



# **AKC Canine Health Foundation Active Grants Portfolio**

**9/15/17**



## General Canine Health Research Program Area

### **02353-A: Characterization of Naturally-Occurring Neuropathic Pain in Dogs**

Principal Investigator: Paulo Steagall DVM, PhD; University of Montreal

Total Grant Amount: \$14,752.80; Grant Period: 10/1/2017 - 9/30/2018

Clinical experience demonstrates that canine patients commonly suffer from neuropathic pain and little is known to address this issue. The researchers will investigate different tools for the recognition, diagnosis and treatment of neuropathic pain using a multidisciplinary approach. A two-phased study will include client-owned healthy dogs as controls and dogs with naturally-occurring neuropathic pain (Neuropathic Group-NG). In a prospective, randomized, masked clinical trial using appropriate inclusion and exclusion criteria, dogs in the NG group will be assigned to receive treatment with a drug for neuropathic pain (gabapentin), or gabapentin in combination with an anti-inflammatory drug (meloxicam) in a cross-over design (dogs will receive both treatments during the study). Placebo will be administered between treatments to assess placebo effect; additional analgesics will be administered if needed. Quality of life, pain scores, client-specific outcome measures, biomarkers of inflammation and quantitative sensory testing will be evaluated and compared with controls for observation of treatment effect, and blood concentrations of gabapentin will be measured. This multidisciplinary research may have a timely, immediate impact in veterinary medicine, canine health and welfare by providing important insight into diagnosis and therapeutic options for neuropathic pain.

### **02242: Understanding the Genetics of Adverse Drug Reactions in Sighthounds**

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

Total Grant Amount: \$150,000; Grant Period: 2/1/2016 - 1/31/2018

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 concern for dog owners and veterinarians. Research conducted at Washington State University has enabled development of a simple cheek swab test (the MDR1 gene test now being used to identify dogs that should either avoid or have reduced doses of certain drugs used to treat cancer and parasite infections. Using a similar strategy the investigators hope to identify the cause of extremely slow recovery from anesthesia (up to several days) in a high proportion of greyhounds and other sighthound breed dogs, such as Scottish Deerhound, Borzoi, Whippets, etc.. The investigators have recently discovered a mutation in a gene known to be essential for metabolism of commonly used anesthetic drugs (such as propofol), as well as many other drugs used in dogs. In addition to sighthound breeds, this gene mutation is also found in other breeds such as Border Collies. The purpose of this research project is to prove that this mutation can cause decreased drug metabolism, while also determining which drugs and which dog breeds are likely to be most impacted. The ultimate goal of the study is to develop a genetic test to guide the safe use of these drugs in dogs with the gene mutation.



## **02322: 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 - 1/31/2019

Following the attacks of September 11, 2001 on the World Trade Center and Pentagon, the AKC Canine Health Foundation funded 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.

The AKC Canine Health Foundation is proud to have funded Dr. Otto through all 16 years of this important work on behalf of Search and Rescue dogs from its inception in 2001.

## **Blood Disease Research Program Area**

### **02343-A: Recognizing and Removing Lipemic Interferences for Accurate Laboratory Testing**

Principal Investigator: Unity Jeffery, VetMB; Texas A&M University

Total Grant Amount: \$9,113; Grant Period: 5/1/2017 - 4/30/2018

Over thirty percent of Miniature Schnauzers have primary hyperlipidemia, a disease in which fats (also termed lipids) are increased in the blood. Lipids are also increased in dogs who have recently eaten or are affected by disorders that alter lipid handling (e.g. diabetes, hypothyroidism, Cushing's disease). Blood samples collected from affected patients are milky and opaque due to large numbers of lipid droplets. Many blood tests rely on measuring a color change or light transmission through a sample, but lipid droplets absorb light and cause random light scatter preventing accurate measurement of these changes. High blood lipids may prevent clinically important tests from being performed or render their results inaccurate. Incorrect diagnosis or treatment may occur, or unnecessary invasive and expensive tests may be performed because of these inaccurate results. Two techniques are commonly



used to reduce lipids before analysis: high-speed centrifugation or addition of a lipid extraction solution. For human samples, centrifugation is insufficient to remove lipid for some tests, but the lipid extraction solution produces inaccuracies in others. In dogs, the effect of the two techniques on subsequent analyses has not been well-established, preventing selection of the most appropriate lipid removal technique. The investigators will establish which biochemistry tests are altered by high lipids and determine the best means to remove the lipid interference, thereby improving the accuracy of laboratory testing and veterinary care.

### **02355-A: Hyperlipidemia in the Miniature Schnauzer: A Combined Metabolomic and Genomic Approach**

Principal Investigator: Christopher O'Callaghan, MD, PhD; University of Oxford

Total Grant Amount: \$14,958; Grant Period: 8/1/2017 - 7/31/2018

Miniature Schnauzers can be affected by a condition causing too much circulating lipid (fat) in the bloodstream, known as hyperlipidemia. The problem appears to worsen as dogs get older and may affect up to 3 in every 10 dogs. Affected dogs are more likely to suffer from other serious health conditions including pancreatitis and diabetes, and liver and kidney problems. Whilst an underlying genetic cause is suspected, the gene or genes responsible for this condition in Miniature Schnauzers have not been identified. At present, routine blood tests only allow veterinarians to measure 2 types of lipid - triglyceride and cholesterol. In contrast, in human lipid disorders, improved diagnosis and personalized treatment options have been achieved by measurement of a much wider variety of lipids in the bloodstream, combined with genetic testing. The investigators will measure over 2000 substances including lipid- and metabolism-related markers in the blood of Miniature Schnauzers to improve understanding of hyperlipidemia that may guide more specific treatment options. The researchers will also examine differences between genes of affected and unaffected Miniature Schnauzers, to try to identify important mutations associated with hyperlipidemia. These studies may lead to a genetic screening test and/or new targets for treatment of this condition.

