causal abnormalities.

*Funding for the research is provided through the efforts and generosity of the Yorkshire Terrier Club of America and the Yorkshire Terrier Club of America Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*



## Identification of Genetic Risk Factors Contributing to Gastrointestinal Motility Disorders

**Principal Investigator:** Leigh Anne Clark, PhD; Clemson University

**Total Grant Amount:** \$57,930

**Grant Period:** 2/1/2020 - 7/31/2022

**Project Abstract:** Gastrointestinal motility disorders affect the nerves and muscles of the esophagus, stomach, and/or the intestines, causing digestive disturbances. Congenital idiopathic megaesophagus (CIM) is an esophageal motility disorder of dogs wherein contractility is reduced and leads to an enlargement of the esophagus. Affected puppies regurgitate after eating and survivors are susceptible to life-threatening complications. The highest incidences of CIM occur in the Great Dane and German Shepherd Dog breeds. Gastric dilatation-volvulus (GDV or bloat) is characterized by dilatation and twisting of the stomach, cutting off blood and oxygen to the organs. Based on a previous study for CIM in Great Danes, the investigators will 1) study a narrow region of chromosome 6, shown to be a major risk factor for CIM; 2) seek additional genomic regions that contribute to CIM, and 3) determine association between CIM and GDV based on shared genetic risk factors that impact gastrointestinal motility. The investigators hope to establish a pattern of transmission and develop a genetic test to reduce the incidence of CIM, and potentially GDV, in Great Danes.

## Assessing Microvasculature for Intestinal Viability in Obstructed Small Intestines and Effects of Resection and Anastomosis Techniques

**Principal Investigator:** Penny Regier, DVM, MS; University of Florida

**Total Grant Amount:** \$8,677

**Grant Period:** 1/1/2020 - 12/31/2022

**Project Abstract:** Intestinal foreign body ingestion is one of the most common causes for emergency surgery in dogs. Frequently, the foreign material causes damage to the intestines requiring surgical removal (resection) of the dead portion of intestine and surgical connection or re-attachment of the remaining intestine ends (anastomosis). At present, assessment of the need to resect and anastomose bowel is subjective and there may be areas of intestine with questionable health or viability. Recently, a hand-held, non-invasive device, GlycoCheck™, capable of imaging the smallest blood vessels (microvasculature) of tissues has been extensively studied in human medicine for quantification of vascular health. This study will use the GlycoCheck™ to assess areas of questionable intestinal health in dogs with foreign body obstruction and establish reference ranges that will aid the surgeon's ability to assess small intestinal viability intra-operatively. This study has the potential to aid in reducing complications associated with foreign body surgery.

## Acute Gastrointestinal Injury in Dogs

**Principal Investigator:** Tracy Hill, DVM, PhD; University of Minnesota

**Total Grant Amount:** \$13,964

**Grant Period:** 2/1/2021 - 7/31/2022

**Project Abstract:** Acute gastrointestinal (gut) injury (AGI) occurs in people and in dogs with critical illness. In people, AGI as a complication of serious illness occurs in 60% of intensive care patients and is associated with an increased risk of death. The rate that AGI occurs in dogs is unknown, although evidence suggests that dogs do show signs of this complication, which can result in failure to deliver nutrition by mouth and potentially fatal complications. With this study, investigators aim to better define AGI in dogs, and to determine the occurrence of some complications that occur with AGI, including gastrointestinal ulcers and changes in intestinal motility. To do this, investigators will use video capsule endoscopy, a vitamin-sized capsule with tiny cameras, which is given to the dog orally to see inside the dog's gastrointestinal tract. Investigators hope that this work will eventually allow for improved care and survival in critically ill dogs.

## **Cardiovascular Complications of Acute Pancreatitis in Dogs**

**Principal Investigator:** Harry Cridge, MVB, MS; Michigan State University

**Total Grant Amount:** \$14,897

**Grant Period:** 1/1/2021 - 6/30/2022

**Project Abstract:** Acute pancreatitis is the most common disease of the exocrine pancreas in dogs. The exact prevalence is unknown, but a recent study documented that 37% of dogs had evidence of acute or chronic pancreatitis at necropsy, with increased risk in certain breeds like Yorkshire Terriers, Miniature Schnauzers, Silky Terriers, and Toy Poodles. Despite its common occurrence, targeted therapeutic options do not exist and current therapy is primarily supportive in nature. Mortality rates are reported to be as high as 27-58%, which is often the result of systemic complications. Cardiovascular complications including conduction abnormalities, echocardiographic abnormalities, and elevated cardiac biomarkers occur in approximately 50% of humans with acute pancreatitis, and many of these complications are reversible with therapy. In addition, the presence of cardiac conduction abnormalities in canine pancreatitis has been correlated with outcome in a prior study but general knowledge in this area remains limited. This study will address this important knowledge void by identifying and characterizing the full range of cardiovascular abnormalities that occur in naturally occurring acute pancreatitis in dogs. These abnormalities could be associated with disease severity and outcome. More importantly, they may represent therapeutic targets that could improve outcomes in this common and frequently deadly disease for dogs.

## How do Maternal, Environmental, and Genetic Factors Contribute to Acquisition and Evolution of the Enteric Microbiome in Dogs?

**Principal Investigator:** David Williams, PhD; University of Illinois

**Total Grant Amount:** \$107,825

**Grant Period:** 4/1/2021 - 3/31/2024

**Project Abstract:** The enteric microbiome is the consortium of microbes, primarily bacteria, living in the intestinal tract of all mammals. The enteric microbiome is critical to the health of the host animal and numerous diseases are associated with abnormalities in its composition. Mammals first acquire their enteric microbiome at the time of birth from contact with microbes living on and in their mothers. As the host animal grows and matures, so does the enteric microbiome which eventually settles upon an adult-like configuration. Whereas the enteric microbiomes of neonates is unstable and lacks diversity, the adult microbiome is stable and highly diverse. Studies in humans and other animals have found that factors that interrupt or delay maturation of the microbiome are associated with the development of diseases including asthma, allergies, chronic intestinal diseases, and obesity. Very little is known about how the enteric microbiome of dogs matures during the first year of life. The purpose of this study is to follow the acquisition and subsequent maturation of the enteric microbiome during the first 14 months of life in three common breeds of dogs: German Shepherd Dogs, Labrador Retrievers, and Golden Retrievers. Investigators will collect samples from mothers during pregnancy and from puppies starting at the day of birth through 14 months of age. The research team will use advanced DNA sequencing technologies to understand how the microbiome matures during this critical developmental window and determine how the genetics of the host animal influences this process. This study has the potential to inform the development of novel preventative and therapeutic approaches to optimize canine health from birth to adulthood.

## Optimizing Storage Conditions of Canine Feces for Fecal Microbiota Transplantation

**Principal Investigator:** Arnon Gal, DVM, PhD; University of Illinois

**Total Grant Amount:** \$21,883

**Grant Period:** 6/1/2021 - 5/31/2023

**Project Abstract:** Clinical signs of acute and chronic enteropathies are common reasons for seeking veterinary care. There is increasing evidence that intestinal dysbiosis plays a major role in the pathogenesis of many of these conditions. Fecal microbiota transplantation (FMT) of frozen human stool from donors with high fecal microbiome diversity is a safe and effective treatment to restore reduced diversity and abundance of gut microbiota of human patients with recurrent *Clostridium difficile* infections, and perhaps other enteropathies. FMT has also shown promise in restoring normobiosis to dysbiotic dogs. Still, lack of standardization in dogs regarding the selection, processing, and storage of feces of FMT donors, route of FMT administration, and definition of clinical indications potentially contribute to reported varying success rates. There have been no studies to determine fecal donor characteristics and storage protocols that may facilitate the selection of donor feces with optimal microbial viability, composition, and diversity. The goals of this study are to assess stability of fecal microbial viability, composition, and diversity under varying duration of time and storage conditions, and to determine donor fecal characteristics that could enhance the viability, composition, and diversity of stored donor feces. Investigators will utilize a targeted genomic approach to test the stability and optimization of feces storage, which is essential for future studies addressing the efficacy of FMT in dogs.

# General Canine Health

02322

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## Analysis of the Health, Behavioral, and Longevity Data Collected in the 9/11 Medical Surveillance Longitudinal Study

**Principal Investigator:** Cynthia Otto, DVM, PhD; University of Pennsylvania

**Total Grant Amount:** \$37,672

**Grant Period:** 2/1/2017 - 1/31/2022

**Project Abstract:** Following the attacks of September 11, 2001 on the World Trade Center and Pentagon, the AKC Canine Health Foundation awarded funds to the only lifetime longitudinal study tracking the medical and behavioral impacts of a major national disaster on the health and behavior of search & rescue (SAR) dogs. On June 6, 2016, the last study dog was laid to rest and data collection for the 9/11 Medical Surveillance Study was concluded. With 15 years of data, including annual radiographs, bloodwork, and handler surveys (health, performance, and behavior), the opportunity for in-depth analysis and discovery of new best practices and protocols for SAR dogs has never been greater. Data collected from deployed dogs will be compared to data collected from control SAR dogs that underwent similar training and careers, but did not deploy to 9/11. The investigators will explore three key areas of data: behavior, occupational hazards, and longevity related to health and work. Critical information gleaned from this study will have major implications applicable to the development, training, and care of our nation's SAR dogs, other working canines, and even companion dogs. Results will improve our understanding of traits of successful SAR dogs and thus influence dog selection. Importantly, following characterization of trait heritability, this data could be critical to a focused breeding program. The complete analysis of the occupational hazards of SAR dogs will shape preventive practices to allow these dogs to safely and effectively fulfill their mission of saving human lives.

## **Understanding the Genetics of Adverse Drug Reactions in Sighthounds: Phase II**

**Principal Investigator:** Michael Court, BVSc, PhD; Washington State University

**Total Grant Amount:** \$229,085

**Grant Period:** 6/1/2018 - 3/31/2023

**Project Abstract:** Life-threatening unanticipated reactions to drugs with a narrow margin of safety, such as those used for anesthesia and to treat cancer, are a common yet serious concern for dog owners and veterinarians. Investigators at Washington State University have been conducting research to identify the cause of extremely slow recovery from anesthesia in a high proportion of Greyhounds, as well as in other sighthound breed dogs, including Italian Greyhounds, Scottish Deerhounds, Borzois, Irish Wolfhounds, Salukis, Afghan Hounds, and Whippets (among others). In previous work funded by the AKC Canine Health Foundation (#02242), the investigators discovered several mutations that were shown by cell-based testing to significantly decrease the function of genes responsible for breaking down (metabolizing) commonly used anesthetic drugs, as well as many other drugs used in dogs. The goal of this next phase of research is to develop a novel drug sensitivity test using saliva, blood or urine samples to identify dogs within a breed (or specific breeds) that metabolize drugs very slowly, thus creating a “personalized” or individual dog approach to drug selection. This test will then be used to confirm that the identified gene mutations are the cause of slow drug metabolism in sighthound dog breeds – as well as identify other breeds and individual dogs that could suffer from similar adverse drug reactions.



## Evaluation of the Transversus Abdominis Plane Block to Control Pain Associated with Abdominal Surgery in Dogs

**Principal Investigator:** Alonso Guedes, DVM, MS, PhD; University of Minnesota

**Total Grant Amount:** \$14,999

**Grant Period:** 3/1/2019 - 2/28/2023

**Project Abstract:** Abdominal surgery is common in veterinary practice, and opioids are the mainstay of pain management. Although effective painkillers, opioids are not free of adverse effects, and challenges stemming from the opioid epidemic in humans increased the urgency for developing alternative pain control strategies. In dogs, local nerve blocks are well-described for limb surgery, but comparatively little information exists for abdominal surgery. This study will determine whether surgical abdominal pain in dogs can be effectively and safely managed with a nerve block technique known as transversus abdominis plane (TAP) block; assessors will be blinded to treatment groups. The investigators will also examine whether a new long-acting form of the local anesthetic bupivacaine can provide long-lasting pain control for abdominal surgery. The results will advance canine health by providing evidence-based information of an alternative strategy to manage surgical abdominal pain in dogs.

## **Scientific and Clinical Assessment of Fecal Microbiota Transplant in Obese Dogs: SLIM Study**

**Principal Investigator:** Jenessa Winston, DVM, PhD; The Ohio State University

**Total Grant Amount:** \$94,989

**Grant Period:** 6/1/2020 - 5/31/2022

**Project Abstract:** Obesity is a growing epidemic in companion animals. Obesity results from a prolonged positive energy balance leading to excessive fat accumulation, which promotes dysregulation of metabolic, hormonal, and inflammatory responses. Ultimately these changes lead to physical impairment, comorbidities, and reduced quality of life. Evidence is mounting that the intestinal microbiota (collection of microorganisms that live in the intestines) contributes to obesity, and rational manipulation of this ecosystem may confer a health benefit. This study will provide a comprehensive scientific and clinical assessment of the efficacy of fecal microbiota transplantation (FMT) as an adjunctive therapy for canine obesity management. The investigators hypothesize that FMT (the transfer of feces from a healthy, lean donor dog into an obese dog) will amplify weight loss in obese dogs compared to the use of standard dietary obesity management. A randomized, placebo controlled clinical trial in client-owned obese dogs consisting of three groups: diet alone, diet + FMT, diet + placebo will provide data on weight loss and characterize the intestinal microbiota and metabolic function. Success of this study will benefit obese dogs by providing a microbial intervention to augment current strategies for canine obesity management aimed at promoting weight loss, normalizing metabolic status, and improving quality of life.

## Do Dog Breeds Differ in Pain Sensitivity?

**Principal Investigator:** Margaret Gruen, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$104,147

**Grant Period:** 3/1/2020 - 2/28/2023

**Project Abstract:** The investigators' recent comprehensive survey found that both veterinarians and members of the public believe that dog breeds differ in their sensitivity to pain, yet this has never been fully investigated. Beliefs about breed differences in pain sensitivity could negatively impact clinical recognition and treatment of pain in dogs and result in unnecessary pain, particularly for breeds viewed as less sensitive to pain. The investigators hypothesize that dogs have similar pain sensitivity thresholds, regardless of breed, but that human perceptions about breed-based differences in pain sensitivity affect the clinical recognition and treatment of pain. This study will endeavor to answer whether dog breeds differ in pain sensitivity, and whether breed affects veterinarians' treatment of pain in dogs. If breed differences in pain sensitivity exist, future work would be performed to understand genetic associations, and advance our understanding of effectively treating pain in a breed-specific manner. If no differences exist, then the impact of the human perception of breed differences must be understood to ensure that dogs of every breed receive appropriate pain management.

## Genetics of Adverse Reactions to Anesthetic and Sedative Drugs in Chow Chows

**Principal Investigator:** Michael Court, BVSc, PhD; Washington State University

**Total Grant Amount:** \$16,924

**Grant Period:** 3/1/2020 - 2/28/2022

**Project Abstract:** Moderate to severe adverse drug reactions, including unexpected effects, slow recovery, and even death have been reported following administration of anesthetic/sedative drugs by owners in some Chow Chow dog. Problematic drugs included butorphanol, acepromazine, trazodone, hydromorphone, ketamine and midazolam. In preliminary studies investigators have excluded a role for the MDR-1 deletion mutation found in herding dog breeds, as well as the CYP2B11/POR mutations found in sighthound breed dogs. Gene capture sequencing of DNA from representative Chow Chows identified a novel mutation in the CYP2B11 gene that is predicted to cause a damaging change in the amino acid sequence (CYP2B11-F182). This study will determine if CYP2B11-F182 mutation is responsible for decreased CYP2B11 enzyme function leading to excessive drug exposure and subsequent adverse effects in affected dogs. Previous studies have identified CYP2B11 as the primary enzyme metabolizing ketamine and midazolam. However, the identities of CYPs metabolizing the other problematic drugs are currently unknown. This study aims to ascertain the role of CYP2B11 in metabolism of the implicated drugs, confirm the deleterious effect of the CYP2B11-F182 mutation on enzyme function in vitro, determine the prevalence of the CYP2B11-F182 mutation across the Chow Chow breed, and identify other breeds with this mutation. Evidence from this study will be used to examine the effect of the CYP2B11-F182 mutation in future studies on drug pharmacokinetics and pharmacodynamics in genotyped dogs.

*Funding for the research is provided through the collaborative efforts and generosity of the Chow Chow Club, Inc. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## **Microbial and Cytokine Signatures of Periodontitis in Dogs**

**Principal Investigator:** Santiago Peralta, DVM; Cornell University

**Total Grant Amount:** \$86,532

**Grant Period:** 11/1/2020 - 10/31/2023

**Project Abstract:** Periodontitis is a painful inflammatory disease that affects a vast majority of dogs at some point during their lifetime. Despite its importance, preventive, diagnostic and treatment strategies have not evolved for decades and these strategies lack a mechanistic rationale. Even though the possible causes and mechanisms of disease are not fully understood, they are believed to involve complex interactions between the host animal's defense mechanisms and the microbial communities that are normally present under the gumline. Evidence suggests that the types of microbes present in these communities and their genetic material can influence a dog's defense mechanisms but has yet to be explored. In this study, investigators will use modern molecular techniques to study the genetic material of the microbial communities present under the gumline of dogs with different stages of periodontal disease and determine how these communities relate to the degree of inflammation and destruction of the tissue attached to affected teeth. The results of this study will generate baseline knowledge on how and why periodontal disease occurs in dogs. Furthermore, this work will be important in designing future studies aimed at developing novel and more effective preventive, diagnostic and therapeutic strategies for this highly relevant disease.

# Hepatic Disease

02297-MOU

## Understanding the Genetics of Hepatic Copper Toxicosis in the Dalmatian

**Principal Investigator:** Andrew Mason, PhD; University of Alberta

**Total Grant Amount:** \$107,668

**Grant Period:** 3/1/2017 - 8/31/2022

**Project Abstract:** Copper toxicosis, leading to early death from liver disease, was first described in Bedlington Terriers in 1975, with similar diseases described in other dog breeds including the Labrador Retriever, West Highland White Terrier, Skye Terrier, and Doberman Pinscher. Genes have been linked to copper toxicosis in the Bedlington Terrier and the Labrador Retriever, but the genes differ by breed. In most breeds the genes are not known. Copper toxicosis was considered rare in the Dalmatian but may be more common than previously believed. Symptomatic dogs may be misdiagnosed as having other liver diseases, never appropriately diagnosed or only diagnosed with copper overload at a terminal stage. The investigators aim to identify the faulty gene(s) in Dalmatians using an advanced whole genome sequencing strategy to obtain the genome sequences of carefully selected members of an affected Dalmatian pedigree. Identification of the problem gene is the first step towards genetic testing and to improved breeding practices necessary to eradicate hepatic copper toxicosis from the Dalmatian breed. Gene identification will help raise awareness of copper toxicosis in the Dalmatian breed, lead to more rapid diagnosis of the condition, and support the search for the most effective therapy.

*Funding for the research is provided through the efforts and generosity of the Dalmatian Club of America and Dalmatian Club of America Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## **Medical Resolution of Gallbladder Mucocele Formation in Dogs**

**Principal Investigator:** Jody Gookin, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$220,333

**Grant Period:** 10/1/2019 - 9/30/2023

**Project Abstract:** Gallbladder mucocele formation is a disorder of the gallbladder where excess secretion of abnormally thick mucus results in obstruction to the normal flow of bile or rupture of the gallbladder contents into the abdominal cavity. The disease has a breed predisposition for Shetland Sheepdogs, Border Terriers, Cocker Spaniels, Schnauzers, Pomeranians, Bichons Frises, Chihuahuas, Pugs, and Beagles and uncommonly affects mixed breed dogs. The only curative treatment for the disease is a costly surgery to remove the gallbladder and on average, 27% of dogs will die from post-operative complications. The objective of this study is to establish whether the course of mucocele formation can be reversed by correcting metabolic disturbances documented in affected dogs. Recent published data, generated by support from the AKC Canine Health Foundation (Grant #01986), demonstrated that dogs with mucocele formation are relatively deficient in essential dietary compounds that play key roles in normal metabolism and/or whose biological activity may help to promote normal secretory function of the gallbladder epithelium. This study will determine whether supplementation with such dietary factors will arrest and reverse the course of mucocele formation in dogs. If successful, this research will have an important positive impact by providing an immediate benefit to dogs with this disease.

## **Treatment of Idiopathic Chronic Hepatitis and Copper Associated Hepatopathy in Dogs**

**Principal Investigator:** Sarah Shropshire, DVM, PhD; Colorado State University

**Total Grant Amount:** \$22,500

**Grant Period:** 1/1/2020 - 6/30/2022

**Project Abstract:** This study will evaluate treatment regimens for two common and important liver diseases in dogs: idiopathic chronic hepatitis (ICH) and copper associated hepatopathy (CAH). Treatment of these diseases is essential to prevent progression to liver failure and death. At present, there are no standardized treatments for ICH or CAH in dogs. Dogs will be treated for ICH and CAH with two separate therapeutic regimens. The investigators will evaluate treatment efficacy by monitoring several hepatic parameters, including clinical signs, liver values, serum drug levels and copper levels. These parameters will be correlated to remission or relapse of disease to identify factors associated with treatment response. The findings of this project may significantly improve veterinarians' ability to treat dogs with ICH and CAH.



## Characterizing Immunohistopathology and Serum Immunoglobulin G in Immune-Mediated Chronic Hepatitis Dogs

**Principal Investigator:** Sarah Shropshire, DVM, PhD; Colorado State University

**Total Grant Amount:** \$14,999

**Grant Period:** 2/1/2022 - 1/31/2023

**Project Abstract:** Immune-mediated chronic hepatitis (ICH) is a highly prevalent liver disorder in dogs that can progress to liver failure if undiagnosed or untreated. An autoimmune cause is suspected because the disease responds to immunosuppressive medications such as cyclosporine. However, the immune features and pathogenesis of ICH have not been fully characterized and the diagnostic criteria have not been established yet. Thus, the diagnosis of ICH continues to be a challenge. Furthermore, the effects of cyclosporine treatment on the immune system in ICH dogs needs to be articulated. This study aims to characterize the unique immune features of ICH and how they respond to cyclosporine treatment. These features will serve as biomarkers of disease diagnosis, treatment response to cyclosporine, and prognosis.

## Evaluation of Serum C-reactive Protein as a Noninvasive Biomarker of Inflammation and Disease Severity in Dogs with Gallbladder Mucocele

**Principal Investigator:** Cynthia Leveille-Webster, DVM; Tufts University

**Total Grant Amount:** \$14,863

**Grant Period:** 2/1/2022 - 1/31/2024

**Project Abstract:** In the last 20 years a condition called gallbladder mucocele has emerged as a very common disease of the liver in dogs. This disease is associated with the accumulation of congealed bile in the gallbladder that subsequently cannot empty properly into the intestine. The result of this is often irreversible damage to the gallbladder wall with rupture and inflammation. This complication is life threatening and requires emergency surgery, which has a high mortality rate (20-40%). There is an increased incidence in several breeds of dogs including Shetland Sheepdogs, Border Terriers, Miniature Schnauzers, Cocker Spaniels, Pomeranians and Bichons, but it can occur in any breed or mixed breed dog. Some dogs can live with the abnormal gallbladder and can be managed medically with low-fat diets and drugs to increase bile flow. Some of these medically managed dogs have an abrupt onset of decompensation that can be fatal, however, veterinarians and researchers do not fully understand how to recognize which dogs this will happen to. Investigators hypothesize that this decompensation may be related to the onset of inflammation which triggers the development of clots in the gallbladder wall that compromise blood flow and lead to necrosis (death) of the wall. In this study, researchers will determine if measurement of C-reactive protein in the blood can serve as a marker of impending decompensation and the need for gallbladder removal.

# Immunology & Infectious Disease

## The Impact of Lidocaine Administration on Natural Killer Cell Populations in Canine Sepsis

**Principal Investigator:** Mandy Wallace, DVM, MS; University of Georgia

**Total Grant Amount:** \$14,896

**Grant Period:** 11/1/2017 - 4/30/2022

**Project Abstract:** Sepsis is a life-threatening condition that results from an excessive systemic inflammatory response to infection. This can occur due to infections in various parts of the body including the chest, abdomen, or bloodstream. Dogs and humans with sepsis have up to a 50% mortality rate, with most dying from organ system failure. This high mortality rate has been linked to the dysfunction of several types of immune cells. One of these cell types, Natural Killer (NK) cells, plays a critical role in the killing of bacteria within the body, but their role has not been evaluated in dogs with sepsis. Administration of the local anesthetic lidocaine, a drug that can decrease pain and correct cardiac arrhythmias, has been shown to increase survival in dogs with sepsis. This study seeks: 1) to determine the relationship between NK cell numbers and phenotype in the blood and disease severity in dogs with abdominal sepsis; and 2) to evaluate if the administration of lidocaine during surgery changes NK cell numbers or affects survival rates in dogs with sepsis from abdominal infection.

## Standardization of rLiNTPDase2 and Derived Chimeras as Antigens on Immunochromatographic Assay for Diagnosis of Canine Visceral Leishmaniasis

**Principal Investigator:** Juliana Fietto, PhD; Federal University of Viçosa

**Total Grant Amount:** \$41,040

**Grant Period:** 4/1/2021 - 3/31/2023

**Project Abstract:** Canine visceral leishmaniasis (CVL) corresponds to the most aggressive and lethal form of leishmaniasis manifesting in dogs. In North, Central and South America, CVL is caused by *Leishmania infantum* and diagnostic techniques currently used to identify infected animals have important limitations in sensitivity and specificity. The main goal of this study is to develop new and accessible technologies for CVL diagnosis based on more specific antigens. This accomplishment has high potential to create a positive impact on diagnostic strategies all over the globe and to increase the amount of correctly diagnosed and treated dogs.

## Antigen Discovery for an Improved Serologic Diagnostic of *Trypanosoma cruzi* Infection in Dogs

**Principal Investigator:** Eric Dumonteil, PhD; Tulane University

**Total Grant Amount:** \$199,025

**Grant Period:** 11/1/2020 - 10/31/2022

**Project Abstract:** Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) parasites and it represents a major public health problem in the Americas. Dogs play a key role in parasite transmission cycles and can develop severe cardiac disease following infection with the parasite. Infection in dogs has been reported in multiple states in the U.S., but the lack of accurate diagnostic tests complicates disease surveillance and reliable identification of cases. There is a critical need for a more reliable test that can detect all infections with this parasite. This study aims to develop a new test for the accurate diagnostic of *T. cruzi* infection in dogs. The availability of a new diagnostic test may facilitate timely identification of Chagas disease cases and improve veterinary care. The test may also be broadly applicable to other species and could lead to an improved test for humans.

02980

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## Canine Systemic Insecticides as a Novel Intervention to Protect Dogs from Triatomine Insect Vectors of Chagas Disease

**Principal Investigator:** Sarah Hamer, DVM, PhD; Texas A&M AgriLife Research

**Total Grant Amount:** \$65,713

**Grant Period:** 2/1/2022 - 1/31/2024

**Project Abstract:** Across the southern U.S., dogs are exposed to triatomine insects, also known as kissing bugs, which can transmit the parasite that causes Chagas disease. Chagas disease causes different types of cardiac disease in dogs, often leading to death. Any dog that encounters the insect vectors distributed across at least 28 southern states is at risk. Further, congenital transmission can occur, and infected dogs may travel, so Chagas disease is not limited to the southern states. There are no currently approved vaccines or anti-parasitic treatments. Prevention relies on vector control. A prior study (CHF grant #02448) developed a network of kennels and quantified a remarkably high disease incidence of disease; over 25% of dogs enrolled as negative seroconverted to Chagas-positive over one-year. Investigators will now utilize this network and offer a novel intervention to reduce the transmission cycle by assessing the effectiveness of four canine systemic insecticides, commonly used for tick and flea prevention, on the survivorship of local kissing bugs to determine if systemic insecticides could play a role in vector management and as a preventive measure against Chagas disease. Kissing bugs will be collected around the areas of treated dogs to identify what animals they are feeding on and understand the impact of systemic insecticides on kissing bug populations. These insects will be tested for the Chagas disease parasite (*Trypanosoma cruzi*) and subjected to blood meal analysis using next generation sequencing to identify their natural hosts, which will provide new insight into which wildlife species to consider in management efforts. Together, these study aims intend to provide solutions for Chagas disease at the vector-dog interface in the southern U.S.

## Enhanced Detection and Characterization of Spotted Fever Group *Rickettsia* Species in Dogs and Ticks with Focus on a Novel *Rickettsia* Species Infecting Clinically Ill Dogs in the U.S.

**Principal Investigator:** Barbara Quorllo, DVM, MS; North Carolina State University

**Total Grant Amount:** \$68,524

**Grant Period:** 1/1/2022 - 12/31/2023

**Project Abstract:** Dogs carry the burden of high exposure to tick-borne diseases, often alerting us to new and emerging pathogens before people are infected. Recently, investigators identified a new tick-borne spotted fever group *Rickettsia* (SFGR) species infecting seven clinically ill dogs in the U.S. All infected dogs had clinical signs like Rocky Mountain spotted fever. Genetic analysis of the novel *Rickettsia* sp., designated *Rickettsia* st. 2019-CO-FNY, showed this to be a distinct and new *Rickettsia* sp. These findings shed light on how little is known about SFGR in dogs and people, due in part to diagnostic limitations and the need for modalities that can more rapidly identify and speciate active SFGR infections. The focus of this study is to investigate the genetic, epidemiologic, and ecological features of emerging SFGR in dogs. Researchers will develop an advanced molecular diagnostic test, attempt to isolate *Rickettsia* st. 2019-CO-FNY from naturally infected dogs for whole genome sequencing, and characterize tick species harboring SFGR species. If successful, investigators will be able to 1) benefit canine health by providing an improved SFGR diagnostic test to better detect the most prevalent group of tick-transmitted pathogens in the US, and 2) share new information on a medically relevant, potentially zoonotic novel SFGR, including its genomic sequence and how it is transmitted. This information will ultimately shed light on how SFGR infections impact canine health and how different *Rickettsia* spp. are maintained in the environment, better preparing us for detection and prevention of disease in dogs and people.

# Kidney & Urological Disease

02437-MOU

## Characterization of Renal Disease in American Boxer Dogs

**Principal Investigator:** Jessica Hokamp, DVM, PhD; The Ohio State University

**Total Grant Amount:** \$56,694

**Grant Period:** 3/1/2018 - 2/28/2022

**Project Abstract:** Chronic kidney disease (CKD) is often a progressive and fatal disease in dogs. Boxer dogs appear to have a predisposition for development of CKD, suggesting that kidney disease in this breed might be heritable. Studies in Europe report an increased frequency of Boxers with kidney and urinary tract maldevelopments leading to CKD, termed "juvenile nephropathy". The investigators' International Veterinary Renal Pathology Service (IVRPS) recently found that juvenile nephropathies are a main underlying cause of CKD in young Boxer dogs; however, there are no published studies that have determined the predominant cause(s) of CKD in Boxer dogs in the United States. The investigators hypothesize that pedigreed Boxers in the U.S. may be afflicted by several causes of CKD, including but not exclusive to juvenile nephropathies. To assess the most common causes of CKD in Boxers, the investigators will perform detailed examination of medical records and archived tissue samples to retrospectively reveal the predominant cause(s) and prevalence of kidney disease in Boxers and will also prospectively collect and analyze tissue and fluid samples from pedigreed families of Boxers afflicted by the predominant types of kidney diseases. This work will determine if certain types of kidney disease in Boxers follow a heritable pattern and might be related to genetic mutations, allowing for future studies on genetic analysis if an inheritance pattern of disease is determined.

*Funding for the research is provided through the efforts and generosity of the American Boxer Charitable Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*



## Characterization of Renal Disease in Greyhound Dogs

**Principal Investigator:** Jessica Hokamp, DVM, PhD; The Ohio State University

**Total Grant Amount:** \$36,182

**Grant Period:** 6/1/2020 - 5/31/2022

**Project Abstract:** Kidney disease is common in dogs, and some dog breeds seem to be more predisposed to development of kidney disease than other breeds. Greyhounds appear to be predisposed to proteinuric renal diseases; however, a complete characterization of the most common types of kidney diseases in this breed has not been performed to date, and potential genetic causes of kidney diseases in Greyhounds remain unknown. Preliminary data based on evaluation of cases through the International Veterinary Renal Pathology Service and Ohio State University has revealed that kidney disease in Greyhounds is likely from a variety of causes; including primary glomerular diseases and glomerular damage secondary to hypertension. For this study, samples will be prospectively collected to identify Greyhounds with kidney disease, determine which are proteinuric, localize the origin of proteinuria (glomerular versus tubulointerstitial), and identify dogs for further evaluations and monitoring. Prospective examination of kidney samples from pedigreed Greyhounds using advanced techniques (transmission electron microscopy and immunofluorescence) will ensure accurate diagnosis of renal injury. Detailed review of archived = samples from Greyhounds will allow identification of common renal lesions. DNA will also be banked for future genomic studies if a hereditary component to kidney disease is detected in the breed.

*Funding for the research is provided through the collaborative efforts and generosity of the Greyhound Club of America. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

03021

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## Judicious Antibiotic Use in Cases of Canine Pyelonephritis

**Principal Investigator:** Marilyn Dunn, DVM, MS; University of Montreal

**Total Grant Amount:** \$45,097

**Grant Period:** 4/1/2022 - 3/31/2024

**Project Abstract:** This research project aims to investigate different duration of antibiotic treatment in cases of canine pyelonephritis (bacterial kidney infection). Currently, no scientific veterinary data is available on the ideal duration of antibiotic treatment for pyelonephritis. In human medicine, pyelonephritis is usually treated with a short course of antibiotics (between 5 and 14 days). Due to the lack of current literature on the subject, veterinarians currently treat pyelonephritis with a 4-6 week course of antibiotics. A traditional 6-week course of antibiotics will be compared to a newer recent recommendation of a 2-week treatment. If the results of this study are in concordance with our hypothesis, it will dramatically change the treatment of pyelonephritis and significantly decrease exposure to antibiotics. This decreased exposure will lower the risk of multi-resistant bacteria which in turn will also improve global human health by decreased exposure to multi-resistant bacteria.

## Lung & Respiratory Disease

### Characterizing Developmental Lung Disease as a Cause of Sudden Death in the Norwich Terrier

**Principal Investigator:** Kurt Williams, DVM, PhD; Michigan State University

**Total Grant Amount:** \$116,076

**Grant Period:** 3/1/2018 - 8/31/2022

**Project Abstract:** Neonatal respiratory distress syndrome has been attributed to more than 60% of deaths early in life in puppies. The underlying cause(s) of this apparently common problem is poorly understood.

Despite the high frequency of respiratory-related mortality in neonatal puppies, there are no reports describing the underlying lung pathology in affected individuals. In human medicine the classification, management and evaluation of diffuse interstitial lung diseases in infants are well described. The most severe neonatal lung diseases in humans develop as a result of abnormal development of the lung, and often result in death soon after delivery. The investigators recently documented microscopic evidence of striking abnormal lung development in puppies of various breeds who died suddenly, suggesting that developmental lung disease (DLD) is an important and unrecognized cause of early death in young puppies. Breeders of Norwich Terriers (NT) report that sudden death of puppies early in life is common. Through preliminary studies, a high incidence of DLD in NT puppies associated with sudden death has been identified. The identification of a breed-association with DLD in the NT presents an opportunity to correlate the pathology and genetics to sudden death in NT puppies. Findings could lead to the development of preventive measures to reduce the incidence of DLD in the NT as well as other dog breeds, and may also be applicable to similar developmental lung diseases in children.

## Diagnostic Utility of Thoracoscopy for Localization of Pulmonary Bullae in Dogs with Spontaneous Pneumothorax

**Principal Investigator:** Valery Scharf, DVM, MS; North Carolina State University

**Total Grant Amount:** \$13,829

**Grant Period:** 1/1/2020 - 12/31/2022

**Project Abstract:** Primary spontaneous pneumothorax is defined as the presence of air in the space around the lungs without an obvious precipitating factor. This disease presents as a life-threatening emergency causing shortness of breath, exercise intolerance, and possible collapse or sudden death. The diagnosis of these lesions that cause spontaneous pneumothorax in dogs (known as pulmonary bullae) remains challenging. The accuracy of advanced imaging such as computed tomography (CT) for identifying bulla in dogs with spontaneous pneumothorax is limited. Currently, thorough exploration of the chest through an open surgical approach is the diagnostic standard for primary spontaneous pneumothorax. This strategy, however, requires an invasive surgical approach and weeks of post-operative recovery. In contrast, video-assisted scoping (thoracoscopy) of the chest, known as VATS, is preferred to open surgery for the treatment of spontaneous pneumothorax in human medicine. VATS is associated with fewer complications and reduced post-operative pain, making it a desirable alternative to the current standard in veterinary medicine, but its reliability in correctly identifying pulmonary bullae associated with spontaneous pneumothorax in dogs has not yet been proven. This study aims to prospectively evaluate the ability of thoracoscopy to identify and localize pulmonary bullae in dogs with primary spontaneous pneumothorax, thus facilitating minimally invasive treatment options for dogs with this disease.

## **The Pathologic Link between Lung and Gut: Diagnosis of Aerodigestive Disorders in Dogs**

**Principal Investigator:** Carol Reinero, DVM, PhD; University of Missouri

**Total Grant Amount:** \$83,801

**Grant Period:** 7/1/2020 - 6/30/2022

**Project Abstract:** Chronic unintentional inhalation (aspiration) of small amounts of gastrointestinal contents into the respiratory tract can cause or worsen respiratory disease in dogs. Gastroesophageal reflux (GER) is reported in up to 90% of people with certain respiratory diseases but is under-recognized and therefore, not treated in dogs in large part because they cannot report signs of heartburn. The investigators will use an advanced imaging technique, fluoroscopy, in dogs with respiratory disease in order to document GER and other swallowing abnormalities. Results may provide new avenues of treatment for canine respiratory disease by targeting underlying GER/swallowing abnormalities.

# Musculoskeletal Conditions & Disease

02107

## **Landmark Clinical Trial to Establish the Evidence-Based Use of Regenerative Medicine to Treat Tendon Injury in Dogs**

**Principal Investigator:** Jennifer Barrett, DVM, PhD; Virginia-Maryland College of Veterinary Medicine

**Total Grant Amount:** \$254,509

**Grant Period:** 7/1/2014 - 11/30/2022

**Project Abstract:** This study will evaluate the effectiveness of Platelet-Rich Plasma (PRP) and stem cells in the treatment of the most common sporting injury in dogs: supraspinatus tendinopathy (similar to the rotator cuff injury in humans). Tendon injuries in dogs often progress undiagnosed and result in chronic lameness and pain. Ultimately, unassisted tendon healing results in scar formation and reduced function of the joint and surrounding muscle tissue. PRP and stem cell therapies aim to accelerate and promote healing through tissue regeneration and reduced scarring. The investigators will conduct a randomized, placebo-controlled clinical trial evaluating the effectiveness of PRP, adipose-derived, cultured stem cells (ASC) and commonly used stromal vascular fraction (SVF) cells to directly compare efficacy of intratendinous injection of ASC versus SVF, both of which are currently commercially available despite having limited scientific evidence of efficacy. The investigators hope to identify an effective treatment to supraspinatus tendon injury.