### **01988: Identification of a Safe Storage Time for Canine Blood Used In the Treatment of Anemia**

Principal Investigator: Mary Beth Callan, VMD.; University of Pennsylvania

Total Grant Amount: \$113,499; Grant Period: 1/1/2014 - 12/31/2017

Red blood cells (RBCs) can be refrigerator stored for up to 35 - 42 days in humans and dogs. Given that blood is a precious and limited resource, both human and veterinary blood banks typically dispense the oldest RBC units first to reduce waste. However, accumulating evidence suggests that transfusion of RBCs stored >14 days is associated with increased rates of complications and death in human patients. Preliminary data from a study of more than 2000 dogs receiving RBC transfusions suggest that administration of older RBCs to dogs with certain types of anemia negatively impact survival. Dr. Callan's goal is to conduct a randomized clinical trial in which dogs with anemia in need of RBC transfusions will receive either "fresh" RBCs (stored <7 days) or "old" RBCs (stored 21-28 days). If they document that administration of older RBCs is associated with increased inflammation and poorer



outcome in dogs with anemia, the results of this study will have a significant impact on canine health and veterinary blood banks by changing current transfusion practices; that is, by providing fresh rather than older RBCs to anemic canine patients.

## Cardiology Research Program Area

### **02368: Identification of Mitral Valve Disease DNA variants in Miniature Schnauzers**

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

Total Grant Amount: \$56,635; Grant Period: 8/1/2017 - 7/31/2019

Mitral valve degeneration is the most common heart disease in the dog, and is particularly common in small breed dogs. Miniature Schnauzers are one of the most commonly affected breeds. Although some dogs live comfortably with the disease, many affected dogs die of congestive heart failure and sometimes sudden death due to rupture of a weakened heart. Mitral valve degeneration is thought to be an inherited disease in the dog although the causative mutation(s) have not been identified. Failure to understand the underlying cause of canine mitral valve degeneration has slowed the development of effective treatment and prevention plans. The investigators will identify genetic variants that lead to the development of mitral valve degeneration in Miniature Schnauzers, and use this information to develop treatment and prevention plans for dogs with high-risk DNA variants.

### **02327-MOU: Identification of Genetic Markers for Familial Subvalvular Aortic Stenosis in Bullmastiffs**

Principal Investigator: Joshua Stern, DVM, PhD; University of California, Davis

Total Grant Amount: \$55,173; Grant Period: 4/1/2017 - 3/31/2019

Subvalvular Aortic Stenosis (SAS) is a heart defect characterized by a fibrous ridge located below the aortic valve. Affected dogs are at risk of developing heart valve infections, congestive heart failure or sudden death. Severely affected dogs have an average lifespan of 19 months. SAS is an inherited heart problem reported in Bullmastiffs and other breeds. Studying this disease in Bullmastiffs has the potential to identify a genetic mutation and develop a test for this condition. Ultimately the identification of a mutation in Bullmastiffs would aid breeders in making decisions to reduce the prevalence of this condition. The objective of this study is to identify the genetic cause of SAS in Bullmastiffs. The investigators have collected DNA samples from affected and unaffected Bullmastiffs and will study inheritance to identify genetic variants associated with SAS.

Funding for the research is provided through the collaborative efforts and generosity of the American Bullmastiff Association. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.



## **02388-MOU: Genetic Markers for Familial Subvalvular Aortic Stenosis in Newfoundlands**

Principal Investigator: Joshua Stern, DVM, PhD; University of California, Davis

Total Grant Amount: \$58,949; Grant Period: 9/1/2017 - 8/31/2019

Subvalvular Aortic Stenosis (SAS) is a heart defect characterized by a fibrous ridge located below the aortic valve. Affected dogs are at risk of developing heart valve infections, congestive heart failure or sudden death. Severely affected dogs have an average lifespan of 19 months. A previous study identified a single gene mutation associated with a cohort of Newfoundland dogs with SAS, however this mutation does not explain all SAS in the breed and requires further evaluation. Studying this disease in Newfoundlands has the potential to identify causative genetic mutations and develop a reliable genetic test for this condition to further aid breeders to reduce the prevalence of this condition. The investigators will study pattern of inheritance and use the most modern genetic techniques to identify the genetic cause of SAS in Newfoundlands, further expanding our understanding of this disease in dogs.

## **02389-MOU: Characterization of Ventricular Arrhythmias in Rhodesian Ridgebacks**

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

Total Grant Amount: \$26,919; Grant Period: 9/1/2017 - 8/31/2020

The investigators recently identified a genetic mutation associated with heart arrhythmias in Rhodesian Ridgebacks. Dogs with the mutation appear to be at the most risk of developing an arrhythmia and suffering sudden death between 12-24 months of age, however, this timeline is variable, and some dogs appear to outgrow the arrhythmia. Due to the lack of knowledge of the specific at risk age, owners of dogs with the mutation must repeat the Holter monitor (a test to monitor heart rhythm) every few months to identify when their dog is at greatest risk and may need treatment. The objective of this study is to repeatedly perform regular Holter monitor testing on dogs with the mutation (including dogs with one copy and with two copies) every 4 months from 6-24 months of age with a final evaluation at 36 months to narrow in on the age when the arrhythmias appear to be the most severe. Gaining this increased clinical understanding of the disorder will decrease the risk of sudden death by helping owners and veterinarians in monitoring and providing treatment intervention for their dogs, and will further inform breeders and owners by characterizing the clinical and genetic manifestations of the disorder.

## **02046: Using a Novel Combination of Drugs to Treat Arrhythmia and Heart Failure in Dogs**

Principal Investigator: Janice Bright, DVM, BSN; Colorado State University

Total Grant Amount: \$33,060; Grant Period: 1/1/2014 - 6/30/2018

Atrial fibrillation is a common heart rhythm abnormality (arrhythmia) in dogs. This arrhythmia affects all dog breeds and frequently coexists with heart failure causing worsening of disease and high



mortality. Atrial fibrillation may be managed by administering drugs to slow heart rate or by restoring normal rhythm (cardioversion). Dr. Bright will evaluate dogs with naturally occurring atrial fibrillation and heart failure for their responsiveness to two drugs -- amiodarone, an antiarrhythmic agent, and ranolazine, a drug used in humans with coronary heart disease. She will determine whether ranolazine given with amiodarone prolongs normal rhythm compared to amiodarone alone and whether ranolazine also improves heart function. Results will validate combined ranolazine/amiodarone administration as an improved new treatment for atrial fibrillation in dogs with heart failure, extending their quality of life.