## Embracing Polygenicity of Common Complex Disease in Dogs: Genome-wide Association of Cruciate Ligament Rupture

**Principal Investigator:** Peter Muir, BVSc, PhD; University of Wisconsin, Madison

**Total Grant Amount:** \$154,116

**Grant Period:** 5/1/2019 - 10/31/2022

**Project Abstract:** Cruciate ligament rupture (CR) is a common disabling, degenerative condition of the knee. It places a large financial burden on the American public. Inflammation of the stifle and fraying of cruciate ligament fibers, particularly in the cranial cruciate ligament, eventually leads to ligament rupture with associated stifle instability in affected dogs. CR is a moderately heritable, complex disease with genetic and environmental risk. CR is common in certain breeds, such as the Labrador Retriever, and rare in other breeds. There is a critical gap in knowledge regarding the genetic contribution to CR, as the number of genes influencing disease risk has never been studied in detail. Our main goal is to comprehensively analyze the genetic features of the disease across the genome and use this knowledge to develop a genetic test for CR disease risk using genomic prediction. We aim to robustly estimate heritability, analyze the genetic architecture of CR, and advance genetic testing using genomic prediction in the Labrador Retriever, the most common purebred dog breed. The rationale for this work is that detailed knowledge of the genetic features of CR will advance development of a genetic test for CR risk using genomic prediction. This work will fundamentally advance knowledge of the genetic architecture of CR, a very common canine disease. Consequently, such knowledge will provide an invaluable guide to future research into other canine complex diseases. CR genetic testing would enable early identification of at-risk dogs for precision medical care, and selective breeding to reduce the disease burden.

## **The Effect of a Modified Approach on Early Weight Bearing in Dogs Following a Tibial Plateau Leveling Osteotomy for Cranial Cruciate Ligament Rupture**

**Principal Investigator:** Dominique Sawyere Hansford, BVSc, MS; Virginia-Maryland Regional College of Veterinary Medicine

**Total Grant Amount:** \$14,939

**Grant Period:** 1/1/2020 - 12/31/2022

**Project Abstract:** A tibial plateau leveling osteotomy (TPLO) is a common procedure performed to address stifle (knee) instability secondary to cranial cruciate ligament rupture in dogs. During the surgical approach for a TPLO, some of the tendons of muscles attaching to the inside of the tibia are cut. These tendons are referred to as the medial crural fascia. The medial crural fascia is important for the normal function of the dog's stifle. As opposed to the rapid gain in wound strength displayed in skin and the GI tract, restoration of fascia integrity is relatively prolonged. In the first week of healing, fascia incisions have no inherent strength; therefore, the repair is entirely dependent on the suture material, making it prone to inadequate healing. The clinical implication of this on early weight bearing and limb use following TPLO surgery is unknown. Additionally, tendons only reach 50-80% of their original strength at one year following reconstruction. In humans, deficiency in the repair of the medial crural fascia has been associated with decreased rotational stability, increased meniscal injury, and continued knee instability following surgery for cranial cruciate ligament rupture. If these findings are consistent in dogs, lack of attention to medial crural reconstruction and its inability to return to the original strength may contribute to continued subluxation of tibia and latent meniscal injury. In this study, dogs who undergo a modified approach to a TPLO will be evaluated to determine normalization of gait parameters and thigh circumference as compared to dogs who undergo a standard approach to a TPLO. Results from this study may change the approach to TPLO surgery in the future and provide canine patients with improved short- and long-term outcomes.



## **Development and Validation of a New Body Condition Scoring System for use in Canine Athletes**

**Principal Investigator:** Cristina Hansen, DVM, PhD; University of Alaska, Fairbanks

**Total Grant Amount:** \$15,000

**Grant Period:** 6/1/2021 - 5/31/2023

**Project Abstract:** Evaluating and monitoring body condition in canine athletes is critically important. Athletic dogs can markedly increase their metabolic rates during exercise, are often in negative energy balance, and can quickly lose body condition. Existing body condition scoring (BCS) systems were not developed with the canine athlete in mind; they were developed using dogs in stable body condition and are only validated for quantifying relative differences in body fat. This proposed study will develop a new BCS system that will better allow canine and veterinary professionals to estimate both fat and muscle loss in dogs. The results of this study will benefit military working dogs, sled dogs, field trial dogs, and any other working dogs through accurate assessment of the amount of metabolic reserve in these dogs and the changes in metabolic reserve resulting from exercise.

## Development of Regional Anesthesia Techniques to Treat Chronic Painful Conditions of the Stifle and Elbow in Dogs

**Principal Investigator:** Diego Portela, DVM, PhD; University of Florida

**Total Grant Amount:** \$8,527

**Grant Period:** 7/1/2021 - 6/30/2023

**Project Abstract:** Knee and elbow osteoarthritis (OA) is a common cause of chronic pain in dogs, significantly impacting quality of life. Traditional management mainly involves the use of oral medications such as non-steroidal anti-inflammatory drugs (NSAIDs). However, long-term use of NSAIDs may be associated with significant gastrointestinal side effects, often leading to treatment discontinuation and resulting in inadequate pain management or euthanasia. Interventional pain medicine is a medical subspecialty employing advanced techniques, such as nerve blocks, to improve the quality of life of patients suffering chronic pain. Nerve blocks apply local anesthesia close to nerves responsible for the transmission of pain sensation in different parts of the body (i.e., elbow). Some nerves are difficult to localize, especially when instruments to guide the needles are not used (i.e., blind technique). The use of ultrasound guidance allows practitioners to direct needles to precise locations where the target nerves are located. No studies are currently available describing how to selectively approach sensory nerves participating in the transmission of pain from knee and elbow in dogs. The present study aims to identify and develop a reliable technique to approach the sensory nerves of the knee and elbow using both blind and ultrasound-guided techniques. A detailed anatomical study will be performed to describe the surface and ultrasonographic landmarks necessary to perform the blocks. This project will set the basis for future clinical studies aimed to evaluate the clinical effectiveness of desensitizing the sensory innervation of elbow and knee joints to provide pain relief in dogs suffering from osteoarthritic pain. Injections of different drug combinations aimed at extending the duration of sensory blockade could be used in the future to improve the quality of life in dogs suffering from chronic and debilitating stifle and elbow OA.

## **Characterization of Mesenchymal Stromal Cell Properties of Canine Culture-expanded Articular Chondrocytes**

**Principal Investigator:** John Kisiday, PhD; Colorado State University

**Total Grant Amount:** \$14,973

**Grant Period:** 3/1/2021 - 8/31/2022

**Project Abstract:** Osteoarthritis is a debilitating, incurable disease that is prevalent in dogs. Mesenchymal stem cells (MSCs) are believed to be strong candidates for the treatment of osteoarthritis based on their robust propensity for immunomodulation. To date, MSCs administered to diseased canine joints via intra-articular (IA) injection has demonstrated limited and sometimes temporary symptom-modifying effects. Therefore, there is a critical need to improve upon the effectiveness of therapies designed around IA injection of MSCs. This study seeks to do so through the innovative approach of using culture-expanded chondrocytes instead of MSCs from conventional tissues such as fat or bone marrow. Existing publications and preliminary data from the investigator's lab demonstrate that adult chondrocytes adopt MSC properties with isolation and expansion and possess an atypical ability to survive in suspension. The ability of chondrocytes to thrive in suspension is expected to overcome a major limitation of MSCs, which have been shown to persist in the joint for only a short period of time after IA injection. If successful, this study will be a critical step in improving the effectiveness of cell therapies for osteoarthritis in dogs.

# Neurology

02589

## **Genetic Basis of Canine Spinal Abnormalities**

**Principal Investigator:** Kari Ekenstedt, DVM, PhD; Purdue University

**Total Grant Amount:** \$112,993

**Grant Period:** 4/1/2019 - 9/30/2022

**Project Abstract:** This study will identify potential genes and risk alleles to better understand the genetic basis of canine spinal abnormalities using comparisons between affected and unaffected dogs. The identification of these genes and risk alleles will advance knowledge with an ultimate goal to develop genetic tests and/or a genetic risk model to help predict healthy spines for good health in breeds with tightly curled tails such as Pugs, French Bulldogs, English Bulldogs, Boston Terriers and Basenjis.

## Characterization of Sensory Neuronal and Muscle Pathology in Canine Degenerative Myelopathy to Identify Targets for Therapeutic Intervention

**Principal Investigator:** Joan Coates, DVM, MS; University of Missouri

**Total Grant Amount:** \$153,360

**Grant Period:** 6/1/2019 - 5/31/2023

**Project Abstract:** Many dog breeds, including mixed breeds, carry genetic mutations for degenerative myelopathy (DM), a late adult-onset disease that begins with loss of coordination and progressive hind limb paralysis. The disease is particularly prevalent in Boxers and Pembroke Welsh Corgis, and is similar to amyotrophic lateral sclerosis (ALS) in people. Approximately two to three years after first signs of DM appear in dogs, loss of muscle function spreads, resulting in complete paralysis. Although the investigators have found that almost all cases of DM in dogs are associated with mutations in a gene called SOD1, it is not understood how these mutations lead to the progressive paralysis that characterizes DM. Ultimately, the research team hopes to develop a treatment that will prevent the onset and progression of disease. In order to do so, a better understanding of the earliest pathology in the muscles and nerves of affected dogs, and how this pathology spreads over time, is needed. This will enable the identification of targets for therapeutic intervention. In this study, the research team hopes to identify the earliest biochemical and structural changes in the central nervous system, muscles and nerves of dogs, and to characterize these changes as the disease progresses.

## Characterization of the Fecal Microbiome in Dogs with Spinal Cord Injury Secondary to Intervertebral Disc Disease (IVDD)

**Principal Investigator:** Kari Foss, DVM, MS; University of Illinois

**Total Grant Amount:** \$14,958

**Grant Period:** 4/1/2020 - 9/30/2022

**Project Abstract:** Intervertebral disc disease (IVDD) is a common cause of spinal cord injury (SCI) in dogs and can significantly impact quality of life in this patient population. Surgery is the current standard of care and focuses on relieving the actual spinal cord compression. In addition to compression, the spinal cord also suffers from contusive injury for which there are not established treatments. As such, even with surgery, the contusive injury can lead to progressive damage of the spinal cord. Studies in mice and people have shown that gut dysbiosis (bacterial imbalance in the gut) occurs as a result of SCI but can also contribute to further injury and damage. This is because the dysbiosis is thought to cause further inflammation within the spinal cord tissue. Not only is the dysbiosis a result of the SCI, but it further contributes to the injury in a vicious cycle. Studies in dogs have shown that spinal cord inflammation contributes to significant injury to the spinal cord secondary to disc disease. Therefore, targeting gut dysbiosis could play an important role in the management of SCI. This study will focus on determining the presence of gut dysbiosis in dogs with SCI secondary to IVDD when compared to healthy dogs.

**Neurofilament Light Chain Concentration in Dogs with Meningoencephalitis (MUE)****Principal Investigator:** Christopher Mariani, DVM, PhD; North Carolina State University**Total Grant Amount:** \$9,473**Grant Period:** 2/1/2020 - 7/31/2022

**Project Abstract:** Meningoencephalitis of unknown etiology (MUE) is a common and devastating disorder that is most prevalent in small and toy breed dogs such as Pugs, Maltese and Chihuahuas. Although dogs frequently respond to anti-inflammatory or immunosuppressive therapy, many dogs suffer relapses or worsen in the face of such therapy, and this condition is ultimately fatal in most cases. Currently available diagnostic tests including magnetic resonance imaging (MRI) and spinal fluid (CSF) analysis are necessary to make a diagnosis of MUE but are not helpful in predicting the course of disease or likelihood of survival. In addition, these tests are expensive and their role in monitoring the response to therapy is uncertain. There is a critical need for novel biomarkers that will help predict responses to therapy and to monitor ongoing therapy, ideally using a blood sample. Neurofilament light chain (NF-L) is a protein found in neurons and released into the CSF and blood after injury to the central nervous system. NF-L has emerged as a promising biomarker of brain inflammation in humans, largely due to the development of a sensitive assay that can detect very small concentrations of this protein. This study will measure NF-L within the CSF and serum of dogs with MUE and compare these concentrations with control samples. The investigators will evaluate the utility of NF-L to predict patient response to therapy and prognosis.

## **Clinical Trial of *Prevotella histicola* Supplementation to Ameliorate Meningoencephalomyelitis of Unknown Origin (MUO)**

**Principal Investigator:** Nick Jeffery, BVSc, PhD; Texas A&M AgriLife Research

**Total Grant Amount:** \$40,180

**Grant Period:** 3/1/2020 - 2/29/2024

**Project Abstract:** Meningoencephalomyelitis of unknown origin (MUO), also known by a number of other abbreviations such as MUE, MUA and, sometimes, GME (granulomatous meningoencephalomyelitis), is the name given to a group of closely-related inflammatory diseases of the brain and spinal cord. These conditions are common, about 25% or more of the neurologic cases treated by veterinary neurologists, and are severe and often fatal. MUO is considered an ‘autoimmune’ disease, in which the immune system attacks part of the body, in this case the nervous system, resulting in neurologic signs including seizures, loss of balance and inability to walk steadily. Current treatment relies on immunosuppressive drugs, such as corticosteroids, cyclosporine, cytarabine, azathioprine and others, many of which have detrimental side effects. A large proportion of affected dogs will die despite treatment or suffer long-term neurologic impairments. MUO has striking similarities to multiple sclerosis in people and a disease called ‘EAE’ in rodents. It is now known that the immune system is regulated by bacteria in the gastrointestinal (GI) tract. GI bacteria in people with multiple sclerosis, rodents with EAE and dogs with MUO are different from those in unaffected individuals. Recent evidence suggests altering bacteria in the GI tract of mice with EAE prevents or reduces severity of disease. In this study, the investigators will test whether giving an oral supplement of a specific harmless bacteria to dogs will reduce the severity of MUO. If successful, this could improve treatment to control disease and reduce reliance on immunosuppressive drugs.



## Identification of Genetic Risk Factors in Degenerative Myelopathy in German Shepherd Dogs

**Principal Investigator:** Kerstin Lindblad-Toh, PhD; Broad Institute

**Total Grant Amount:** \$90,000

**Grant Period:** 7/1/2021 - 6/30/2022

**Project Abstract:** Canine degenerative myelopathy (DM) is a naturally occurring progressive adult-onset neurodegenerative disease that is fatal. Performing genome-wide association studies (GWAS) in Pembroke Welsh Corgis (PWC), investigators identified an association to a SOD1 variant, coding for the E40K amino acid substitution, that occurs in >180 dog breeds. The vast majority of breeds with DM have the same SOD1 mutation. Using PWC with the SOD1 disease allele, comparisons were made between early onset cases and elderly healthy dogs that also were homozygous for the DM risk allele. A mutation in SP110 was found to predispose to early onset DM. Given that the frequency of the SOD1 mutation and disease is variable within and across breeds investigators believe that multiple additional genes may affect at what age the disease starts. Overall, the research team has collected DNA from >20,000 German Shepherd Dogs (GSD) and genotyped them for the standard SOD1 mutation to demonstrate the disease is frequent and the allele frequency is 35% for this breed. This study will perform health updates for already collected and novel GSDs and look at the age of onset distribution for DM. As with the PWC studies, comparisons will be made between old healthy GSDs and GSDs with the earliest onset of DM (both categories having two copies of the SOD1 mutation). This should identify novel modifier genes determining if SOD1+ dogs get the disease early or late (or not at all). Finally, whole-genome sequencing of three GSDs with the disease, but lacking the known SOD1 mutation, will help identify additional risk factors. Together, these aims may develop more accurate genetic tests for DM in GSDs and other breeds.

*Funding for the research is provided through the collaborative efforts and generosity of the German Shepherd Dog Club of America. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

# Oncology - General

02487

One  
Health

## **OX40 Checkpoint Molecule Targeted Antibodies for Cancer Immunotherapy in Dogs**

**Principal Investigator:** Steven Dow, DVM PhD; Colorado State University

**Total Grant Amount:** \$168,905

**Grant Period:** 3/1/2018 - 2/28/2022

**Project Abstract:** Checkpoint molecules play a key role in regulating T cell immunity against cancer (T cells are one type of immune cell called lymphocytes). Clinical trials of antibody therapeutics that target checkpoint molecules such as PD-1 in human oncology (e.g., Opdiva, Keytruda) have demonstrated remarkable results in inducing tumor regressions and cures, against a variety of different cancer types. This new era of cancer immunotherapy also has tremendous potential for treatment of cancer in dogs. The investigators will begin development of a new, second-generation immunotherapy targeting the canine checkpoint molecule OX40 (CD134). Development of the first generation PD-1 antibodies for canine oncology is already underway, and the investigator's laboratory has been involved in evaluating immune responses to these antibodies. Studies in rodent models indicate that targeting the OX40 checkpoint molecule may be more effective than PD-1 for cancer immunotherapy. The investigators will use antibodies generated in their lab against the canine OX40 checkpoint molecule to investigate its role in regulating cancer immunity in dogs, as a first step in advancing OX40 antibodies to clinical trials in dogs with cancer. In this project, they will characterize canine OX40 antibodies, determine which immune cells express OX40 in dogs, determine how OX40 antibodies activate effector T cells in dogs, and how these antibodies trigger immune activation in tumor tissues to help accelerate development of OX40 checkpoint molecule targeted antibodies as next generation cancer immunotherapeutics for dogs.

## **Tumor-educated Platelets: A Novel Minimally Invasive Liquid Biopsy for Early Cancer Diagnosis**

**Principal Investigator:** Unity Jeffery, VetMB, PhD; Texas A&M AgriLife Research

**Total Grant Amount:** \$14,999

**Grant Period:** 1/1/2020 - 12/31/2022

**Project Abstract:** Platelets are a vital part of the blood clotting system and interact closely with tumor cells. These interactions promote tumor growth and spread, but also alter the RNA content of platelets. These altered platelets are described as tumor-educated platelets because they carry this tumor signature to distant sites as they circulate through the body. tRNA from platelets routinely collected in blood samples can be sequenced to identify the genes of origin. This platelet RNA profile can reliably distinguish human cancer patients with a wide variety of tumors from healthy people and patients with inflammatory disease. This study will perform RNA sequencing to determine the platelet RNA profile for dogs with several types of cancer versus healthy dogs and dogs with infectious disease. This will be a first step in developing a platelet-based minimally invasive cancer screening test.

## **Optical Coherence Tomography for Margin Evaluation of Canine Skin and Subcutaneous Neoplasms**

**Principal Investigator:** Laura Selmic, BVetMed, MPH; The Ohio State University

**Total Grant Amount:** \$43,443

**Grant Period:** 3/1/2020 - 2/28/2022

**Project Abstract:** Skin cancer is common in older dogs and often requires surgery to treat. For these tumors, the best chance of cure is offered if the surgeon can fully remove both visible and microscopic traces of the tumor. Currently surgeons must rely on pathologist's assessment of tissues after surgery and the success of the procedure will not be known until several days later. This result is important as residual cancer may need further surgery or other treatments like radiation therapy. Additional treatments such as these can result in further risk and discomfort for dogs as well as be an emotional and financial cost for owners. Optical coherence tomography is an emerging diagnostic imaging tool that uses light waves to generate real-time, high-resolution images of tissue at a microscopic level. These images can be used to evaluate for residual disease at the time of surgery giving immediate feedback to the surgeon. This study will focus on validating this technology for the imaging of surgical margins of canine skin and subcutaneous tumors. If successful, this technology could be used to assess for residual cancer intra-operatively to benefit patients by guiding accurate treatment recommendations.

## Bladder Carcinogen Exposures in Pet Dogs

**Principal Investigator:** Lauren Trepanier, DVM, PhD; University of Wisconsin, Madison

**Total Grant Amount:** \$149,145

**Grant Period:** 3/1/2020 - 2/28/2023

**Project Abstract:** Bladder cancer is an aggressive cancer that affects ~ 20,000 dogs per year, and often leads to euthanasia. Certain breeds have a higher incidence of bladder cancer but genetic studies even in the highest risk breeds have been inconclusive and still indicate influence from environmental exposures. The investigators propose that specific household environmental chemical exposures contribute to the risk of bladder cancer in dogs. In this study, they will measure urinary concentrations of five different chemicals that are known or suspected to be bladder carcinogens, in dogs with bladder cancer compared to unaffected dogs. The investigators will determine whether the presence of certain chemicals is associated with household exposures, based on owner questionnaires and household proximity to industrial sites. Finally, they will determine whether urinary chemical concentrations are linked to early DNA damage in the urinary cells of healthy dogs that do not have bladder cancer. The overall goal of this study is to provide veterinarians and dog owners with evidence-based bladder cancer prevention strategies.

## Transcriptional Profiling of Canine Soft Tissue Sarcoma

**Principal Investigator:** Andrew Miller, DVM; Cornell University

**Total Grant Amount:** \$132,759

**Grant Period:** 3/1/2020 - 2/28/2023

**Project Abstract:** Soft tissue sarcomas account for 10-15% of all skin and subcutaneous cancers in dogs. Traditionally, biopsy and subsequent histology have been the primary means of diagnosing these cancers. The histology is assigned to one of three grades ranging from low (grade I), intermediate (grade II), and high (grade III). Histologic grade is currently the key criterion for guiding treatment and determining patient outcome. However, in human medicine and pathology, soft tissue sarcomas are diagnosed with a hybrid approach that involves both histologic features and genetic analysis of the tumor sample. This genetic analysis guides further treatment, aids in developing accurate follow-up information, and has been shown to have a positive effect on patient outcome and survival. Despite how common soft tissue sarcomas are in the dog, current veterinary care still relies solely on the histologic grade, which is subjective at best, and does not incorporate genetic data into the diagnostic plan. This study will perform transcriptome analysis on 300 canine soft tissue sarcomas in order to establish the transcriptome profile of canine soft tissue sarcoma and correlate this transcriptome to patient follow-up. This will allow for the formation of a hybrid diagnostic approach that will provide more accurate information to inform the prognosis for dogs afflicted with soft tissue sarcoma.

## Repurposing Drugs to Modulate Myeloid-derived Suppressor Cells (MDSCs) in Canine Soft Tissue Sarcomas

**Principal Investigator:** Sita Withers, BVSc, PhD; Louisiana State University

**Total Grant Amount:** \$13,782

**Grant Period:** 3/1/2021 - 2/28/2023

**Project Abstract:** This study addresses the need for a deeper understanding of how canine soft tissue sarcomas (STS) inhibit the anti-tumor immune response. The overarching goal is to define the effects of a particularly immunosuppressive cell type, myeloid-derived suppressor cells (MDSCs), on the host immune response within canine STSs, and to explore the ability of readily-available drugs to alter this activity. Investigators will address this goal by pursuing two objectives: 1) to define the types of immune cells and their function within the tumor and peripheral blood of dogs with STS; 2) to determine the ability of repurposed drugs (sildenafil, all-trans retinoic acid, and ranolazine) to decrease the immunosuppressive forces provided of tumor-infiltrating MDSCs in canine STS. Investigators hypothesize that MDSCs will concentrate within the canine STS tumor and will display greater immunosuppressive functions in the tumor compared to peripheral blood, and that repurposed drugs can be used to decrease the immunosuppressive activity of MDSCs. Findings will be directly translatable to future clinical trials for canine STS.

## **Investigation of Mechanisms of Resistance to Immunotherapy in Dogs with Spontaneous High-grade Glioma**

**Principal Investigator:** Susan Arnold, DVM; University of Minnesota

**Total Grant Amount:** \$13,375

**Grant Period:** 3/1/2021 - 8/31/2022

**Project Abstract:** This study will investigate the underlying reasons why French Bulldogs with high grade gliomas (HGG) respond poorly to immunotherapy-based treatment. The research team will investigate novel immunotherapies designed to alter the interaction between the immune system and high grade gliomas (HGG) and includes anti-tumor vaccine-based therapy and other therapies to stimulate the immune response against tumors. All forms of immunotherapy that have been investigated by this research team have yielded comparable or superior survival times to conventional therapy in all breeds other than French Bulldogs. While other breeds have a median disease-related overall survival time of 267 days when treated with any form of immunotherapy, in comparison, French Bulldogs experience a median disease-related overall survival time of only 48 days. All dogs enrolled in the studies had similar HGG features on biopsies, raising questions about differences in genetic fingerprints of the tumors and immune responses of this breed compared to others. The first aim is to identify genetic differences in French Bulldog HGG cells compared to HGG cells of other dog breeds. The second aim is to characterize the immune system profile of French Bulldogs with HGG by comparing their immune cells to those of healthy French Bulldogs as well as other breeds with HGG. This project is highly impactful for advancing canine health and may reveal breed-specific immunotherapeutic targets that increase the success of immunotherapy for canine HGG.



## Use of CRISPR-based Genome-wide Approach for Identification of Vulnerabilities in Canine Oral Melanoma

**Principal Investigator:** Maciej Parys, DVM, PhD; R(D)SVS and Roslin Institute, University of Edinburgh

**Total Grant Amount:** \$94,794

**Grant Period:** 3/1/2021 - 2/28/2023

**Project Abstract:** Oral melanoma is a frequently occurring cancer in dogs. Currently there are few effective therapies for this disease. This study aims to identify the genes necessary for cancer cells to grow using a tool called CRISPR whole genome knock-out library. This tool has the capacity to shut down nearly 18,000 out of 21,000 genes in the canine genome. The tool's library contains viral vectors that can infect melanoma cell lines while being able to turn off a single gene in a single cell. After cells grow in culture, investigators will perform sequencing to see which of the virus sequences remain (cells are still alive, gene is not necessary) and which of the virus sequences are not present (cells are dead, thus the gene was needed). This process distinguishes the genes specific for melanoma development. Subsequently investigators will validate the genes found using drugs, specifically targeting these genes. Through an in-depth analysis of melanomas, this study may find novel approaches for treatment of this devastating disease with drugs specifically targeting the genes needed in the presence of melanoma cells.

## Enhanced Surgical Margin Imaging with Polarization-sensitive Optical Coherence Tomography in Canine Soft Tissue Sarcoma and Mammary Tumors

**Principal Investigator:** Laura Selmic, BVetMed, MPH; The Ohio State University

**Total Grant Amount:** \$49,227

**Grant Period:** 6/1/2021 - 5/31/2023

**Project Abstract:** Surgery is the most common treatment used for skin and mammary cancer in dogs. Currently, a pathologist determines whether surgery has removed all cancer cells many days after the procedure. However, rapid and accurate testing during surgery is needed to detect residual cancer to decrease cancer recurrence and the necessity for repeated surgery or treatments. Polarization-sensitive optical coherence tomography (PS-OCT) is a new imaging technology that uses near-infrared light waves to generate real-time, high-resolution images of the microscopic structure of tissues, specifically looking at the organization of the tissues. Investigators have performed initial evaluations using this optical coherence tomography for detection of residual cancer (including CHF grants 02758 and 02204-T), which has had very encouraging results. This study will focus on assessing whether PS-OCT could help us improve the accuracy to detect residual cancer in dogs following soft tissue sarcoma or mammary cancer removal. This project will open the door to veterinarians having the technology to allow accurate, real-time interpretation of surgical margins to minimize the necessity for additional surgeries or other treatments and to decrease tumor recurrence.

## Ultrasound-guided Histotripsy Ablation of Canine Brain Tumors through an Acoustically Transparent Cranial Window

**Principal Investigator:** John Rossmeisl, DVM, MS; Virginia-Maryland Regional College of Veterinary Medicine

**Total Grant Amount:** \$106,783

**Grant Period:** 6/1/2021 - 5/31/2023

**Project Abstract:** Meningiomas and gliomas account for 85% of canine primary brain tumors (PBT). These neoplasms are common in several dog breeds and a significant cause of morbidity and mortality. Surgery remains a mainstay of treatment for canine PBT, with the goal of achieving gross total surgical resection. However, significant proportions of canine PBT are considered inoperable due to their location in the brain, local recurrence after surgery remains a major mode of treatment failure, and conventional surgical techniques are invasive and often associated with adverse neurological events. As such, the clinical evaluation of the biologic effects of novel approaches for managing non-resectable or recurrent PBT is warranted to improve quality and quantity of life in dogs. In this study, the safety and feasibility of using ultrasound-guided histotripsy, a non-invasive and non-thermal acoustic method of tissue ablation, to treat canine primary brain tumors (PBT) will be investigated. This proof-of-concept of study will provide foundational data necessary to further evolve histotripsy technology towards completely non-invasive transcranial applications. Results have the potential to cause a clinical practice paradigm shift that will allow precision image-guided, non-invasive treatment of PBT using ultrasonographic equipment and techniques that are currently in use in the majority of small animal veterinary practices.

## Open-Label, Phase-2 Clinical Trial of Chlorambucil and Toceranib for Canine Mast Cell Tumors

**Principal Investigator:** Kristen Weishaar, DVM, MS; Colorado State University

**Total Grant Amount:** \$72,156

**Grant Period:** 3/1/2021 - 2/28/2023

**Project Abstract:** Mast cell tumor (MCT) is the most common malignant canine skin tumor. Although all breeds are at risk, Boxers, Boston Terriers, Bulldogs, Labrador Retrievers, Beagles, Vizslas, Rhodesian Ridgebacks, Weimaraners, Shar-Peis and Schnauzers are predisposed. While surgery remains the main treatment for most MCT, a subset have a high potential for eventual recurrence and metastasis and require systemic medical therapy following surgery in an attempt to delay or prevent eventual metastasis. Furthermore, many dogs may present with disease that is not amenable to resection owing to size, location, or multifocal or disseminated disease. Medical therapy for these patients remains suboptimal. Both low dose continuous (metronomic) chlorambucil (MC) and toceranib (TOC, Palladia) have some reported activity against MCT, and the combination is sometimes utilized clinically. However, (1) there have been, to date, no studies demonstrating that the empirically determined dose of MC exerts biologic effects; (2) the combination of MC and TOC has not been systematically evaluated for safety or efficacy in any tumor type. Investigators will address these critical knowledge gaps with a clinical trial to characterize the antitumor effects and adverse effect profile associated with chlorambucil/TOC in dogs with measurable MCT and quantify changes in circulating Treg and MDSC following MC and subsequent TOC treatment in the patient population. Lastly, they aim to describe alterations in previously identified biomarkers of TOC exposure and interrogate novel MC/TOC exposure biomarkers through gene-expression profiling of peripheral blood mononuclear cells. They hypothesize that a combination of MC and TOC will be well tolerated and will cooperatively reduce circulating regulatory T cells (Treg) +/- myeloid-derived suppressor cells (MDSC) in dogs with MCT, with the potential to translate into improved tumor control.

## Continued Investigation into Tumor-permissive Collagen Signatures in Canine Mammary Gland Tumors: Development of Prognostic Markers and Targeted Therapies for Improved Outcomes

**Principal Investigator:** Susan Volk, VMD, PhD; University of Pennsylvania

**Total Grant Amount:** \$197,473

**Grant Period:** 3/1/2021 - 2/28/2023

**Project Abstract:** Canine mammary gland tumors (CMT) are the most common malignancies in intact female dogs with the resulting morbidity and premature death having a profound impact on a large number of dogs, their owners and the veterinarians that treat them. While genetic alterations within tumor cells can promote their uncontrolled growth and ability to spread to distant sites (metastasis), normal, non-cancerous cells and networks of proteins including collagens found outside the cells also regulate tumor growth and metastasis. The researchers' recent study has identified specific cancer-associated collagen networks (signatures) in CMT biopsy samples that predict clinical outcome better than commonly used markers, potentially improving the ability of veterinary oncologists to accurately predict which dogs need immediate aggressive treatment to improve survival. They have also identified physiologic responses that drive the formation of cancer-associated collagen signatures that promote cancer progression and are optimizing a collagen-containing (Col3) biomaterial that prevents invasive and metastatic behavior of cancer cell lines in the laboratory. The goals of this project are to 1) improve the ability to predict which dogs will develop metastasis by including collagen signatures in a new predictive panel, 2) determine how tumor-permissive collagen signatures develop so that their formation can be stopped or reversed and 3) optimize the formulation of Col3 biomaterials for use during tumor-removal surgery to improve healing and inhibit residual tumor growth and metastasis. Based on studies using biopsy samples and cells in the laboratory, investigators predict that identifying and targeting tumor-permissive collagen signatures will improve both diagnosis and treatment of dogs with malignant CMT and change the trajectory of clinical care for patients. This work expands on Dr. Volk's previous AKC CHF-funded study #02489.

02987

New

One  
Health

## It's All in the Genes: The Mutational Landscape of Acute Myeloid Leukemia in Dogs

**Principal Investigator:** Tracy Stokol, BVSc, PhD; Cornell University

**Total Grant Amount:** \$109,183

**Grant Period:** 1/1/2022 - 12/31/2023

**Project Abstract:** Acute myeloid leukemia is a cancer of the blood. Although uncommon, it is a highly aggressive form of cancer and often kills dogs quickly, particularly because there are not many drugs that can treat leukemia. Great strides have been made in humans with acute myeloid leukemia, which is similar to the disease seen in dogs, and now there are new treatment options, longer patient survival, and the disease can be more accurately divided into subtypes to better inform treatment and prognosis. In fact, treatments are often tailored to the specific subtype of leukemia in the patient, which is known as precision medicine. All of these improvements in the diagnosis, treatment and prognostication of acute myeloid leukemia in humans have been made possible by genetic testing and identification of specific genetic defects or mutations that are responsible for the tumor. However, unlike humans, very little is known about the genetic mutations that underlie acute myeloid leukemia in dogs. In this multi-institutional study involving blood cancer specialists in veterinary and human medicine, investigators will perform in-depth genetic analysis of 50 dogs with acute myeloid leukemia by sequencing the genes within the tumor. Relevant genetic mutations will be identified by comparing gene sequences of the cancer cells to those of normal tissue. From this genetic analysis, investigators hope to identify mutations in acute myeloid leukemia in dogs that would be responsive to newer treatments or that could be targeted for development of new drugs. In turn, they could more accurately classify affected dogs into subtypes, which would help veterinarians better inform owners of prognosis and treat the dogs with more appropriate therapy, thereby prolonging their life, just as accomplished in humans.

03009

New

One  
Health

## Evaluating Accuracy for Identification of Sentinel Lymph Nodes in Dogs with Cutaneous MCT: A Comparison

**Principal Investigators:** Judith Bertran, DVM, MS and Natalie Worden, DVM; University of Florida

**Total Grant Amount:** \$49,982

**Grant Period:** 3/1/2022 - 12/31/2023

**Project Abstract:** Mast cell tumors are the most common skin cancer in dogs, and they often spread to nearby lymph nodes (LNs). Finding evidence that cancer has spread to a LN is necessary to determine how much longer the dog will live and whether the dog will require systemic cancer treatment, such as chemotherapy, in addition to surgical removal of the tumor. Also, the removal of cancerous LNs at the time of the tumor excision allows dogs to live longer. Therefore, it is important to accurately identify which LNs are the most likely to contain cancerous cells. This study will assess the use of an affordable, advanced imaging system that has been used in published human studies and can be used for veterinary surgical oncology purposes to find potential LNs that mast cell tumors of the skin may have spread to in dogs. Investigators will compare this advanced imaging system (near-infrared fluorescence imaging, or NIRF) to an established imaging system that is used for this purpose but must be done before surgery (indirect computed tomography lymphography, or CTL). They will compare the LNs identified by these imaging systems to the LNs that are traditionally sampled by veterinarians without access to advanced imaging. Two sampling methods (cytology and histopathology) on both the identified and traditionally sampled LNs will be tested to compare how well these methods detect cancerous cells in these LNs. The findings from this study will reveal more about the nature of mast cell tumors and validate the use of NIRF for detecting the draining LNs.

## Molecular Characterization of Canine Soft Tissue Sarcomas Using Spatially Defined Proteomics and Transcriptomics

**Principal Investigator:** Enni Markkanen, Dr. med. vet., Dr. sc. nat.; University of Zurich

**Total Grant Amount:** \$50,841

**Grant Period:** 3/1/2022 - 8/31/2023

**Project Abstract:** Soft-tissue sarcomas (STS) are frequent cancers that affect dogs of all breeds and can occur almost anywhere in the body. Therapy usually consists of surgical removal of these tumors, but it is difficult for the surgeon to distinguish the exact borders of the tumor, which often leads to incomplete removal and regrowth of the tumor in many affected patients. Novel approaches, such as specific anticancer drugs or tumor-cell-specific dyes to improve visualization during surgery have great potential to improve care for STS patients. However, the development of such approaches for STS is frustrated by a striking lack of molecular data to illuminate what mechanisms drive the growth of these tumors or to define targets that specifically differentiate tumor cells from the normal peritumoral tissue (PTT). This lack of knowledge on an extremely relevant canine tumor type warrants further investigation.

Using an innovative approach to analyze specific areas of archival patient samples by laser-capture microdissection (LCM), RNAsequencing, and proteomics, the overarching goal of this project is to gain a detailed molecular understanding of the very frequent STS subtypes: perivascular wall tumors (PWT) and peripheral nerve sheath tumors (PNST). This information will complement preliminary data for fibrosarcoma (FSA) and may enable development of future novel diagnostic and therapeutic modalities for patients, including identification of specific anticancer drugs and targeted tumor visualization strategies to guide surgical excision of STS. As complete tumor removal with clean margins is the most important factor to influence recurrence, metastasis, and survival in patients, precise visualization of STS has tremendous potential to improve the currently available options to treat these canine cancers. The knowledge gained through the study will work to improve the care and therapy of dogs with STS. Additionally, as canine STS are considered good models to better understand STS in humans, these results also have the potential to significantly impact human health from a One Health perspective.