### **02163-MOU: Is Hypothyroidism a Contributor to Progression of Arrhythmogenic Right Ventricular Cardiomyopathy?**

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

Total Grant Amount: \$50,857; Grant Period: 1/1/2015 - 12/31/2017

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. Dr. Meurs' research group identified a causative mutation in the cardiac Striatin gene that is highly associated with the development of Boxer ARVC. They 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 that individual dog which could include genetic or other more external factors including diet, exercise and hormonal levels. Genetic factors could include common variants in the nucleotide sequence of other cardiac modifying genes that have been shown to influence the severity of cardiac diseases. In addition, endocrine issues like hypothyroidism complicate ARVC and may play a role in disease progression. Dr. Meurs hypothesizes that low thyroid levels and/or other genetic variants may lead to the development of the more severe form of Boxer ARVC. Understanding the role of these factors in the severity of disease will greatly improve the ability to manage the common and sometimes fatal heart disease of ARVC.

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 administration of funds and scientific progress reports.

### **01760-T: 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 - 2/28/2019

Dilated cardiomyopathy (DCM) is the second most common cause of heart disease in dogs, and medical management of the secondary signs is the only current 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, the investigators 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 dogs 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.

## **Dermatology and Allergic Disease Research Program Area**

### **02176-A: Intralymphatic Immunotherapy for the Treatment of Canine Atopic Dermatitis**

Principal Investigator: Andrea Lam, DVM; Tufts University

Total Grant Amount: \$12,113; Grant Period: 7/1/2015 - 1/31/2018

Atopic dermatitis (AD) is a genetically predisposed inflammatory skin condition affecting approximately 10% of dogs globally and is probably the most prevalent skin disease in all canines. Affected dogs manifest with itchy skin and ears and secondary infections. Clinical features are associated with IgE antibodies produced against indoor/outdoor environmental allergens. Current treatment options include antihistamines, corticosteroids, cyclosporine, oclacitinib, and allergen-specific immunotherapy (ASIT), as well as adjunctive topical and antimicrobial therapy. Antihistamines are effective in about 25% of dogs. Corticosteroids are extremely efficacious; however, side effects are common, thus long-term use is strongly discouraged. Cyclosporine is effective in many dogs with few serious adverse effects, but cost can be a limitation. Oclacitinib has been shown to have good efficacy, but long-term side effects have not been studied. ASIT appears as the only treatment that is able to induce a clinical cure. However, the percentage of atopic dogs that respond to this treatment is only 60-70% and in many, the response is only partial. It has been proposed that efficacy of subcutaneous ASIT is limited by the skin's ability to stimulate the immune system. This study will test an alternative route of administration using ASIT for canine atopic dermatitis.

### **02241: The City Dog Study: Dermatologic and Respiratory Disease among Inner-City Dogs Living in the Homes of Children with Asthma**

Principal Investigator: Meghan Davis, DVM, MPH, PhD; Johns Hopkins University

Total Grant Amount: \$158,367; Grant Period: 2/1/2016 - 1/31/2019

Children who live in inner-city households of low economic means suffer disproportionately from skin and lung diseases, including asthma. This study will evaluate the burden of skin and respiratory disease among the dogs who live with them. These dogs often can be hard to study because their owners may not have the means or access to take them to the veterinarian. As an adjunct to a funded public health





research effort targeting 200 children with asthma, Dr. Davis and her team will enroll 100 dogs and follow their health at three home visits over six months, and perform two additional evaluations. First, they will study the microbial (bacterial) communities on the dogs to determine how these change over time, and if the changes are associated with skin or respiratory diseases in the dogs. Then, the investigators will look at how the children and dogs share bacteria (i.e. microbiome). Early life exposures to dogs may protect children against the development of asthma, so next will be to investigate if dogs also have a beneficial impact when the children are older and have existing disease. This study will provide knowledge needed to help understand disease in underserved dogs in urban neighborhoods, providing data to support keeping dogs and keeping them healthy to benefit both dogs and their owners.

## Endocrinology Research Program Area

### **02366-A: Individualization of Pharmacological Interventions in Diabetic Dogs**

Principal Investigator: Nicolas Villarino, Med.Vet.; Washington State University

Total Grant Amount: \$14,435; Grant Period: 5/1/2017 - 4/30/2018

Diabetes mellitus is a disease of middle-aged to older dogs which means many affected dogs will develop other diseases such as arthritis, infections, and behavior disorders, all requiring drug therapy. Poor control of glucose levels in diabetic dogs can alter how drugs behave in the body, which can result in drug toxicities. This is an area of intense investigation in diabetic humans, but such effects have not been investigated in canine medicine, and prescribed treatments may result in individual dogs being under- or overdosed. The investigators intend to move from a 'one dose fits all' strategy to an individualized medical approach to ensure each patient receives optimal pharmacological therapy. Completion of this study is the first step toward establishing an in vitro method for evaluating the many drugs used in diabetic dogs. The long-term goal is to develop a free downloadable application for mobile devices (smartphones and tablets), for use by clinicians to make treatment selection, and to avoid drugs that may cause problems in diabetic patients. This research stands to play a substantial role in the clinical management of dogs with diabetes mellitus.

### **02342-A: Molecular Analysis of Giant Schnauzer-Type Congenital Hypothyroidism**

Principal Investigator: John Fyfe, DVM PhD; Michigan State University

Total Grant Amount: \$14,900; Grant Period: 6/1/2017 - 5/31/2018

Isolated congenital hypothyroidism (CH) is a condition occurring at or near birth characterized by insufficient thyroid hormone production. The disorder in purebred dogs is usually inherited and leads to dwarfism and mental dullness. CH in giant schnauzers (GS) was first described in 1991 (Greco, et al) as a likely autosomal recessive disorder due to failed activity of the hypothalamus or pituitary gland. Since then the investigators have studied GS CH in three widely separated families and found pituitary failure of thyroid stimulating hormone (TSH) production beginning at birth in most affected dogs, but not until several months of age in a few. A genetic locus was mapped to a region of dog chromosome 28. The researchers will now perform DNA sequencing of affected dogs and their parents and



candidate variants will be further assessed further in all available members of the three families, as well as a large number of GS DNA samples available in the OFA CHIC repository. A successful outcome will lead to a reliable genetic test for GS CH, increased understanding of an essential pituitary function, and illumination of a highly similar condition reported in miniature schnauzers.