03019

New

One  
Health

## Evaluation of a Targeted Anti $\alpha\beta 3$ Integrin Near-InfraRed (NIR-) Dye for Controlled Resection of Naturally Occurring Soft Tissue Sarcomas in Dogs

**Principal Investigator:** Mirja Nolff, Dr. med. vet., DVM; University of Zurich

**Total Grant Amount:** \$135,272

**Grant Period:** 3/1/2022 - 2/29/2024

**Project Abstract:** Soft tissue sarcomas are among the most common neoplasias of the skin and underlying tissue encountered in dogs. While they rarely spread to other organs, they tend to invade the surrounding tissues and grow unpredictably. Unfortunately, the surgeon cannot delineate these extensions by vision or touch, making it practically impossible to determine the true tumor borders during surgery. This is a very important limitation, as complete removal is essential to prevent regrowth. In order to compensate for the inability to define the true borders, the tumors are removed with a safety margin of 3 cm of surrounding tissue, which frequently result in very invasive surgeries. Nevertheless, complete resections are still not achieved in up to 30% of cases. If the surgeon would be able to actually see the true tumor borders while removing it, this important drawback could be addressed. Precise delineation of the tumor would enable the surgeon to remove the complete tumor while reducing the need for overly aggressive resections in unaffected regions. This study will evaluate the usefulness of a fluorescent dye that offers the chance to mark soft tissue sarcomas in dogs and make them shine under near-infrared (NIR) lighting during surgery. This study is designed to assess if the dye consistently and reliably marks tumor cells by comparing NIR-based resections with the standard approach to find out if 1) usage of the dye will increase the chance for complete resections and 2) the visible extension of the tumor under NIR light truly represents the histological extension of the tumor. As soft tissue sarcomas also occur in humans, the result of this study might not only serve to improve sarcoma treatment in dogs in the future but could also help to pave the way for improved treatment in humans.

# Oncology - Hemangiosarcoma

02510-T

## Identification of Novel Synthetic Lethal Partners to Optimize PI3K Targeted Therapies in Canine Hemangiosarcoma

**Principal Investigator:** Cheryl London, DVM, PhD; Tufts University School of Medicine

**Total Grant Amount:** \$168,857

**Grant Period:** 3/1/2018 - 2/28/2022

**Project Abstract:** Hemangiosarcoma (HSA) is a cancer of the cells lining the blood vessels that is very aggressive and has nearly always spread by the time it is diagnosed. HSA accounts for 5-7% of all cancers in dogs resulting in approximately 25,000-50,000 new cases per year. The treatment of choice is surgical removal followed by chemotherapy administration. Unfortunately, despite aggressive therapy, the majority of dogs diagnosed succumb to their disease within 6-8 months. Virtually no improvements in outcome for affected dogs have occurred in the past 30 years despite multiple clinical trials and research efforts. More recently a new therapy has been developed targeting two receptors on HSA cells. However, the majority of dogs still died by 10-12 months after treatment. The molecular pathway known as the PI3K/AKT/mTOR pathway has previously been implicated in the pathogenesis of many forms of cancer including HSA. Indeed, inhibitors of PI3K/AKT/mTOR pathway have some activity against HSA cell lines in the laboratory. The investigators have generated data showing that a subset of canine HSA tumors possess genetic mutations in PI3K that are known to activate the pathway in cancer cells. In this study, they will fully characterize the expression and function of PI3K in canine HSA tumor cell lines and tumor samples. This information will then be leveraged to identify new ways to block the PI3K/AKT/mTOR pathway using a combination of small molecule inhibitors that are most effective at killing tumor cells. These data will then be used to design future clinical trials in dogs with HSA.

## Prevalence of *Bartonella* spp. Infection in Dogs with Cardiac and Splenic Hemangiosarcomas Within and Between Geographic Locations

**Principal Investigator:** Edward Breitschwerdt, DVM; North Carolina State University

**Total Grant Amount:** \$219,026

**Grant Period:** 2/1/2018 - 1/31/2023

**Project Abstract:** Splenic masses comprise ~50% of all canine splenic disease. Despite advances in imaging and pathologic definition, the etiology and medical relevance of splenic lesions in dogs are often ambiguous. While some splenic tumors are benign, approximately two-thirds are highly malignant and carry a poor prognosis. Hemangiosarcoma (HSA) accounts for the majority of canine malignant splenic tumors and occurs in many large dog breeds, including mixed breeds. A less common site of HSA localization is the heart (cardiac HSA). Risk factors for both cardiac and splenic HSA remain unclear, confounding development of preventative strategies. The investigators recently reported a high prevalence of species of the bacterial genus *Bartonella* in dogs with HSA from North Carolina, suggesting a potential role in the initiation and/or progression of this cancer. *Bartonella* species exist worldwide and are transmitted by blood-sucking arthropods (e.g. ticks, fleas) and their presence in splenic tissue could potentially be explained by the fact that the spleen is primarily responsible for removal of blood-borne parasites from the systemic circulation. The investigators will perform a comprehensive examination of the potential association between *Bartonella* infection and HSA by comparing the prevalence of *Bartonella* DNA in tumor and blood samples from both splenic and cardiac HSA cases, and also within and between distant geographical locations in the US. Ultimately, demonstration of a robust association between *Bartonella* infection and the development of HSA may lead to new opportunities for improved diagnosis, treatment and prevention of this devastating cancer.

## **Clinical Trial for Evaluation of Propranolol and Doxorubicin in the Treatment of Canine Hemangiosarcoma**

**Principal Investigators:** Erin Dickerson, PhD and Antonella Borgatti, DVM, MS; University of Minnesota

**Co-Investigators:** David R. Brown, PhD; University of Minnesota, Michael O. Childress, DVM, MS; Purdue University, Jennifer Mahoney, DVM and Pascale Salah; University of Pennsylvania

**Total Grant Amount:** \$334,306

**Grant Period:** 7/1/2019 - 6/30/2022

**Project Abstract:** Canine hemangiosarcoma is a largely incurable cancer in dogs, and treatment approaches to improve outcomes have remained relatively stagnant over the past few decades. Treatment remains a challenge partly because the cancer is frequently detected at an advanced stage and because these tumors are often resistant to chemotherapies. Recently published reports showed that propranolol, a drug used to treat heart disease in humans and dogs, substantially increased the survival time of human angiosarcoma patients when used in combination with standard of care treatments. Propranolol was also shown to sensitize hemangiosarcoma cells to doxorubicin, providing a more effective way to kill tumor cells. Because angiosarcoma is strikingly similar to canine hemangiosarcoma, this multi-institutional clinical trial has been designed to determine the efficacy of propranolol in dogs with hemangiosarcoma when used in combination with surgery and chemotherapy. The main goal of the study is to establish whether propranolol in combination with doxorubicin following surgery improves outcomes for dogs when compared to the use of chemotherapy and surgery alone. The investigators will also evaluate the plasma concentrations of propranolol achieved during dosing to assess whether the levels of propranolol correlate to survival times. If successful, the findings from this approach will be rapidly conveyed to the veterinary community, and the guidelines provided to clinicians for the use of propranolol and doxorubicin for the treatment of canine hemangiosarcoma.

## Evaluation of Serum miRNA as a Diagnostic Tool for Canine Splenic Hemangiosarcoma

**Principal Investigator:** Janet Grimes, DVM, MS; University of Georgia

**Total Grant Amount:** \$15,000

**Grant Period:** 3/1/2020 - 8/31/2022

**Project Abstract:** Cancer is the leading cause of death in adult dogs, with hemangiosarcoma (HSA) being the most common tumor of the spleen. Affecting predominantly large-breed dogs, HSA is associated with a nearly 100% death rate with most dogs surviving 3-6 months with treatment. Other splenic masses occur in dogs which may have better prognoses; however, there are not currently available methods to accurately differentiate HSA from other masses of the spleen prior to surgery and histopathology. There is a critical need to identify the presence of HSA earlier than is currently possible. MicroRNA are short segments of RNA that control gene expression and have been shown to be involved in cancer progression. Studies have shown that microRNA present in dogs with HSA are different than those present in normal dogs and dogs with other tumors. The objectives of this study are to identify microRNA present in the blood of dogs with HSA before and after removal of the spleen, and to determine a panel of microRNA that can discriminate dogs with HSA from dogs with other masses of the spleen. This will allow for earlier diagnosis, leading to improved prognosis, and also assist with more accurate monitoring for disease recurrence/progression.

## Reprogramming the Tumor Immune Niche in Canine Hemangiosarcoma

**Principal Investigator:** Jong Hyuk Kim, DVM, PhD; University of Minnesota

**Total Grant Amount:** \$150,000

**Grant Period:** 7/1/2020 - 6/30/2022

**Project Abstract:** Hemangiosarcoma (HSA) is a common, devastating disease of dogs. The malignant tumor is seen frequently in older Golden Retrievers, German Shepherd Dogs, Portuguese Water Dogs, Labrador Retrievers, and Schnauzers, but it can occur in any dog of any breed at any age. Survival times of dogs with the tumor are short, even with surgical removal and standard of care treatment. Inflammation within the tumor tissue is common in canine HSA, and the immune response may contribute to tumor heterogeneity and prognosis for the dog. Yet, the immunological features in the context of the HSA niche are virtually unknown. The investigators have found that HSA cells have a strong capacity to promote proliferation and differentiation of hematopoietic stem and progenitor cells, with increased inflammatory cytokines, suggesting a niche regulatory function of HSA cells. This study will focus on understanding the functional relationships between HSA cells and immune cells that contribute to the tumor niche to identify molecular mechanisms that regulate critical signaling pathways in canine HSA. This approach will improve our understanding of the tumor immunity and heterogeneity, as well as aid in patient selection for novel immunotherapies.

## Strategic Prevention of Canine Hemangiosarcoma: Lifetime Follow-Up

**Principal Investigator:** Jaime Modiano, VMD, PhD; University of Minnesota

**Total Grant Amount:** \$269,238

**Grant Period:** 8/1/2020 - 7/31/2024

**Project Abstract:** The Shine On project is designed to utilize complementary technologies to reduce the impact of hemangiosarcoma in companion dogs. This novel, potentially disruptive approach is the first of its kind where artificial intelligence applied to the results of a blood test will be used to assign dogs to a risk category for the development of hemangiosarcoma. The test, called the Shine On Suspicion (SOS) Test is designed to detect hemangiosarcoma at its earliest stages of development before it becomes a clinically-detectable disease. Dogs that are considered to be at high risk based on the SOS Test results will be eligible to receive the drug eBAT for strategic prevention; that is, to eliminate emergent hemangiosarcoma tumors before they form. eBAT is a rationally designed drug developed in the laboratory to attack the cells that initiate and maintain the cancer, as well as to make the environment inhospitable for their growth. For the initial phase of the Shine On project, investigators developed and refined the SOS Test and the artificial intelligence methods to assign dogs to specific diagnostic categories and started to establish the utility of the test in early detection in a group of 209 presumably healthy, pedigreed Golden Retrievers, Boxers, and Portuguese Water Dogs, 6 years of age or older. In this continuation phase of the Shine On project, this group of dogs that had the SOS Test will be followed for their lifetimes to identify any diagnosis of cancer or another chronic disease, the cause of death, and date of death. In addition, a subset of dogs determined to be at high risk using the SOS Test will receive eBAT in the setting of prevention and also followed over their lifetime to establish their outcomes. This project expects to develop firm proof of concept to support larger clinical trials, and eventual deployment of this approach to the veterinary community setting for all dogs at risk of developing hemangiosarcoma.

*Funding for the research is provided through the collaborative efforts and generosity of the American Boxer Charitable Foundation, the Golden Retriever Foundation, the Portuguese Water Dog Foundation and the AKC Canine Health Foundation, which will oversee grant administration and scientific progress.*

## **Luteinizing Hormone Receptor Activation in Canine Hemangiosarcoma Cells**

**Principal Investigator:** Michelle Kutzler, DVM, PhD; Oregon State University

**Total Grant Amount:** \$11,718

**Grant Period:** 2/1/2021 - 7/31/2022

**Project Abstract:** Hemangiosarcoma is an aggressive, silent cancer that sometimes snares its victims without any sign of illness. In the U.S., hemangiosarcoma is believed to be responsible for the deaths of tens of thousands of dogs each year. German Shepherd Dogs, Golden Retrievers, and Labrador Retrievers are most commonly affected but this cancer affects all dogs. While there is no cure, early surgical intervention and chemotherapy treatment may prolong the lives of dogs afflicted with hemangiosarcoma. Additional treatment options are needed to increase life expectancy and possibly even prevent the development of this deadly disease. Several studies have shown that spayed female dogs have a two- to ten-fold increase for developing hemangiosarcoma compared to intact female dogs. This may be due to overproduction of luteinizing hormone (LH) following spay or neuter. Investigators have previously demonstrated that hemangiosarcoma tissues collected from dogs have binding sites for LH. The proposed research will determine if LH binding to these sites increases cancer cell growth. The results of this research may allow for a better understanding of the relationship between spaying or neutering and the development of hemangiosarcoma. In addition, future development of a method to reduce LH secretion in spayed or neutered dogs may lower the risk for some breeds to develop hemangiosarcoma.



## Genome-wide Molecular Interrogation of Canine Hemangiosarcoma

**Principal Investigator:** Scott Coonrod, PhD; Cornell University

**Total Grant Amount:** \$102,549

**Grant Period:** 9/1/2021 - 8/31/2022

**Project Abstract:** Hemangiosarcoma (HSA) is often called a “silent killer” because dogs with splenic forms of this disease usually do not show clinical signs until it is too late. Importantly, once at the hospital, it can be very difficult for clinicians to tell with certainty whether the mass is malignant or benign without removing the spleen (which is expensive) and performing histopathological analysis on the mass (which can take days to complete). Given this inability to distinguish HSA from benign disease at the time of initial presentation, there is an urgent need to develop new biomarkers that can rapidly differentiate HSA from non-life-threatening conditions so that owners can make informed decisions regarding the course of treatment. Investigators have recently begun to analyze gene expression in HSA tumors using a novel genome-wide technique called Chromatin-Run-On sequencing (ChRO-seq) in order to better document the molecular nature of HSA. In this study, investigators will expand on these initial studies and now use ChRO-seq and other cutting-edge genome-wide technologies to identify gene signatures and molecular features of these tumors. Outcomes from this study may also speed up the development of early detection screening tools for high risk dogs and help with prognosis in dogs with HSA.

## Towards Curative Outcomes in Canine Hemangiosarcoma

**Principal Investigator:** Chand Khanna, DVM, PhD; Ethos Discovery

**Total Grant Amount:** \$348,559

**Grant Period:** 11/1/2021 - 10/31/2026

**Project Abstract:** Canine hemangiosarcoma is the most aggressive cancer seen in all dogs, but disproportionately affects older, large breed dogs. Despite aggressive treatment with surgery and chemotherapy, more than 50% of dogs die due to metastatic spread of their cancer within 6 months and no significant advancements in the treatment of hemangiosarcoma have occurred in over 30 years. Several challenges have hindered improvements in outcomes including limitations in the capabilities of current imaging and/or blood-based markers to detect early relapse/metastasis, gaps in current understanding of the molecular biology of hemangiosarcoma, and a lack of effective therapies that effectively alter the aggressive metastatic behavior of this cancer. A nation-wide clinical trial for 400 dogs with splenic hemangiosarcoma seeks to deliver curative outcomes for dogs with this disease. With collaboration from AKC Canine Health Foundation, dog-owning families from outside the geographical enactment of 30 Ethos veterinary hospitals can join the trial to receive care, and to conduct genomic correlative studies with two internationally recognized scientific teams to gain critical new knowledge necessary to propel the field to this future goal of curative outcomes. Researchers will utilize a patient-forward approach and leverage genomic insight into hemangiosarcoma to propose and answer the following questions: (1) are there molecular biomarkers that predict prognosis in dogs with this disease?; (2) can we define genomic subgroups of dogs with hemangiosarcoma who are most likely to benefit from specific anticancer drugs?; (3) can we define new therapeutic approaches to prevent the spread of this cancer? This approach follows the road map used in transforming childhood leukemia from a fatal diagnosis to a commonly cured disease. By leveraging the strengths of a unique and multidisciplinary team of clinicians and scientists, this canine hemangiosarcoma study will lay the foundation for accelerating drug development and improving patient outcomes for dogs with this devastating disease.

## Suppression of Extracellular Glutamate Efflux & mGluR1 Signaling to Impede Canine Hemangiosarcoma Cell Growth

**Principal Investigator:** Timothy Fan, DVM, PhD; University of Illinois

**Total Grant Amount:** \$170,087

**Grant Period:** 3/1/2022 - 2/28/2025

**Project Abstract:** Canine splenic hemangiosarcoma (cHSA) is a highly malignant solid tumor that results in near universal fatality. The occult nature of splenic cHSA poses a clinical dilemma, as all too often, pet dogs harboring this deadly disease behave normally until the manifestation of overt, and often catastrophic, clinical symptoms. While emergent medical interventions including splenectomy and whole blood transfusions can often be life-saving, therapeutic benefit is usually transient given the remarkable proliferative capacity of cHSA cells. Unfortunately, most pet dogs will eventually decompensate from the rapid reseeding or metastases progression of cHSA within the peritoneal cavity or lungs.

Attempting to improve outcomes in dogs diagnosed with cHSA, most veterinary investigations have focused on evaluating systemic treatment options that might exert cytoreductive activities, yet survival times in dogs treated with cytotoxic combinations remain disappointing. An alternative strategy that has received limited attention is the exploitation of metabolic vulnerabilities that might exert cytostatic effects; and in the setting of non-terminal tumor burden, cytostasis of cancer cells might afford the opportunity for maintaining high quality-of-life and prolongation in survival times.

This investigation explores extracellular glutamate efflux and metabotropic glutamate signaling in sustaining cHSA cell proliferation. If the investigators' hypothesis is true, current FDA drugs can be repurposed to inhibit glutamate efflux and serve as novel adjuvant strategies for curbing the explosive outgrowth of cHSA cells. Targeting the metabolic dependency of glutamate efflux and consequent paracrine metabotropic signaling could improve the clinical management and survival of pet dogs afflicted with this deadly vascular malignancy.

## A GD3 Nano-scaled Liposomal Cancer Vaccine Clinical Trial for Canine Hemangiosarcoma

**Principal Investigator:** Rowan Milner, BVSc, MMedVet, PhD, Keijiro Shiomitsu, DVM, and Sandra Bechtel, DVM; University of Florida

**Total Grant Amount:** \$156,065

**Grant Period:** 3/1/2022 - 2/29/2024

**Project Abstract:** Hemangiosarcoma, a cancer of the lining of blood vessels, is a devastating cancer of numerous large breed dogs. Hemangiosarcoma (HSA) often presents as an emergency in otherwise healthy individuals with evidence of acute abdominal bleeding or bleeding around the heart. Owners are often distraught by the unexpected news. HSA is rarely diagnosed in the early stages as imaging of the abdomen is often not included in the yearly physical examinations. Sadly, most dogs die within 2-3 months of treatment because of the advanced stage (splenic rupture) of the cancer and spread to the lungs and other organs. The addition of chemotherapy improves survival times for dogs with splenic HSA, but only on average 145-180 days. Regrettably, there has been little improvement in survival in dogs with HSA in the last 30 years. However, modifying how the body's immune system reacts to the cancer offers new hope for improved survival. This research proposes to use a vaccine that investigators have extensive experience with in canine melanoma and bone cancer (osteosarcoma). New preliminary research indicates that the vaccine target, GD3, is present in HSA, similar to melanoma and osteosarcoma use. All dogs will get standard-of-care treatment and randomized into two groups - one getting the GD3-based vaccine and the other group receiving placebo. Since melanoma and osteosarcoma have shown promising results, investigators hope that dogs with HSA respond similarly to the vaccine.

# Oncology - Lymphoma

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## Precision Medicine for Canine Lymphoma

**Principal Investigator:** Nicola Mason, BVetMed, PhD; University of Pennsylvania

**Total Grant Amount:** \$86,400

**Grant Period:** 3/1/2018 - 2/28/2022

**Project Abstract:** The clinical response of dogs with lymphoma to multi-agent chemotherapy is highly variable. Although up to 85% of dogs respond initially, some relapse within weeks, while others enjoy remission times of two years. This heterogeneity in clinical response is in part explained by the recognition that "lymphoma" is not a single disease entity, but consists of different subtypes that can be characterized on a molecular level by mutations in specific genes. As in human medicine, it follows that different lymphoma subtypes, driven by different molecular mechanisms, may respond better to therapies that are specifically selected to inhibit the driver mechanisms within that patient's tumor. Recent work using sophisticated genetic sequencing tools (next-generation sequencing (NGS)) has begun to shed light on the different molecular subtypes of canine B cell lymphoma, and specific therapies aimed at targeting patient-specific driver genes and pathways are being developed. To enable targeted therapies to move into the clinic, a personalized diagnostic tool must be developed that can rapidly and cost-effectively determine the mutational profile of a patient's cancer allowing selection of the most effective drug for that patient. The investigators aim to develop a NGS diagnostic test that can be employed on standard biopsy samples to identify molecular drivers of a patient's lymphoma (personalized diagnostics), enabling the most appropriate targeted therapy to be selected for that patient. In addition, they aim to determine whether specific mutational profiles within canine lymphoma identified by their NGS panel are predictive of clinical outcome.

## Identifying Early Stage Ultra-rare Mutations as Predictive Biomarkers of Lymphoma in High-risk versus Low-risk Breeds Within the Dog Aging Project

**Principal Investigator:** Daniel Promislow, PhD; University of Washington

**Total Grant Amount:** \$75,600

**Grant Period:** 3/1/2020 - 6/30/2022

**Project Abstract:** The most common type of cancer in dogs is lymphoma, with ~80,000 cases diagnosed annually in the United States. Breeds vary in their risk of lymphoma, but it is unclear why there is variation despite considerable effort to identify the genetics of cancer risk and progression in dogs. Cancer typically arises from the accumulation of non-inherited (i.e. somatic) mutations. However, variation among breeds in cancer risk could be due to breed-specific variation in the types of mutations, the rate of accumulation of mutations, or the downstream effects of mutations in healthy dogs. This study will use novel sequencing technology to test the hypothesis that breed-specific lymphoma risk is due to variation in the frequency and type of rare precancerous mutations. Normally, measuring these low-frequency mutations has been beyond the range of standard sequencing technology, which is limited to detecting mutations present in >1% of cells. The new technology applied here represents a >10,000-fold improvement in accuracy, enabling the investigators to accurately detect a precancerous mutation present at a single site at a frequency of just one out of every 10 million DNA base pairs. By determining if mutation frequency in blood of healthy high-risk and low-risk dogs can predict lymphoma risk, this work could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma. This work has the potential to shed light on the mechanisms that underlie breed-specific variation in lymphoma risk, and in the long term, could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma.

## Exposure to Environmental Chemicals in Boxers with Lymphoma

**Principal Investigator:** Lauren Trepanier, DVM, PhD; University of Wisconsin, Madison

**Total Grant Amount:** \$108,751

**Grant Period:** 3/1/2021 - 2/28/2023

**Project Abstract:** Lymphoma is a common and deadly cancer in dogs, and Boxers are one of several high-risk breeds. While breed-related risk for canine lymphoma is likely inherited, there is also epidemiologic evidence for environmental and potentially modifiable risk. Lymphoma correlates with areas of higher industrial activity in both humans and dogs, but the specific chemicals putting dogs at risk are not understood. Previous studies have reported epidemiologic associations between canine lymphoma and air pollution, herbicides, and chemical solvents. Investigators recently found that lymphoma in Boxers was associated with living within 2 miles of a chemical supplier or an active crematorium. This study will use direct environmental and urine monitoring to evaluate exposures to several high-risk chemicals in dogs with lymphoma and compare them to unaffected matched controls. While a single breed study will decrease bias from inherited risk factors and possible breed-associated differences in chemical disposition, successful completion of these aims may support evidence-based avoidance or remediation recommendations to owners of Boxers and may be generalizable to other breeds at high risk for lymphoma.

## Characterizing the LINE-1 Transcriptome in Canine High-grade Peripheral T-cell Lymphoma by RNAseq to Gain Insight into Mechanisms of Drug and Immune Resistance

**Principal Investigator:** Paul Hess, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$33,234

**Grant Period:** 3/1/2021 - 8/31/2022

**Project Abstract:** High-grade lymphomas are common cancers of white blood cells in dogs. T-cell lymphoma is a particularly aggressive form associated with poor outcomes. Chemotherapy ultimately fails in T-cell lymphoma patients because of a tiny subpopulation of cancer cells – so-called minimal residual disease (MRD) – that resists most drugs, and eventually takes over, leading to short survivals. Researchers will investigate the role of “jumping genes,” a set of genes able to copy and paste themselves into new places in DNA, in T-cell lymphoma. Genes jumping to new spots is disruptive to the integrity of the genetic code, and is permitted only under certain circumstances but can occur when cells become cancerous. Investigators found that jumping genes are unusually active in canine T-cell lymphoma. When cancer cells can suppress jumping gene activity, they can better tolerate chemotherapy drugs and evade immune detection. Researchers hypothesize that MRD emerges during chemotherapy because that subset of cells hijacks a system normally used by reproductive cells to inhibit jumping genes. Investigators plan to use next-generation genetic techniques to define the currently unknown world of active jumping genes in T-cell lymphoma and investigate the molecular causes and consequences of their activity. A successful study will begin characterizing an unexplored pathway used by lymphoma cells, which could be an important new treatment target in a canine cancer that desperately needs novel therapies.



**Adoptive Natural Killer (NK) Cell Immunotherapy for Canine Lymphoma****Principal Investigator:** William Kisseberth, DVM PhD; The Ohio State University**Total Grant Amount:** \$149,979**Grant Period:** 9/1/2021 - 8/31/2023

**Project Abstract:** Natural killer (NK) cells are immune cells whose function are to eliminate virus infected and cancer cells from the body. In this clinical trial investigators will test the feasibility, safety and immunologic and biologic activity of adoptive NK cell therapy combined with chemotherapy to treat dogs with lymphoma. NK cells from healthy dogs will be isolated from their normal blood donations, expanded in the laboratory and cultured under conditions that enhance their function. These cells will then be given to dogs with lymphoma in combination with chemotherapy. Concentrations of NK cells in blood and lymph nodes and immunologic effects in treated dogs will be assessed using correlative assays. Successful completion of this trial will inform the development of future clinical trials using adoptive NK cell therapies for the treatment of lymphoma and other cancers and diseases.

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## Whole-Exome and Transcriptome Sequencing of Canine Small Cell B Cell Lymphoma and Comparative Analysis to Diffuse Large B Cell Lymphoma

**Principal Investigator:** Anne Avery, DVM, PhD; Colorado State University

**Total Grant Amount:** \$58,136

**Grant Period:** 3/1/2022 - 2/29/2024

**Project Abstract:** Lymphoma is a common canine tumor. Diffuse large B cell lymphoma is the most common type of B cell lymphoma in dogs, but there are less common B cell lymphoma types that have aggressive behavior and little is known about the mechanisms driving these tumors. The goal of this study is to genetically characterize these less common forms and compare these findings to diffuse large B cell lymphoma. This will inform whether targeted therapies for diffuse large B cell lymphoma, including ones developed for human patients, are useful for other B cell lymphoma types. Investigators will also study patient outcome in these cases to identify prognostic factors and work to develop tests that more accurately diagnose clinically important subtypes of B cell lymphoma. This work will improve our understanding of canine B cell lymphoma to advance diagnosis and prognosis and develop better therapies to improve outcomes.

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Health

## Chimeric BiTE-redirected Anti-viral T Cells for Fratricide of Minimal Residual Disease in T-cell Malignancies

**Principal Investigator:** Paul Hess, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$89,484

**Grant Period:** 3/1/2022 - 2/29/2024

**Project Abstract:** T cells are front-line soldiers deployed throughout the body to defend against infections and cancer. Sometimes, a rogue T cell itself can become cancerous. These resulting blood cancers (lymphoma or leukemia) are unusually resistant to traditional chemotherapy drugs. Immunotherapy has revolutionized the treatment of chemo-resistant B-cell blood cancers, but targeting T cells must be far more precise, to avoid creating AIDS-like conditions in the process. This study proposes to develop an agent that can target only designated “brigades” of the T-cell army that contain the cancerous soldiers. The remaining 90% of normal T cells are unaffected, and the defenses of blood cancer patients against infections are unharmed. Most importantly, this off-the-shelf agent contains no toxin or drug, and does not involve expensive cell-based therapy. Instead, the agent tricks normal T cells into seeing their cancerous cousins as infected, so they are killed to eliminate the sham infection. Further, vaccination against normal infections can boost these cancer-killing effects. This technology will re-direct T cells to police their own rogue elements to help improve outcomes for dogs, and their human counterparts, with deadly T-cell blood cancers.

# Oncology - Osteosarcoma

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## Identifying the Genes That Confer Risk for Osteosarcoma

**Principal Investigator:** Carlos Alvarez, PhD; The Research Institute at Nationwide Children's Hospital

**Total Grant Amount:** \$120,000

**Grant Period:** 1/1/2012 - 12/31/2021

**Project Abstract:** Osteosarcoma (OSA) is the most common cancer of the bone in both dogs and humans. A prime candidate for investigation of the genetic component of OSA is the Greyhound, which has the highest risk of OSA of any breed. However, despite significant effort, classical genetic approaches have not identified any Greyhound variant that accounts for most OSA cases in that breed. Dr. Alvarez proposes that Greyhound OSA variants have been directly or indirectly selected for in racing performance, consistent with the vastly elevated incidence in racing vs. show Greyhounds. If this is true and all racers carried an OSA mutation on both chromosomes, then this could not be detected using classical approaches (which require different genetic markers to distinguish cases v. controls). Here Dr. Alvarez proposes an innovative genetic approach that is impervious to the limitations described above, and enables genome-wide discovery of Greyhound variation with large effects on OSA risk. Such findings would lead to rapid development of therapies and clinical trials in dogs, and translation to human medicine.

## Defining the Functional Consequences and Therapeutic Vulnerability of Dystrophin Alterations in Canine Osteosarcoma

**Principal Investigator:** Cheryl London, DVM, PhD; Tufts University

**Total Grant Amount:** \$94,605

**Grant Period:** 4/1/2020 - 3/31/2023

**Project Abstract:** Osteosarcoma is the most common primary bone tumor in dogs, predominantly occurring in large and giant breed dogs such as the Great Dane, Irish Wolfhound, Rottweiler, Greyhound and Golden Retriever, among others. While surgery and chemotherapy help improve outcome for patients, over 90% of dogs will develop chemotherapy resistance and die due to disease progression within one year. Therefore, new treatment approaches are needed for dogs with osteosarcoma. The investigators previously performed whole genome sequencing of canine osteosarcoma tumors and identified large deletions in DMD, the gene that encodes the dystrophin protein. Loss of this protein is associated with more aggressive cancers in people, however virtually nothing is known about the role of dystrophin in canine osteosarcoma. This study will determine the incidence of DMD gene deletions across a larger number of osteosarcoma tumors and validate a targeted sequencing panel to rapidly identify these deletions in client-owned dogs with osteosarcoma. Additionally, the investigators will characterize the role of DMD deletions in tumor biology to determine the best way to treat osteosarcoma tumors with DMD deletions. This work will lay the groundwork for future prospective clinical trials targeting genetic mutations in dogs with osteosarcoma.

## Genetic Contribution to Early-onset Osteosarcoma

**Principal Investigator:** Susannah Sample, DVM, MS, PhD; University of Wisconsin, Madison

**Total Grant Amount:** \$161,718

**Grant Period:** 3/1/2020 - 2/29/2024

**Project Abstract:** Osteosarcoma is a devastating disease that affects many dog breeds. Although osteosarcoma is typically a disease of older dogs, in some breeds, such as the Irish Wolfhound, dogs can be affected at a young age. This study will investigate the genetic basis of early-onset osteosarcoma in the Irish Wolfhound breed. Osteosarcoma is responsible for ~20% of deaths in the Irish Wolfhound breed, with many dogs being diagnosed before 5 years of age. Consequently, there is a critical need to advance understanding of the genetic basis of early-onset osteosarcoma in the Irish Wolfhound. State-of-the-art DNA sequencing will provide insight into an osteosarcoma-associated genetic variant that strongly influences disease risk in young Irish Wolfhounds. This discovery, with subsequent development of a genetic screening test, will contribute to selective breeding decisions to decrease osteosarcoma prevalence in the Irish Wolfhound breed. Findings can then be applied to other osteosarcoma-predisposed breeds. The genomic research approach will also have substantial impact in method development for other genetic studies of rare diseases in dogs or diseases in rare breeds.

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## Investigating Extracellular Crosstalk Between Canine Osteosarcoma and Macrophages

**Principal Investigator:** Erika Gruber, DVM, PhD; North Carolina State University

**Total Grant Amount:** \$14,544

**Grant Period:** 3/1/2022 - 2/28/2023

**Project Abstract:** Osteosarcoma is the most common bone tumor in dogs, with increased risk for large and giant breeds. Osteosarcoma is an aggressive disease, and most dogs succumb to metastatic disease in the lungs, regardless of treatment. Tumors survive and thrive by reprogramming the host immune response to ignore abnormal tumor cells and even promote their growth and spread to other sites. How tumors, including osteosarcoma, exert control over the immune system is not well-understood. Preliminary work from the investigators' laboratory shows that canine osteosarcoma cells reprogram macrophages to increase the production of signals that have been associated with aggressive and metastatic human cancers. This study will determine whether canine osteosarcoma cells induce these changes through classic signaling molecules called cytokines, or through small lipid-bound cellular fragments called extracellular vesicles. Next, they will determine whether the signals released by macrophages stimulate growth or aggressive behavior in the osteosarcoma cells themselves. These studies will provide important insight into how canine osteosarcoma controls macrophages, with potential implications in the development of new therapies that slow or even halt tumor growth and spread.

## Evaluating Magnetic Resonance Imaging for Quantifying Histotripsy Ablation in Canine Osteosarcoma

**Principal Investigator:** Joanne Tuohy, DVM, PhD; Virginia-Maryland College of Veterinary Medicine

**Total Grant Amount:** \$25,380

**Grant Period:** 3/1/2022 - 2/28/2023

**Project Abstract:** Osteosarcoma (OS) is a devastating and common cancer in dogs. Large and giant breed dogs are predisposed to OS with Scottish Deerhounds, Rottweilers, Irish Wolfhounds, and Greyhounds associated with increased incidence of OS due to genetic factors contributing to increased risk inheritance in these breeds. Current standard-of-care treatment of canine OS involves resection of the primary tumor either via limb amputation or limb-salvage surgery, followed by adjuvant chemotherapy to delay metastatic disease. Limb salvage surgery is associated with high complication rates and not all dogs are appropriate candidates for limb amputation. Despite various permutations in chemotherapeutic regimens, the median survival for canine OS remains at 10-12 months and improved treatment options are needed. Histotripsy is a precision non-thermal focused ultrasound method that mechanically disintegrates tissues. Histotripsy can also potentially induce immune activation towards an anti-tumor immune response. These properties translate into a unique and exciting potential for histotripsy to be an effective non-surgical limb salvage treatment for the primary tumor, and serve as an immunotherapeutic capable of inducing an anti-OS immune response against metastatic disease to increase survival. With CHF-funding (grant #02773), the investigators have acquired data suggesting immune activation in response to histotripsy ablation of canine OS. Complete ablation of OS lesions using histotripsy can achieve non-surgical limb salvage and also potentially stimulate an anti-tumor immune response against metastatic disease. Thus histotripsy is uniquely poised to accomplish the ultimate goal of OS therapy – to target both the primary tumor and metastatic development. Accurately quantifying the degree of histotripsy ablation to confirm complete OS ablation is critical to the success of histotripsy. This study will evaluate the use of magnetic resonance imaging (MRI) to quantify the degree of histotripsy ablation in canine OS and represents a vital step towards translating histotripsy into clinical use as a standard-of-care therapy for dogs with OS.



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## Characterizing the Immunomodulatory Response of Histotripsy in Canine Osteosarcoma

**Principal Investigator:** Joanne Tuohy, DVM, PhD; Virginia-Maryland College of Veterinary Medicine

**Total Grant Amount:** \$54,918

**Grant Period:** 3/1/2022 - 2/28/2023

**Project Abstract:** Osteosarcoma (OS) is a devastating and common cancer in dogs. Large and giant breed dogs are predisposed to OS, with Scottish Deerhounds, Rottweilers, Irish Wolfhounds, and Greyhounds are associated with increased incidence of OS due to genetic factors contributing to increased risk inheritance in these breeds. Current standard-of-care treatment of canine OS involves resection of the primary tumor either via limb amputation or limb-salvage surgery, followed by adjuvant chemotherapy to delay metastatic disease. Limb salvage surgery is associated with high complication rates and not all dogs are appropriate candidates for limb amputation. Despite various permutations in chemotherapeutic regimens, the median survival for canine OS remains at 10-12 months and improved treatment options are needed. Histotripsy is a precision non-thermal focused ultrasound method that mechanically disintegrates tissues. Histotripsy can also potentially induce immune activation towards an anti-tumor immune response. These properties translate into a unique and exciting potential for histotripsy to be an effective non-surgical limb salvage treatment for the primary tumor, and serve as an immunotherapeutic capable of inducing an anti-OS immune response against metastatic disease to increase survival. Investigators will conduct an in-vitro study to further understand the effect of histotripsy on immune cells and on metastatic development in canine OS. This study will complement and broaden the canine patient immune evaluation the research team has previously performed, and advance the progress to develop histotripsy as an immunotherapeutic in OS. Harnessing the immunomodulatory potential of histotripsy could overcome metastatic disease and offer a major breakthrough in survival expectations for OS in dogs.

## The Immune and Molecular Landscape of Canine Osteosarcoma at the Single-Cell Level

**Principal Investigator:** Jaime Modiano, VMD, PhD; University of Minnesota

**Total Grant Amount:** \$161,903

**Grant Period:** 3/1/2022 - 2/29/2024

**Project Abstract:** The focus of this project is bone cancer(osteosarcoma), and the results will be especially relevant to large and giant dog breeds, and to mixed breed dogs with these breeds in their ancestry. Large dogs have an elevated risk to develop osteosarcoma, but there is little information available that can predict the speed at which the disease will progress or help guide treatment. Until recently, the prevailing dogma was that the immune system either ignored or was excluded from bone tumors. Using sensitive genomic methods, investigators showed that dogs (and children) that have immune cells present in their tumor survive longer than dogs (and children) where immune cells remain outside of their tumor. This is part of a growing body of evidence indicating that the immune system plays an important role in combating this disease. Two important questions regarding the role of the immune system in bone cancer remain unanswered: 1) “What is the precise identity of the immune cells that enter the tumor and benefit the patient?” and 2) “Where in the tumor do those cells need to be located to achieve this potential benefit?” By answering these questions, this project will inform the development of tests to guide treatment as well as new treatments to activate or enhance anti-tumor immune responses. The hypothesis is that bone tumors are segregated into 'neighborhoods' where productive anti-tumor immune responses are most likely to be initiated and sustained. Investigators will establish the extent to which tumor cells have followed separate evolutionary paths and created different subpopulations with unique behavior and susceptibility or resistance to treatment. They will identify, quantify, and characterize the diverse constituents of the bone cancer microenvironment (tumor cells, immune cells, supporting cells) at single-cell resolution, defining the 3-dimensional, spatial relationship of the cells in the bone cancer microenvironment using advanced methods. The 3-dimensional maps generated will help determine if and how immune cells are able to penetrate regions where the cancer cells reside, or if they are compartmentalized or excluded from these spaces. This project is the first to apply these technologies to bone cancer, and the information gained will further advance our understanding of how bone tumors form, aiding in earlier detection and prevention strategies.