## **02298-MOU: Using OFA Testing to Assess Progression of Canine Autoimmune Thyroiditis**

Principal Investigator: Brian Petroff, DVM, PhD; Michigan State University

Total Grant Amount: \$35,630; Grant Period: 8/1/2016 - 12/31/2017

Hypothyroidism may be the most common endocrine disorder in adult dogs. As currently understood, a majority of cases are caused by autoimmune thyroiditis (AIT), a disorder in which the body's own immune system attacks the thyroid gland. This causes progressive, irreversible destruction of thyroid gland cells resulting in loss of thyroid hormone production. This disorder has similarities to Hashimoto's thyroiditis, an important cause of hypothyroidism in people. In dogs with AIT, low circulating concentrations of thyroid hormones are often seen in conjunction with increased autoantibodies against thyroglobulin, a large protein made by thyroid cells. Detection of thyroglobulin autoantibodies (TgAA) are the first marker of early stage of AIT, long before there is complete loss of thyroid function. Identification of elevated TgAA results with otherwise normal thyroid hormone concentrations is referred to as 'subclinical thyroiditis.' Dogs with subclinical thyroiditis are considered at risk of progression to hypothyroidism. It is assumed that while dogs with subclinical thyroiditis have increased TgAA, the rate of progression to hypothyroidism varies, and not all animals with increased TgAA will become hypothyroid. The investigators will study dogs with subclinical thyroiditis to better define what proportion develop hypothyroidism, and the timeline to disease progression.

Funding for the research is provided through the efforts and generosity of the Orthopedic Foundation for Animals. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.



## Epilepsy Research Program Area

### **02323: Efficacy of Cannabidiol (CBD) for the Treatment of Canine Epilepsy**

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

Total Grant Amount: \$356,022; Grant Period: 10/1/2017 - 9/30/2020

Epilepsy is the most common neurologic condition in dogs. Approximately 20-30% of dogs receiving standard therapy remain uncontrolled for their seizures. Additionally, the side effects of the antiepileptic drugs (AED) are often unacceptable. Thus, there is a need for an AED that is efficacious with minimal side effects. Cannabidiol (CBD), a prominent non-psychotropic component of the Cannabis sativa plant, has been shown to have anti-convulsant properties. While CBD offers promise as a treatment for canine epilepsy, controlled studies are needed to prove its effectiveness. In this randomized, double-blinded, placebo-controlled, crossover clinical trial, client-owned dogs with uncontrolled epilepsy will be enrolled following a full seizure evaluation, including bloodwork and magnetic resonance imaging. The canine patients will first receive either a placebo or CBD in addition to their standard AED protocol and then the opposite drug in this crossover designed study. Seizure frequency and medication side effects will be monitored by owners using a seizure log and questionnaire. Regular CBD plasma concentrations, routine bloodwork and serial physical examinations will be monitored by the investigator. The primary goal of the study is to determine the efficacy of CBD in the treatment of canine epilepsy. If CBD is effective in decreasing seizure frequency, it has the potential to improve the quality and length of life for dogs with uncontrolled epilepsy, and add a much-needed tool for veterinarians in the treatment of canine epilepsy.

### **02131: Neurostimulation: A Groundbreaking New Treatment for Canine Epilepsy**

Principal Investigator: Sam Long, BVSc, PhD; The University of Melbourne

Total Grant Amount: \$116,000; Grant Period: 10/1/2014 - 9/30/2017

Epilepsy is a debilitating condition that affects a large number of dogs, resulting in premature death and distress for their owners. For many dogs the underlying cause is unknown. In people, advances in some types of imaging have identified subtle abnormalities, including abnormal development and shrinkage of particular regions in the brain of some people with epilepsy that can be surgically removed to improve the control of seizures. This project will apply the same advanced techniques to the brains of dogs with epilepsy to determine whether those same abnormalities exist in dogs. In those dogs in which no abnormalities can be found, this project will investigate a new form of treatment, known as neurostimulation which has been shown to reduce the frequency of seizures dramatically in human clinical trials. This involves surgically implanting a new, highly sophisticated device called the Brain Radio that can provide controlled electrical stimulation to parts of the brain while simultaneously recording the brain's activity. This device is one of the very first that could potentially provide successful therapy only when needed to treat imminent seizures and if it proves successful in dogs it will enter clinical trials in people with epilepsy.



## **02248: Identification of a Novel Juvenile Myoclonic Epilepsy Gene and Its Underlying Disease Mechanism**

Principal Investigator: Hannes Lohi, PhD; University of Helsinki and the Folkhälsan Institute of Genetics  
Total Grant Amount: \$82,240; Grant Period: 5/1/2016 - 10/31/2017

Epilepsy is the most common neurological disease in dogs and affects almost all breeds. Genetics is likely to play a major role in seizure risk, and gene discovery remains as an important goal to better understand the disease and its treatment. However, genetic breakthroughs have been rare partially due to incomplete clinical diagnostics to identify true cases and controls, or to distinguish specific syndromes for genetic analyses. The investigators have recently utilized an advanced wireless video-EEG approach in clinical studies to identify juvenile myoclonic epilepsy (JME) in Rhodesian Ridgebacks with characteristic epilepsy phenotype, age of onset and photosensitivity. The pedigree established using the JME cases suggests a strong genetic contribution and is supported by preliminary genetic data that proposes a novel disease locus and a deleterious mutation in a neuronal candidate gene. These promising early findings necessitate further electroclinical and genetic studies for confirmation. In this study, the investigators' objectives are to: i) further characterize EEG, imaging and disease features of JME, ii) confirm the presence and segregation of an epilepsy gene, iii) investigate the breed-specificity, prevalence and penetrance of the mutation, iv) conclude the inheritance model, and v) define the pathogenicity of the mutation. The confirmation of the genetic defect would allow for development of a genetic test for breeding purposes and also to understand how myoclonic seizures develop. This could ultimately lead to improved treatments for canine epilepsy.