## Genetics of Primary Angle Closure Glaucoma in American Cocker Spaniels

**Principal Investigator:** Sara Thomasy, DVM, PhD; University of California, Davis

**Total Grant Amount:** \$40,000

**Grant Period:** 5/1/2017 - 10/31/2023

**Project Abstract:** Glaucoma is a leading cause of irreversible blindness and globe removal (enucleation) in dogs. Primary angle closure glaucoma (PACG) is the most common form of glaucoma in dogs whereby acute blockage of the iridocorneal angle leads to a rapid increase in intraocular pressure. Consequently, PACG is painful, demands immediate medical attention, and often causes incurable vision loss. The American Cocker Spaniel (ACS) has the highest reported prevalence of any canine breed for PACG. The investigators will study the genetics of PACG in the ACS to identify potential disease-causing loci and variants. Dogs will be extensively phenotyped as PACG cases or controls using advanced imaging equipment used to investigate glaucoma in human patients. Identification of genetic markers associated with PACG in ACSs will facilitate the development of a genetic test to inform breeding programs. Furthermore, identification of the molecular basis of PACG may help elucidate novel therapeutic or testing strategies in the management of this blinding disease that may be translatable to the human condition.

## **Microphthalmia and Delayed Growth Syndrome in the Portuguese Water Dog**

**Principal Investigator:** Margret Casal, DVM, PhD; University of Pennsylvania

**Total Grant Amount:** \$12,960

**Grant Period:** 11/1/2017 - 10/31/2022

**Project Abstract:** Microphthalmia and delayed growth syndrome (aka "puppy eye syndrome") has been reported by Portuguese Water Dog breeders dating as far back as 1986. However, there is no information in the scientific literature and the majority of data concerning this syndrome has been obtained from records of breeders, which have anecdotal reports of the disease and little, if any, medical diagnostics. Affected dogs present with microphthalmia of varying severity, other eye abnormalities, short stature and other findings. To date, the investigators have been able to collect DNA from 24 affected dogs. Males and females can be affected, although females predominate (about 70%). Preliminary pedigree studies suggest an autosomal recessive inheritance. Human literature reports numerous syndromes associated with microphthalmia, and many genes have been identified as having a causative role. The goals of this investigation are to better characterize the clinical syndrome seen in Portuguese Water Dogs, confirm a suspected mode of inheritance, obtain additional samples for investigation into the genetic mutation, and develop a mutation based, genetic test for breeders to eliminate this syndrome from the Portuguese Water Dog breed.

*Funding for the research is provided through the collaborative efforts and generosity of the Portuguese Water Dog Foundation, Inc., and the Portuguese Water Dog Club of America. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## **Genetics of Primary Angle Closure Glaucoma in the Siberian Husky**

**Principal Investigator:** Gillian McLellan, BVMS, PhD; University of Wisconsin, Madison

**Total Grant Amount:** \$121,740

**Grant Period:** 3/1/2018 - 2/28/2023

**Project Abstract:** Glaucoma is a very painful and rapidly blinding disease that leads to irreversible sight loss in many thousands of dogs in the USA and worldwide each year. Current medical and surgical treatments that target the damaging high pressure in the eyes of affected dogs are not able to cure the disease but only control it. In many dogs with glaucoma, surgical removal of both eyes is needed to control pain. Past research reveals that the Siberian Husky is one of the more commonly affected breeds in both North America and Europe. With improvements in canine DNA sequencing tools, it is now possible to carry out very detailed sequencing of DNA of individual dogs, and these techniques have identified mutated genes responsible for several dog diseases. The investigators in this study will analyze DNA from Siberian Huskies with glaucoma and compare it to DNA from dogs without glaucoma. The goal is to identify the DNA mutation (or mutations) that cause glaucoma and, in turn, develop a genetic test for the disease in this breed and possibly other affected breeds such as the Samoyed and Shiba Inu. A DNA test would provide an important tool in efforts to fight this disease as dog breeders could develop more informed breeding strategies, with a goal to ultimately help eliminate this disease from the dog population.

## Development of a Polygenic Risk Model for Pigmentary Uveitis in Golden Retrievers

**Principal Investigator:** Wendy Townsend, DVM, MS; Purdue University

**Total Grant Amount:** \$89,855

**Grant Period:** 3/1/2019 - 8/31/2022

**Project Abstract:** Pigmentary uveitis (PU) affects 10% of senior Golden Retrievers and often results in blindness due to cataracts and glaucoma. There are no current methods to prevent or reverse the disease. The best options to maintain vision are early detection through annual ophthalmic examinations and early initiation of topical anti-inflammatory therapy. The disease does not develop until eight years of age or older, thus, affected dogs may have already been bred before their PU status is known. Using previous AKC CHF funding, the investigators have established a bank of Golden Retriever DNA and, in an initial genetic analysis, determined that PU involves not just one but multiple genes. The research problem remains to identify involved genes and risk alleles that are associated with PU. The objective of this study is to identify these genes and alleles using genome-wide association studies and whole genome sequencing of severely affected dogs in conjunction with analysis of RNA expression within the iris and ciliary body of affected dogs. Identification of the involved genes and risk alleles will allow for creation of a genetic risk score to quantify an individual's genetic risk for developing PU, allowing identification of high-risk individuals and intervention prior to the onset of clinical signs. In addition, breeders will have the necessary knowledge to decrease the prevalence of PU.

*Funding for the research is provided through the collaborative efforts and generosity of the Golden Retriever Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## Characterization of Retinal Phenotypes and their Association with RPGRIP1 and Modifiers in English Springer Spaniels

**Principal Investigator:** Keiko Miyadera, DVM, PhD; University of Pennsylvania

**Total Grant Amount:** \$99,303

**Grant Period:** 4/1/2019 - 3/31/2022

**Project Abstract:** Advances in molecular techniques have led to the identification of nearly 30 gene mutations that cause inherited retinal diseases in dogs, often leading to loss of vision. While an insert in the RPGRIP1 gene has been linked to a blinding retinal disease first found in Dachshunds, this same mutation is very common in English Springer Spaniels (ESSs). However, retinal diseases are rarely seen in this breed, raising the question as to whether the RPGRIP1 mutation by itself causes retinal disease. Notably, the research team has found similar mismatches between the mutation and the disease in Dachshunds, where the disease presentation varies greatly. In this breed, they found additional genetic factors or ‘modifiers’ that together with the RPGRIP1 mutation, are better able to predict the disease. This study will determine if these factors or additional factors yet to be identified also contribute to retinal disease severity in ESSs by 1) clinically characterize the spectrum of the retinal disease in ESSs, including functional tests to detect the earliest sign of disease in dogs with an apparently normal phenotype, and 2) study the relationship between the RPGRIP1 mutation and the disease status and then search for other genetic contributors specific to ESSs. By determining the role of the RPGRIP1 mutation in ESS retinal disease, a reliable DNA testing platform may be established.

*Funding for the research is provided through the collaborative efforts and generosity of the English Springer Spaniel Field Trial Association Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.*

## **Histologic Characterization of Golden Retriever Pigmentary Uveitis**

**Principal Investigator:** Wendy Townsend, DVM, MS; Purdue University

**Total Grant Amount:** \$11,793

**Grant Period:** 2/1/2019 - 6/30/2022

**Project Abstract:** Pigmentary uveitis affects an estimated 10% of senior Golden Retrievers and often results in blindness due to cataracts and glaucoma. There are no current methods to prevent or reverse the disease. Clinically, a known risk factor for the development of glaucoma in dogs affected by pigmentary uveitis is the presence of an undefined, amorphous material in the eye. On histologic examination of affected eyes, the amorphous material has been noted within uveal cysts, surrounding uveal cysts, coating the lens, and free floating in the anterior chamber. The material blocks fluid flow within the eye resulting in glaucoma. Limited analysis has shown the material is unusual with features of both collagen and an acid. This amorphous material is considered to play a significant role in the pathology of pigmentary uveitis and associated glaucoma, but little is known about its chemical composition, structure, or tissue of origin. The objectives of this study are: 1) determine if all Golden Retrievers with pigmentary uveitis have this amorphous material within their eyes, or if only a specific subset; 2) define the chemical composition of the amorphous material; and 3) determine the ocular tissue producing the material. This information may play an important role to help develop preventative or therapeutic measures for pigmentary uveitis and define phenotype(s) for genetic studies.

*This research is co-funded through the collaborative efforts of the Golden Retriever Foundation and the AKC Canine Health Foundation*



## Identification of Genetic Risk Factors for Primary Closed Angle Glaucoma and Pectinate Ligament Abnormality in the Basset Hound

**Principal Investigator:** Cathryn Mellersh, PhD; University of Cambridge

**Total Grant Amount:** \$29,750

**Grant Period:** 3/1/2019 - 9/30/2022

**Project Abstract:** Primary glaucoma is a painful and blinding disease associated with abnormally high intraocular pressure. Treatment in dogs is usually unsuccessful, and most affected dogs ultimately require removal of their eyes. The most common form of canine primary glaucoma is primary closed angle glaucoma (PCAG) which is significantly associated with pectinate ligament abnormality (PLA), also referred to as pectinate ligament dysplasia (PLD), an abnormality affecting the drainage angle of the eye. PCAG and PLA are prevalent in several breeds, and PLA is highly heritable. Not all dogs with PLA develop glaucoma, however, indicating that the inheritance of PCAG is complex. This complex inheritance and the progressive nature of PLA mean that breeding strategies based on ophthalmic examinations alone are unlikely to be sufficient to eliminate the disease. PCAG and PLA appear to be prevalent in the Basset Hound (BH) both in Europe and the U.S. Two genetic regions have been identified in the BH which are strongly associated with PCAG but not with PLA in European and USA BH dogs. This study will sequence the genomes of European and USA BH dogs with PCAG and PLA to identify variants that segregate with PCAG (and not PLA). Follow-up on PCAG candidate variants in large cohorts of European and USA BH will be performed to confirm association with disease in the breed. The ultimate aim is to develop DNA tests for PCAG in the BH that will reduce disease prevalence when used in parallel with breed-specific guidance for breeders while simultaneously allowing for breeding of BH with PLA that are not at risk of PCAG.

## **Efficacy and Safety of Netarsudil for Canine Corneal Endothelial Dystrophy**

**Principal Investigator:** Sara Thomasy, DVM, PhD; University of California, Davis

**Total Grant Amount:** \$116,640

**Grant Period:** 2/1/2020 - 1/31/2023

**Project Abstract:** The corneal endothelium is primarily responsible for maintenance of corneal dehydration and transparency, which is critical for normal vision. Corneal endothelial dystrophy (CED) is a late-onset disease in dogs whereby the endothelial cells prematurely degenerate resulting in progressive corneal swelling, vision loss and ocular pain due to corneal ulceration. Secondary corneal infection and perforation can occur necessitating eye removal. Currently, the only definitive treatment for CED and a similar disease in human patients, termed Fuchs' endothelial corneal dystrophy (FECD), is corneal transplantation. However, corneal transplants are rarely performed in dogs due to the risk for graft rejection, lack of appropriate donor tissue, and expense. Alternative treatments for canine CED are urgently needed. Preliminary work demonstrated that a rho-associated kinase coiled-coil containing protein kinase (ROCK) inhibitor accelerated corneal endothelial regeneration. Netarsudil 0.02% ophthalmic solution (Rhopressa®) is a topical ROCK inhibitor and norepinephrine transport inhibitor recently approved by the Food and Drug Administration (FDA) for use in patients with glaucoma. Preliminary data suggest that netarsudil accelerates corneal endothelial recovery. This study will investigate the efficacy and safety of netarsudil for the treatment of early canine CED. If successful in dogs, netarsudil findings may translate to use for treatment of FECD in human patients.

## Effect of Glycemic Control on the Onset of Cataract Development in Diabetic Dogs

**Principal Investigator:** Michal Mazaki-Tovi, DVM; The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem

**Total Grant Amount:** \$15,000

**Grant Period:** 8/1/2021 - 7/31/2023

**Project Abstract:** Diabetes mellitus is a common endocrine disorder in dogs. Its most common long-term complication is cataract formation, occurring in about half of the dogs within six months of diagnosis. Diabetic cataracts typically develop quickly, causing blindness and requiring expensive surgery to restore vision. It is generally assumed that tight control of blood glucose levels delays the onset of cataract development, however, indicators of short-term glucose control were not associated with the risk of cataract in dogs. Since tight glucose concentration control with insulin treatment increases the risk for dangerously low glucose concentrations, the potential of such a treatment to prevent or delay cataract formation should be proven before it is routinely recommended. Investigators hypothesize that poorer long-term glucose control is associated with earlier onset, and faster progression of cataract formation in diabetic dogs. Ten newly diagnosed diabetic dogs without ocular disease will be enrolled for six months. Glucose control will be evaluated by continuous glucose monitoring in addition to bi-weekly clinical assessment and blood tests. Ophthalmic examination will be performed bi-weekly, and the presence of a cataract and its stage will be documented. Associations between the time of onset and rate of progression of cataract and measures of glucose control prior to its formation will be determined. If such an association is verified, further study will be warranted to determine whether a strict insulin protocol to achieve tight glucose control might delay the onset of cataracts in diabetic dogs.

## **Late-onset Hereditary Cataract in the Boston Terrier: A Whole Genome Sequencing Approach**

**Principal Investigator:** Kathryn Graves, PhD; University of Kentucky Research Foundation

**Total Grant Amount:** \$8,541

**Grant Period:** 8/1/2021 - 7/31/2022

**Project Abstract:** Identifying mutations for heritable cataracts in dogs has proved to be frustrating; with only one gene (HSF4) being discovered to date that harbors mutations associated with cataract in four breeds of dogs, including the Boston Terrier. At least two types of cataract are found in the Boston Terrier, an early-onset hereditary cataract (EHC) caused by the HSF4 mutation, and a late-onset hereditary cataract (LHC) found in dogs over four years of age. Factors confounding the discovery of additional mutations include inconsistencies in tracking cataract progression, such as age of onset and rate of progression, in addition to the location of the cataract within the lens. Advances in genetic technologies such as whole genome sequencing provide a potential avenue to discovery of the causative mutations for hereditary cataracts in dogs, a vexing problem for breeders and owners. This study aims to take advantage of the significant decrease in the cost of whole genome sequencing of individual animals, coupled with access to a family of Boston Terriers where cataract occurrence and progression has been tracked across generations.

## Genetics of X-linked Progressive Retinal Atrophy in Greyhounds

**Principal Investigator:** Sara Thomasy, DVM, PhD; University of California, Davis

**Total Grant Amount:** \$42,500

**Grant Period:** 11/1/2021 - 10/31/2022

**Project Abstract:** Progressive retinal atrophy (PRA) is a group of heritable retinal diseases characterized by retinal degeneration in both eyes which progresses to blindness and for which no treatment exists. Most forms of PRA occur via an autosomal recessive form of inheritance with only a few X-linked PRAs reported due to degeneration of areas of the retina that express the defective X chromosome. In humans, X-linked PRA causes particularly severe disease, thus canine models of this condition would be particularly beneficial for identifying novel genetic causes and developing new therapies for both species. The research team has identified 72 racing Greyhounds with vision impairment typically initially observed at 1.5-4 years of age and confirmed PRA in 46 dogs. Of the 72 Greyhounds, 69 are male which strongly implicates an X-linked pattern of inheritance. Importantly, this disease appears widespread through selection of daughters of a popular sire. In dogs, two types of X-linked PRAs have been described with mutations in the gene RPGR and sequencing of the exon containing these mutations in one PRA-affected Greyhound determined that they were not present. Investigators have also identified three putative carrier females with PRA, although the phenotype is less severe. This preliminary data suggests that a new X-linked PRA likely exists in racing Greyhounds with the potential to dramatically impact the well-being and adoptability of these visually impaired dogs. Furthermore, AKC registered Greyhounds may also be affected as the frequency of carriers is currently unknown. This study aims to identify the causal mutation, develop a genetic test, define the phenotype in carrier females, and determine the prevalence of the causal variant in Greyhounds.

## **Genetic Basis of non-HSF4 Hereditary Cataracts in a Family of Miniature American Shepherds**

**Principal Investigator:** Kari Ekenstedt, DVM, PhD; Purdue University

**Total Grant Amount:** \$23,874

**Grant Period:** 10/1/2021 - 9/30/2022

**Project Abstract:** Hereditary cataracts (HC) are one of the leading causes of blindness in dogs, observed in almost one hundred breeds. To date, only two genetic tests are available for HC, and they are only applicable to a small number of breeds. Both tests are in the same gene (called HSF4); one applies to Boston Terriers, French Bulldogs, and Staffordshire Bull Terriers and the other applies to Australian Shepherds. However, neither test explains all of the HC in these breeds. Miniature American Shepherds (MAS) are a breed developed out of the Australian Shepherds and investigators have recently collected DNA samples from a family of MAS containing multiple dogs affected with severe, rapidly progressive, adult-onset HC that were not explained by the available HSF4 Australian Shepherd test. The cataracts progressed so quickly that each affected dog required corrective surgery within months of diagnosis. Researchers will use this MAS family to search for new disease-associated genetic variants, via whole-genome sequencing. Once such a disease-associated mutation is identified, they will use additional MAS with known HC status to validate the mutation, and then establish the prevalence of that mutated allele in the breed at large. Investigators will also see if the mutated allele is present in Australian Shepherds. This study's goal is to create a new genetic test for HC to help reduce the frequency of HC in the MAS and potentially other breeds.

## **Genetics of Glaucoma in the Entlebucher Mountain Dog**

**Principal Investigator:** Gillian McLellan, BVMS, PhD; University of Wisconsin System Board of Regents

**Total Grant Amount:** \$29,951

**Grant Period:** 10/1/2021 - 9/30/2023

**Project Abstract:** Glaucoma is a very painful and rapidly blinding disease that leads to irreversible loss of sight in many thousands of dogs in the United States and worldwide every year. Medical and surgical treatments that target the damaging high pressure in the eyes of affected dogs are not able to cure the disease but only control it. In many dogs with glaucoma, surgical removal of both eyes is needed to control pain. Glaucoma has been recognized as an increasing problem in the Entlebucher Mountain Dog in the United States and Europe. Using powerful DNA sequencing tools to compare the DNA sequences of Entlebucher Mountain Dogs with and without glaucoma, researchers will identify mutated genes associated with glaucoma in this breed, with the goal of developing a genetic test for the disease in this breed and possibly other related breeds. Due to the current lack of effective treatments for glaucoma, a DNA test would provide an invaluable resource for breeders seeking to preserve genetic diversity while formulating breeding strategies to eliminate this painful, disabling disease from the dog population.

New

One  
Health

## Corneal Cross-linking as Treatment for Corneal Ulceration - “Using Light to Save Sight”

**Principal Investigator:** Simon Pot, DVM; University of Zurich

**Total Grant Amount:** \$30,000

**Grant Period:** 2/1/2022 - 7/31/2024

**Project Abstract:** The cornea is the transparent front window of the eye, predominately made of highly organized collagen fibers. A corneal ulcer can occur due to corneal injury and is a painful condition that can lead to eye rupture if not treated adequately. Ulcers can deteriorate quickly and turn into ‘melting ulcers’ when bacteria infect the wound, cause inflammation, and lead to the production of enzymes which dissolve the corneal collagen fibers. Brachycephalic dog breeds are particularly susceptible to the development of corneal ulcers but all dogs can be affected. Corneal ulcer treatment aims to eliminate bacteria, stop the melting process, and allow normal healing to resume. First-line treatment involves medical therapy by frequent application of antibiotic and enzyme inhibitor eyedrops, as well as pain relief. Treatment success varies, and antibiotic resistance remains a concerning issue. If intensive medical therapy does not achieve this goal, surgery is often indicated.