## **02249-A: Studying the Role of the Gastrointestinal Tract in Canine Epilepsy**

Principal Investigator: Karen Munana, DVM; North Carolina State University  
Total Grant Amount: \$14,995; Grant Period: 6/1/2016 - 11/30/2017

Epilepsy is the most common nervous system disorder of dogs. Approximately one-third of dogs with epilepsy fail to achieve adequate seizure control with anti-seizure medication, and are considered to have drug resistant epilepsy. The mechanisms that lead to drug resistance are poorly understood. Alterations in the population of intestinal bacteria in the Lactobacillus group are believed to play a role in the development and progression of several human diseases of the nervous system, including anxiety/depression, autism, multiple sclerosis and Alzheimer's disease. An association between epilepsy and both celiac disease and inflammatory bowel disease has been identified in humans, which suggests that changes in intestinal bacterial might play a role in the progression of epilepsy as well. The investigators hypothesize that dogs with epilepsy have an altered population of Lactobacillus species in their gastrointestinal tracts compared to normal dogs, thus influencing the course of disease. Using molecular genetics and bacterial culture techniques, the investigators will determine differences in bacterial populations, and quantify the Lactobacillus component of the feces of untreated epileptic and control dogs, and determine the effect of antiepileptic medication on Lactobacillus growth rates. By providing preliminary information on the role of gastrointestinal tract bacteria in canine epilepsy, information can be gained to further our understanding of epilepsy and drug resistance in dogs, and ultimately lead to more successful management of the disorder.



## **02252: Investigating a Ketogenic Medium-Chain Triglyceride (MCT) Supplement for the Treatment of Drug-Resistant Canine Idiopathic Epilepsy and Its Behavioral Comorbidities**

Principal Investigator: Holger Volk, DVM, PhD; Royal Veterinary College, University of London  
Total Grant Amount: \$107,697; Grant Period: 5/1/2016 - 10/31/2017

Canine epilepsy often requires lifelong medication with anti-epileptic drugs (AEDs). Despite appropriate treatment with available AEDs, seizure freedom may not always be achievable. Over two-thirds of dogs with epilepsy continue to have seizures long-term and around 20-30% remain poorly controlled on standard AEDs. There is an urgent need to develop alternative treatments to improve the quality of life (QoL) of drug-resistant patients. The ketogenic diet, originally characterized as high in fat and low in carbohydrates, has been a successful treatment in children with epilepsy. Recent research has identified a component of the ketogenic diet, a medium-chain fatty acid (MCT) called C10 has direct anti-seizure effects on the brain. The investigators will assess whether dietary supplementation with MCT oil containing C10 for dogs with drug-resistant epilepsy will reduce seizure frequency and/or severity. As epilepsy has multiple impacts on QoL beyond seizure frequency, the researchers will also investigate whether the MCT supplement alters the side effect profile of AEDs, improves behavioral problems associated with epilepsy (e.g. anxiety) and cognition, and improves the stress levels of the affected dog. If successful, MCT supplements could provide a new tool for canine epilepsy treatment.

## **02257: Identification of Genetic Risk Factors for Canine Epilepsy**

Principal Investigator: Gary Johnson, DVM, PhD; University of Missouri, Columbia  
Total Grant Amount: \$97,081; Grant Period: 5/1/2016 - 10/31/2017

Despite strong evidence that genetics is important in determining the risk of idiopathic epilepsy, numerous gene mapping studies have failed to identify a locus that accounts for that risk in either dogs or humans. Seizures occur when excessive activity goes beyond the normal threshold for brain function, many factors contribute to that level of activity, and therefore, mutations in numerous genes may collectively contribute to increased activity until that threshold is exceeded, resulting in epilepsy. Any one of these mutations may be present in non-epileptic dogs, but because it only partially alters activity, it would not produce seizures. Therefore, traditional gene mapping studies might overlook that mutation. Using a whole genome sequencing approach the investigators hope to identify DNA variations in epileptic dogs that could affect the function of genes such as ion channels and neurotransmitter receptors. The frequency of such variations in populations of epileptic and non-epileptic dogs will be directly compared rather than the indirect markers used in traditional mapping studies. The increased power provided by looking for specific gene candidate variations rather than linked markers will aid the identification of epilepsy risk factors, perhaps leading to development of DNA tests to enable breeders to select against such risk factors.



## **Gastrointestinal Disease and Bloat Research Program Area**

### **02338: The Genetics of Bloat in German Shepherd Dogs: The Roles of Immune System Genes and the Gut Microbiome**

Principal Investigator: Michael Harkey, PhD; Fred Hutchinson Cancer Research Center

Total Grant Amount: \$152,270; Grant Period: 6/1/2017 - 5/31/2019

While Gastric Dilatation Volvulus (GDV or bloat) is a serious problem for many large canine breeds, little is known about the causes of this deadly disease. The most significant factors may be genetic, since certain breeds are more susceptible than others, and strong familial predispositions are seen within breeds. The investigators have recently shown a significant association of three immune genes with bloat in Great Danes. For each of the three genes, one allele (variant) is found at unusually high frequency in dogs that have been treated for bloat, and the presence of any one of these "risk" alleles triples the chance that the dog will experience bloat at some time in its life. The research team also showed that the bacterial population living in the gut (the gut microbiome) is altered in dogs with bloat, and in dogs that carry these "risk" alleles, which may predispose these dogs to bloat. It is not known if other breeds show this same association of genetics and microbiome with bloat. The team will investigate whether bloat in German Shepherd Dogs is associated with the same risk alleles and the same microbiome profiles as were seen in Great Danes. The results of this work could lead to genetic tests for at-risk dogs, as well as dietary and probiotic therapies to prevent bloat.

### **01935-B: Abnormalities in the Stomach's Ability to Contract Predisposes Large-Breed Dogs to Bloat**

Principal Investigator: Laura Nelson, DVM; Michigan State University

Total Grant Amount: \$233,774; Grant Period: 1/1/2014 - 6/30/2018

Gastric dilatation-volvulus (GDV or bloat) is common in large and giant-breed dogs. Occurring most frequently in older dogs with a close relative who has also suffered the condition, the stomach becomes both displaced and distended with air. Without emergency medical stabilization and surgical intervention, affected dogs quickly experience shock, damage to the stomach wall, and death. The underlying cause of GDV remains unknown. Abnormalities in the ability of the stomach to contract have been documented in dogs after naturally-occurring GDV. An analogous stomach condition in cattle has been shown to, in some instances, be associated with abnormalities in the motilin gene. Motilin is an important driver of stomach contraction. The investigators will study the relationship between abnormal stomach contraction and GDV, and define the biochemical and genetic alterations that may be associated with these stomach abnormalities. The long term goal is to develop a test to identify dogs at high risk for GDV to allow for early detection, and offer selective breeding as an option to eliminate the condition and determine preventive therapies.



### **01937-B: Evaluating the Complex Genetic Basis of Bloat**

Principal Investigator: Elizabeth Rozanski, DVM; Tufts University  
Total Grant Amount: \$251,097; Grant Period: 1/1/2014 - 12/31/2018

Gastric dilatation and volvulus (GDV), or bloat, common in large and giant breed dogs has an unacceptably high morbidity and mortality rate. There is no known single cause for GDV; its occurrence is multifactorial, with both genetic and environmental factors likely contributing. The investigators will study how risk factors cause GDV through the application of genomic and molecular methods. Samples from purebred dogs with GDV will be analyzed and compared to control dogs of similar age and breed that have not developed GDV to identify differences in genetic makeup, and see which genes are turned on and off in GDV (epigenomics). Researchers will also determine if dogs with GDV have different types or amounts of proteins, hormones and other molecules in their blood and tissues (transcriptomics, proteomics and metabolomics). Using genomic, epigenomic, transcriptomic, proteomic and metabolomic strategies, the investigators hope to understand causes of GDV, and guide more effective preventive and treatment strategies.

### **02233-A: Evaluation of a Novel Technique for Gastric Decompression in Dogs with Gastric Dilatation and Volvulus**

Principal Investigator: J. Case, DVM, MS; University of Florida  
Total Grant Amount: \$12,960; Grant Period: 11/1/2015 - 10/31/2017

Gastric dilatation-volvulus (GDV) is a common medical and surgical emergency that involves severe gas distention and malposition of the stomach in dogs. GDV results in profound distension of the stomach which compresses vital blood vessels and organs within the abdomen, thus reducing oxygen delivery to these organs. The ultimate result is tissue death and toxins in the blood stream. Surgery is necessary to correct the condition, and overall mortality rates range from 10-50% depending on severity and duration of gastric dilatation. Rapid and effective decompression of the stomach is critical for successful treatment of dogs with GDV. Current approaches to decompression have a temporary effect and gas can re-inflate the stomach within minutes. Oftentimes affected dogs are not near a facility with surgical capabilities when they develop signs of GDV. A new, minimally-invasive technique, similar to that used in human medicine, will be tested for its ability to immediately and continuously alleviate gas distention in the stomach of GDV patients using a specialized catheter, thus allowing the patient to be stabilized and/or transported for surgery.

### **02002: Defining the Genetic Basis of Inflammatory Bowel Disease**

Principal Investigator: Karin Allenspach, DVM PhD; Royal Veterinary College, University of London  
Total Grant Amount: \$119,268; Grant Period: 10/1/2014 - 9/30/2017

Inflammatory Bowel Disease (IBD) is a group of disorders in which the intestinal tract has become inflamed. Over time, this inflammation causes the intestine to become less efficient at absorbing nutrients, and weight loss and vomiting or diarrhea often result. Currently, IBD can be controlled, but not cured. The cause of IBD is poorly understood, and it appears genetics, diet, intestinal bacteria, and



abnormalities of the dog's immune system all may play a role. The investigator has recently identified genetic markers known as SNPs (single nucleotide polymorphisms) which she believes contribute to disease susceptibility. Beyond genetics, this research group has mechanistic data showing one of the putative mutations contributes to the inflammation seen in the intestine of dogs with IBD, and will investigate underlying genetic factors that could contribute to disease, thus leading to the development of new diagnostic and therapeutic avenues for canine IBD.

## **Hepatic Disease Research Program Area**

### **02363-A: Platelet Function in Dogs with Chronic Liver Disease**

Principal Investigator: David Panciera, DVM; Virginia-Maryland Regional College of Veterinary Medicine

Total Grant Amount: \$14,904; Grant Period: 6/1/2017 - 5/31/2018

Chronic liver disease is common among adult dogs. Liver biopsy is usually required to identify the underlying cause of liver disease in these patients, and is often recommended to monitor response to treatment. Because dogs with liver disease have abnormal clotting activity, bleeding is a substantial risk of biopsy. Routine screening for clotting abnormalities in dogs with liver disease is accomplished using blood tests including prothrombin time, partial thromboplastin time, and platelet count. Unfortunately, these routine tests do not necessarily correlate with excessive biopsy-induced bleeding, which makes predicting and preventing hemorrhage during liver biopsy difficult. Humans with liver disease have abnormal platelet function that contributes to abnormal coagulation. Because standard diagnostics do not assess platelet function, the investigators will evaluate platelet function to determine if dogs with chronic liver disease have platelet dysfunction and if there is a correlation between platelet function and bleeding after liver biopsy.