Corneal cross-linking (CXL) was introduced in human medicine to increase tissue strength in weakened areas of the cornea and is used as corneal ulcer treatment to resist enzymatic digestion. CXL also effectively kills both antibiotic-resistant and sensitive bacteria. The 15-minute CXL procedure involves the application of Riboflavin (Vitamin B2) drops onto the cornea and illumination with ultra-violet (UV) light. Despite evidence suggesting that CXL helps heal patients with corneal ulcers, it is not clear that CXL works better than or equally well as existing medical therapy in dogs. In this study, 10 animal hospitals have joined efforts to launch a clinical trial to determine whether CXL will allow canine patients to heal more quickly and with a lower risk of deterioration compared to state-of-the-art medical therapy. The research project can lead to CXL becoming a routine clinical treatment modality with the potential to transform corneal ulcer treatment and increase treatment success rates and overall patient welfare.



# Reproductive Conditions

## Prospective View into the Use of Antimicrobials in Canine Pyometra and Prognostic Risk Factors for Postoperative Infection and Hospitalization

**Principal Investigator:** Sari Mölsä, DVM, PhD; University of Helsinki

**Total Grant Amount:** \$47,342

**Grant Period:** 9/1/2020 - 8/31/2024

**Project Abstract:** Increasing antimicrobial resistance is one of the biggest threats to human and animal welfare and refraining from the use of unnecessary antimicrobials is the only way to control this challenge. This study evaluates the use of antimicrobials in canine pyometra and risk factors for postoperative infection and hospitalization. Pyometra is a common disease of the reproductive tract. Ovariectomy or ovariohysterectomy are surgical procedures commonly used to prevent reproduction and uterine infectious diseases in female dogs. Medication is commonly prescribed postoperatively but there are no reliable data on its necessity. Emerging resistance in *Escherichia coli*-bacteria associated with pyometra is known to hamper the antimicrobial effect. Further, some dogs require prolonged hospitalization postoperatively, however, the predictive factors for hospitalization are unknown.

The aims of the study are to identify the dogs that benefit from antimicrobial treatment after surgery of uncomplicated pyometra, and to determine whether there is a difference in occurrences of postoperative surgical site infections (SSIs) or urinary tract infections (UTIs) in dogs that receive a course of antimicrobials postoperatively and those that do not. Further aims are to identify and characterize the bacterial strains; compare the antimicrobial resistance and virulence among bacterial isolates from the uterus and urine as well as the bacteria causing postoperative surgical site infection; and evaluate the utility of a recently validated scoring system in predicting the need for prolonged hospitalization in canine pyometra patients. Based on the findings, the use of antimicrobials may be minimized in patients that would not benefit from the medication and targeted to the patients that actually need it, improving suitability as well as cost in the treatment of canine pyometra.

# Tick-Borne Disease Initiative

02819

One  
Health

## Identification of *Bartonella henselae* In Vivo Induced Antigens for Development of a Reliable Serodiagnostic Assay for Canine Bartonellosis

**Principal Investigator:** Edward Breitschwerdt, DVM; North Carolina State University

**Total Grant Amount:** \$52,317

**Grant Period:** 1/1/2021 - 12/31/2022

**Project Abstract:** *Bartonella*, a genus of gram-negative bacteria, are associated with a wide spectrum of life-threatening diseases in animals and humans. More than 40 *Bartonella* species have been reported to infect mammalian reservoir hosts, and infection often leads to chronic bacteremia. At least ten *Bartonella* species have been implicated in association with serious diseases in dogs, including endocarditis, hemangiosarcoma, myocarditis, peliosis hepatis, polyarthritis and vasculitis. Despite biomedical advances and ongoing research in the field of canine bartonellosis, currently available PCR, culture, and serological based assays lack sensitivity for diagnosis of bartonellosis. Dogs throughout the United States and much of the world are exposed to *Bartonella* species. From a public health perspective there is an increased risk of direct and vector-borne transmission of *Bartonella* species from animals to humans. These factors justify the need for the ongoing development of a reliable serodiagnostic modality and ultimately an effective vaccine for prevention of bartonellosis in dogs. We will employ In-Vivo Induced Antigen Technology (IVIAT) to identify *Bartonella* in-vivo induced antigens, which will allow us to evaluate their potential as diagnostic markers for canine bartonellosis. This proposed study will result in development of a novel and sensitive ELISA assay for diagnosing *Bartonella* infection in dogs and will provide insights into the development of effective vaccine candidates for preventing *Bartonella* infection.

## Mechanisms of NK(T) Cell Mediated Inflammation during Canine Lyme Disease

**Principal Investigator:** Christine Petersen, DVM, PhD; University of Iowa

**Total Grant Amount:** \$160,677

**Grant Period:** 12/1/2020 - 11/30/2022

**Project Abstract:** Tick-borne diseases are found in all 50 states of the United States and are the most common vector-borne disease diagnosed in the U.S. Lyme disease, caused by *Borrelia burgdorferi* and related species (*sensu lato*), is the flagship disease for this in both dogs and people. Another important canine tick-borne disease found in combination with *B. burgdorferi* is anaplasmosis caused by *Anaplasma platys* or *A. phagocytophilum*, which also causes disease in people. Dogs are sentinel species for human tick-borne disease, often reporting disease before humans are found to be infected in an area. Additionally, immune responses and disease outcomes are very similar between people and dogs, meaning that important lessons can be learned by sharing information between human and veterinary medicine (One Health). The University of Iowa research group has a strong record for understanding mechanisms of canine immunity to vector borne diseases. Through these studies, they have developed important immunological tools to follow canine immune responses over time as dogs become infected and get sick. In this study, researchers will establish how the canine immune systems functions or malfunctions during clinical disease caused by *Borrelia burgdorferi* (Lyme disease), compared with healthy dogs that have subclinical infections. These studies will allow investigators to understand which immune cells drive disease versus disease protection. This will also allow investigators to identify how to target the molecules the immune cells produce to alter the course of Lyme disease in all dogs.

**Babesia Species in Thrombocytopenic Dogs in the Upper Midwest, USA****Principal Investigator:** Erin Lashnits, MS, DVM, PhD; University of Wisconsin, Madison**Total Grant Amount:** \$14,805**Grant Period:** 10/1/2021 - 9/30/2022

**Project Abstract:** Canine babesiosis is a severe disease caused by several *Babesia* species and is an important cause of low platelets (thrombocytopenia) in dogs. Since thrombocytopenia and regenerative anemia are common laboratory findings in dogs with babesiosis, it can be difficult for veterinarians to distinguish babesiosis from primary idiopathic autoimmune diseases, particularly immune mediated thrombocytopenia (ITP) and immune mediated hemolytic anemia (IMHA). Because of this, without specific testing for *Babesia* infection, dogs with babesiosis can easily be misdiagnosed and treated with immunosuppressive medications or splenectomy, worsening their prognosis for recovery. The geographic distribution of *Babesia* in dogs and whether *Babesia* spp. are endemic in particular areas of the United States are therefore critical pieces of epidemiologic information for clinicians to correctly diagnose and treat thrombocytopenic dogs. The prevalence – or indeed even existence – of canine babesiosis in the upper Midwest of the United States is unknown. The increasing prevalence of *Babesia* infection in humans and ticks in this region, along with advances in diagnostic testing and treatment, make this an important pathogen to investigate. This straightforward observational study will investigate infection with, and exposure to, *Babesia* species in dogs in this understudied area of the country, allowing improved diagnosis and treatment, as well as prevention, of this potentially fatal disease.

## Genome-wide Identification and Characterization of Peptide Epitopes from *Ehrlichia canis* and *Anaplasma platys* with Potential to be Used as Vaccine Candidates

**Principal Investigator:** Sreekumari Rajeev, BVSc, PhD; University of Tennessee

**Total Grant Amount:** \$100,244

**Grant Period:** 2/1/2022 - 1/31/2024

**Project Abstract:** *Ehrlichia canis* and *Anaplasma platys* are tick-borne canine pathogens and cause serious and debilitating illness in dogs. There is a critical need to improve diagnostics and to develop vaccines and novel treatment strategies. The goal of this study is to identify vaccine candidates and diagnostic markers using an advanced genomic approach. Investigators will identify potential vaccine candidates by exploring all proteins in the pathogen's genome in an approach termed reverse vaccinology. This approach will circumvent the expensive and time-consuming procedures in traditional vaccine design and development. Researchers aim to identify unique and shared molecules that could be used to develop a common vaccine for both agents. After initial prediction using computer analysis of *A. platys* and *E. canis* genomes, they will experimentally validate use through laboratory assays. The long-range goal of this work is to develop vaccines and improve diagnostics and intervention strategies to alleviate *E. canis* and *A. platys* infection in dogs. Tick-borne diseases such as those caused by *E. canis* and *A. platys* have a global distribution. Proactive prevention, accurate diagnosis, and prudent treatment are critical aspects of disease control. Results from this project will have a global impact on influencing the health and wellbeing of dogs and their owners.

# Clinician-Scientist Fellowship Program

The AKC Canine Health Foundation Clinician-Scientist Fellowship Program supports young scientists. Through this effort our mission to prevent, treat and cure canine disease will endure for years to come.

Recipients are selected based upon the following criteria for a resident/graduate student:

- 1) Enthusiasm for pursuing a career in canine health research,
- 2) Research aligns with CHF's mission to advance the health of all dogs,
- 3) Research abides by CHF policies, including our Humane Use of Animals Policy.

## 2022 AKC Canine Health Foundation Clinician-Scientist Fellows

**Sarvenaz Bagheri, DVM; Washington State University**

**Mentor: Yael Merbl, BSc, DVM, DECVN**



03034-E

Dr. Sarvenaz Bagheri is a combined neurology/neurosurgery resident at Washington State University College of Veterinary Medicine. Under the mentorship of Dr. Merbl, she will study effect of N-Acetylcysteine on dogs with spinal cord injury.

*This fellowship is generously sponsored by owners Carolyn and Gary Koch along with breeders Kristy and Kevin Ratliff in honor of "Rumble," GCHP Hill Country's Let's Get Ready To Rumble.*



**Rachel V. Brady, DVM; Colorado State University**



023033-E

Dr. Rachel V. Brady is a doctoral student in the Cell and Molecular Biology Graduate Program at Colorado State University. Under the mentorship of Drs. Duval, Dow, and Thamm, she will study genetic alterations that drive diffuse large B cell lymphoma as well as tumor and immune system interactions in osteosarcoma (bone cancer).

*This fellowship is generously sponsored by the Orthopedic Foundation for Animals (OFA).*

**Mentors: Dawn Duval, PhD; Steven Dow, DVM, PhD, DACVIM; and Douglas Thamm, VMD, DACVIM (Oncology)**

**Lopamudra Kher, BVSc&AH, MVSc, MS; University of Florida**



02924-E

Dr. Lopamudra Kher is a doctoral candidate in the Small Animal Clinical Sciences Department of the University of Florida College of Veterinary Medicine. Under the mentorship of Dr. Domenico Santoro, she will study the effect of signaling molecules associated with canine atopic dermatitis on *S. pseudointermedius* bacteria.

*This fellowship is generously sponsored in part by the Westie Foundation of America.*

**Mentor: Domenico Santoro, DVM, MS, DrSc, PhD**

**2021 AKC Canine Health Foundation Clinician-Scientist Fellows**

**Josephine Dornbusch, DVM; The Ohio State University**



02922-E

Dr. Josephine Dornbusch is a small animal surgery resident at the Ohio State University College of Veterinary Medicine. Under the mentorship of Dr. Laura Selmic, she will evaluate the clinical efficacy of a novel technique for urethral catheterization of female dogs weighing less than 22 pounds (toy breeds and puppies).

*This fellowship is generously sponsored by Rumble's owners, Carolyn and Gary Koch and breeders Kristy and Kevin Ratliff.*

**Mentor: Laura Selmic, BVetMed (Hons), MPH, DACVS-SA, DECVS**



**Skylar Sylvester, DVM; Cornell University**

**Mentors: Cheryl Balkman, DVM, DACVIM (Oncology)  
& Kelly Hume, DVM, DACVIM (Oncology)**



02923-E

Dr. Skylar Sylvester is a medical oncology resident at Cornell University College of Veterinary Medicine. Under the mentorship of Drs. Cheryl Balkman and Kelly Hume, she will lead a clinical trial to evaluate the efficacy of temozolomide in addition to standard doxorubicin therapy in dogs with splenic hemangiosarcoma.

*This fellowship is generously sponsored by the Orthopedic Foundation for Animals.*

## **2020 AKC Canine Health Foundation Clinician-Scientist Fellow**

**Pradeep Neupane, MS; North Carolina State University**

**Mentor: Edward Breitschwerdt, DVM**



02787-E

Mr. Neupane is a doctoral candidate in the Intracellular Pathogens Research Laboratory at North Carolina State University. Under the mentorship of Dr. Edward Breitschwerdt, he is studying serodiagnostic testing options for infection with canine *Bartonella spp.* and evaluating the association between Bartonella infection and hemangiosarcoma in dogs.

*This fellowship is generously sponsored by the American German Shepherd Dog Charitable Foundation, Inc. and Briard Club of America Health and Education Trust.*

**To support this program visit: [akcchf.org/clinsci](https://akcchf.org/clinsci)**



# Theriogenology Residency Program

This program is a collaboration between the American Kennel Club, the AKC Canine Health Foundation, and the Theriogenology Foundation to increase the number of trained practitioners in companion animal theriogenology and clinical genetics. Theriogenology is the branch of veterinary medicine concerned with reproduction, including the physiology and pathology of male and female reproductive systems, and the clinical practice of veterinary obstetrics, gynecology, and andrology.



## 2021 Theriogenology Residents



02845-E

### **Anum Ahmed, DVM**

**Residency Coordinator:** Audrey A. Kelleman, DVM, DACT; University of Florida

**Total Grant Amount:** \$94,717

**Grant Period:** 7/1/2021-6/30/2023

Dr. Ahmed received her bachelor's degree in biology at the University of Central Florida. She completed her veterinary degree at Kansas State University College of Veterinary Medicine and a small animal rotating internship through BluePearl Veterinary Partners in Tampa, FL.



02846-E

### **Nicole Sugai, DVM**

**Residency Coordinator:** Julie Cecere, DVM, MS, DACT; Virginia-Maryland College of Veterinary Medicine

**Total Grant Amount:** \$99,998

**Grant Period:** 7/1/2021-6/30/2024

Dr. Sugai received her bachelor's degree in biology and evolutionary anthropology at the University of Michigan. She completed her veterinary degree, with honors, at the University of Illinois at Urbana-Champaign College of Veterinary Medicine. After two years in general practice, Dr. Sugai is pursuing advanced education in reproductive medicine.

## 2020 Theriogenology Residents



02668-E

### **Alex Horner, DVM**

**Residency Coordinator:** Fiona Hollinshead, BVSc, PhD, DACT;  
Colorado State University  
**Total Grant Amount:** \$100,000  
**Grant Period:** 7/1/2021-6/30/2024

Dr. Horner pursued her veterinary training at the University of Melbourne, in Australia, where she graduated with her DVM. Dr. Horner maintains a strong interest in breeding healthy purebred dogs to preserve their unique histories and working abilities.



02666-E

### **Joanna Koilpillai, BVSc & AH**

**Residency Coordinator:** Marco A. Coutinho da Silva, DVM, PhD; The Ohio State University  
**Total Grant Amount:** \$99,965  
**Grant Period:** 7/1/2020-6/30/2022

Dr. Koilpillai earned her veterinary degree from Madras Veterinary College in Chennai, India. After one year in mixed animal private practice in India, she completed a small animal internship at Veterinary Healthcare Associates in Florida. Her interests are small animal medicine and mixed animal reproductive medicine.

## 2019 Theriogenology Resident



02538-E

### **Jamie Douglas, DVM**

**Residency Coordinator:** Robyn Wilborn, DVM, MS; Auburn University  
**Total Grant Amount:** \$100,000  
**Grant Period:** 7/1/2019 - 6/30/2022

Dr. Jamie Douglas received her DVM from Michigan State University College of Veterinary Medicine in 2014 and her master's degree in animal science (reproduction) from Southern Illinois University Carbondale's College of Agricultural Sciences in 2015. She returned to Michigan State University to complete a veterinary anesthesia internship in 2015.

To support this program visit: [akcchf.org/therio](http://akcchf.org/therio)

## Impact of CHF Funding

### Peer-Reviewed Publications of Research

Jan 1, 2022 - March 15, 2022

- Bell, S. M., Evans, J. M., Evans, K. M., Tsai, K. L., Noorai, R. E., Famula, T. R., Holle, D. M., & Clark, L. A. (2022). Congenital idiopathic megaesophagus in the German shepherd dog is a sex-differentiated trait and is associated with an intronic variable number tandem repeat in Melanin-Concentrating Hormone Receptor 2. *PLOS Genetics*, 18(3), e1010044. <https://doi.org/10.1371/journal.pgen.1010044>
- de Castro, R. B., de Souza, A. C. A., Pavione, N. da R. T., de Moraes, J. V. B., Ribeiro, I. C., de Melo Agripino, J., Bressan, G. C., de Souza Vasconcellos, R., Silva-Júnior, A., & Fietto, J. L. R. (2022). Urea, salts, and Tween 20 influence on adsorption of IgG and Leishmania rNTPDase2 to nitrocellulose. *Analytical Biochemistry*, 114648. <https://doi.org/10.1016/j.ab.2022.114648>
- Hendricks-Wenger, A., Arnold, L., Gannon, J., Simon, A., Singh, N., Sheppard, H., Nagai-Singer, M. A., Imran, K. M., Lee, K., Clark-Deener, S., Byron, C., Edwards, M. R., Larson, M. M., Rossmeisl, J. H., Coutermarsh-Ott, S. L., Eden, K., Dervisis, N., Klahn, S., Tuohy, J., ... Vlasisavljevich, E. (2022). Histotripsy Ablation in Preclinical Animal Models of Cancer and Spontaneous Tumors in Veterinary Patients: A Review. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 69(1), 5–26. <https://doi.org/10.1109/TUFFC.2021.3110083>
- Neupane, P., Maggi, R. G., Basnet, M., Lashnits, E., Andrews, G. P., & Breitschwerdt, E. B. (2022). Bartonella henselae Recombinant Pap31 for the Diagnosis of Canine and Human Bartonellosis. *Pathogens*, 11(2), 182. <https://doi.org/10.3390/pathogens11020182>
- Santoro, D., Archer, L., & Chong, E. (2022). Evaluation of cutaneous and circulating (serum and exosomes) levels of chemokines (CCL17, CCL22, CCL27 and CCL28) in atopic dogs and their correlation with severity of the disease. *Veterinary Dermatology*. <https://doi.org/10.1111/vde.13061>
- Sarver, A. L., Makielski, K. M., DePauw, T. A., Schulte, A. J., & Modiano, J. F. (2022). Increased risk of cancer in dogs and humans: A consequence of recent extension of lifespan beyond evolutionarily determined limitations? *Aging and Cancer*. <https://doi.org/10.1002/aac2.12046>

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