### **01986: Profiling the Metabolic and Lipid Imbalances that are Causative of Gallbladder Disease in Dogs**

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

Total Grant Amount: \$135,354; Grant Period: 1/1/2014 - 12/31/2017

The gallbladder mucocele (GBM) is one of the most common, poorly understood and deadliest biliary diseases of dogs. A GBM develops when the gallbladder secretes abnormal mucus that eventually obstructs or ruptures the gallbladder. GBM formation afflicts all dogs, but especially Shetland Sheepdogs, Miniature Schnauzers and Cocker Spaniels, and in general, dogs with disorders of steroid hormone or lipid metabolism. By the time a diagnosis of GBM is made, emergency surgery to remove the gallbladder is often required. After surgery only 22-50% of dogs survive to be discharged from the hospital. There is a critical need to determine why dogs form a GBM so we can prevent the high cost and lost lives of these dogs. Based on the breeds and diseases that predispose to GBM, Dr. Gookin hypothesizes these dogs have a unique disturbance in cholesterol or lipid metabolism. If the cause of this disturbance can be identified we will be able to understand why GBM form, develop tests for early diagnosis and design diets or drugs to prevent GBM formation.





## **02297-MOU: Understanding the Genetics of Hepatic Copper Toxicosis in the Dalmatian**

Principal Investigator: Andrew Mason, PhD; University of Alberta

Total Grant Amount: \$100,000; Grant Period: 3/1/2017 - 2/28/2019

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. 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 a 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, 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/DCA Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.

## **Immunology and Infectious Disease Research Program Area**

### **02380-A: Estimating Prevalence and Identifying Risk Factors for Canine Leptospirosis in North America**

Principal Investigator: Jason Stull, VMD, PhD; Ohio State University

Total Grant Amount: \$14,990; Grant Period: 5/1/2017 - 10/31/2018

Leptospirosis is an important and re-emerging disease of dogs, humans and other species that is transmitted by contact with infected urine. Infected dogs can develop severe illness, including death. Despite being recognized as a disease that appears to be increasing in frequency in dogs across the United States and Canada, many areas important to dog health are unknown. Regions of greatest canine leptospirosis risk, dog factors that increase risk and the most important prevention methods remain unclear. The investigators will use a large international database to determine the occurrence and changes over time and region of this disease. Current "hot spots" for canine leptospirosis will be determined and further evaluated in detail by enrolling dogs and their owners in a follow-up study to identify key behaviors and practices that can be used to successfully reduce the risk of leptospirosis in dogs. Maps will be created for use by dog owners and veterinarians to identify areas of greatest risk and concern for this disease. Together, maps and risk reduction data will allow for targeted education to individuals with dogs living or traveling to higher-risk areas to protect dogs against leptospirosis.



## **02404-A: 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: 10/1/2017 - 3/31/2019

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.

## **01771: Defining the Unique Genetic Markers in Dogs That Define Immune Function, Disease Resistance and Tissue Transplantation**

Principal Investigator: Beverly Torok-Storb, PhD; Fred Hutchinson Cancer Research Center

Total Grant Amount: \$178,200; Grant Period: 1/1/2013 - 6/30/2018

The Major Histocompatibility Complex (MHC) genes encode proteins critical for a wide range of biological functions, from immune protection against infectious disease to the predisposition to develop diabetes and autoimmune diseases. The MHC genes in the dog are incompletely characterized, thereby severely limiting the ability to fully define causes of many canine diseases. The investigators have developed improved methods for identifying canine MHC genes in a large number of dogs of diverse breeds. The investigators will characterize MHC genetic variation in over 1200 dogs from at least 50 breeds using a high throughput sequencing strategy. The methodology and data gained from this study will enhance the power of association studies between MHC types and canine diseases. Such a database will enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies (stem cell transplants) and other diseases (tissue transplantation). Fully defining the canine MHC will have broad impact across canine health, including oncology, immunology and infectious disease.



## **02245-MOU: Genetic Predisposition to Avian Tuberculosis in Miniature Schnauzers and Basset Hounds**

Principal Investigator: Urs Giger, DVM, PhD; University of Pennsylvania

Total Grant Amount: \$106,858; Grant Period: 5/1/2016 - 10/31/2017

While people and dogs are generally resistant to avian tuberculosis infections, there are individuals that lack proper host defense against these intracellular bacteria. The precise molecular basis is unknown, but there is much interest because of the major morbidity and mortality in susceptible patients. The investigators have recognized that many young adult Miniature Schnauzers (and few Basset Hounds) succumb to systemic avian tuberculosis (referred to as Mycobacterium avium complex or MAC), characterized by enlarged lymph nodes, fever, diarrhea and respiratory signs. Based upon pedigree analysis, this appears to be a simple autosomal recessive trait. Preliminary pedigree and limited molecular genetic data suggest a strong signal for one specific small chromosomal region, which the investigators will substantiate using whole genome sequencing. Identification of the molecular basis of this genetic predisposition will allow for the development of a DNA screening test to identify animals at risk and carriers. As avian tuberculosis is a zoonotic disease, the findings should provide insight into genetic determinants of host microbe interaction and resistance in dogs and people, and thereby could have an impact on comparative medicine.

Funding for the research is provided through the efforts and generosity of the American Miniature Schnauzer Club. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.

## **02299-A: Investigating Recovery of the Skin Microbiota after Surgery**

Principal Investigator: Julie Horvath, PhD; North Carolina Museum of Natural Sciences

Total Grant Amount: \$9,605; Grant Period: 8/1/2016 - 7/31/2018

Collaborative Grant between Triangle Center for Evolutionary Medicine and AKC Canine Health Foundation

Microbes that live on the skin of humans and animals are microscopic organisms including bacteria, Archaea, and fungi. These microbes contribute to the overall health and wellness of animals including humans, and have been shown to influence the wound healing process. Antibiotic resistant bacteria are a growing threat to good health. Therefore, while it is not yet understood how microbes play a role in wound healing, a better understanding would allow potential new treatments to emerge using either the microbes themselves, and/or microbial products. This project brings together collaborators from the NCMNS, North Carolina Central University, North Carolina State University (NCSU) and the NCSU College of Veterinary Medicine to investigate the ecological changes in skin microbe composition of dogs following elective surgery. The dogs in this study receive veterinary care at NCSU's College of Veterinary Medicine, undergoing surgery as part of their care, and are given antibiotics. The study's investigators will assess the presence of antibiotic resistant bacteria on dog skin before and after surgery and evaluate the impacts on wound healing.



## Lung and Respiratory Disease Research Program Area

### **02346-A: Blood Culture and Blood Microbiome as Minimally Invasive Diagnostics for Canine Bacterial Pneumonia**

Principal Investigator: Carol Reiner, DVM, PhD; University of Missouri

Total Grant Amount: \$11,394; Grant Period: 6/1/2017 - 5/31/2018

Canine bacterial pneumonia is a common and serious respiratory infection. Pneumonia can develop from contagious environmental bacteria or from the dog's own bacteria gaining access to the lungs (e.g., after accidentally inhaling food, liquids or vomit). Diagnosis relies on clinical signs, x-rays, and lung fluid (bronchoalveolar lavage fluid or BALF) analysis. Analysis of BALF helps identify the causative bacteria and aids in appropriate antibiotic selection. While key to definitive diagnosis and management of bacterial pneumonia, collection of BALF requires general anesthesia, which can be risky in dogs with severe lung disease. To address the clinical need for a minimally invasive diagnostic test, the first study objective is to determine if blood cultures, acting as a surrogate for BALF analysis, can identify the bacteria causing pneumonia and provide antibiotic susceptibility information. In addition, the investigators will employ molecular means of identification of bacterial populations in samples, so called "microbiome" analysis. Researchers will compare BALF and blood microbiomes to determine sample relatedness and then to the bacteria identified via BALF culture to determine if lung bacteria appear in the blood in minute quantities and whether the predominant cultured bacteria is reflected in the blood microbiome.

### **02232-MOU: Characterization of Upper Airway Syndrome in Norwich Terriers**

Principal Investigator: Bryden Stanley, BVMS; Michigan State University

Total Grant Amount: \$74,496; Grant Period: 11/1/2015 - 12/31/2017

Upper airway issues in Norwich Terriers (NTUAS) can vary from mild airway noise to severe distress with heat and exercise intolerance, and death. Descriptions of NTUAS have focused on everted laryngeal saccules (outpouched laryngeal tissue), however, recent evidence shows changes in the larynx including redundant tissue at the top of the larynx, and narrowing of the larynx behind the glottis. The investigators will characterize NTUAS in detail through comprehensive history, oral examination and upper airway endoscopy in US Norwich Terriers. Results will be used to create a NTUAS severity grading system. A subset of dogs will undergo computed tomography and nasal airflow measurements. Results will be compared for Norfolk Terriers, brachycephalic and mesaticephalic dogs of similar ages from a separately funded study. Identifying the contributory components of NTUAS is the first step in determining prognosis and evaluating treatment options.

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



## Musculoskeletal Conditions and Disease Research Program Area

### **02229-A: TPLO Surgery and Recovery: A Comparison of Arthroscopy and Arthrotomy**

Principal Investigator: Andrea Sundholm-Tepper, DVM; Washington State University

Total Grant Amount: \$12,960; Grant Period: 11/1/2015 - 12/31/2017

Cranial cruciate ligament (CrCL) rupture is the most common stifle (knee) condition in many breeds of dogs. Surgery is recommended to provide stabilization of the stifle and allow the patient to be free of lameness. Although several surgical procedures are available, all require examination and potential manipulation of damaged ligaments and cartilage inside the stifle joint. In human patients, arthroscopy is associated with lower costs and infection rates, and decreased morbidity (patient-related negative effects) compared to arthrotomy. Arthroscopy in dogs can be combined with many CrCL rupture surgeries including the Tibial Plateau Leveling Osteotomy (TPLO). Currently, clinical impressions are that dogs undergoing stifle arthroscopy are more comfortable and using their limbs sooner post-operatively than dogs undergoing arthrotomy for CrCL rupture surgery. This study will objectively measure and compare the recovery of dogs with CrCL rupture treated by TPLO with arthroscopy or arthrotomy. These findings will inform the decision-making process for stifle surgical procedures in dogs.

### **02275: Disease Risks Associated with Spay and Neuter: A Breed-Specific, Gender-Specific Perspective**

Principal Investigator: Benjamin Hart, DVM, PhD; University of California, Davis

Total Grant Amount: \$61,784; Grant Period: 9/1/2016 - 2/28/2018

This study extends the previously completed AKC Canine Health Foundation-funded study of 12 dog breeds to identify differences in the degree to which spay or neuter may be related to joint disorders (hip dysplasia; cranial cruciate ligament tear) and/or cancers (lymphoma; hemangiosarcoma; and mast cell tumor). The original breeds studied were: Labrador Retriever, Golden Retriever, German Shepherd Dog, Rottweiler, Boxer, Bulldog, Doberman Pinscher, Dachshund, Corgi (both breeds), Chihuahua, Yorkshire Terrier and Shih Tzu. The study did not find disease association in the small breeds with spaying or neutering, while in larger breeds disease risk was dependent upon gender, and whether the spay or neuter procedure was performed before or after one year of age (Hart, B.L., et al. 2014. Long-term health effects of neutering dogs: Comparison of Labrador Retrievers and Golden Retrievers. PLoS ONE 9(7): 10.1371/journal.pone.0102241). In this second phase, the following breeds will be studied: Great Dane, Australian Shepherd, Bernese Mountain Dog, Cocker Spaniel, Border Collie, Beagle, St. Bernard, Irish Wolfhound, Jack Russell Terrier, Pug, Maltese, Pomeranian, Miniature Schnauzer, Boston Terrier, Australian Cattle Dog, Shetland Sheepdog, English Springer Spaniel, Cavalier King Charles Spaniel, and West Highland White Terrier. Upon completion, the major publisher, Wiley, has agreed to place the total data set of all 31 breeds on an open access website as a resource for breeders, dog owners, researchers and veterinarians.



## Neurology Research Program Area

### **02387-MOU: Hereditary Deafness in Dogs – Genomic Studies in English Setters Using Full Sibling Pairs**

Principal Investigator: George Strain, PhD; Louisiana State University

Total Grant Amount: \$12,960; Grant Period: 9/1/2017 - 8/31/2018

Hereditary deafness associated with white pigmentation occurs in several dog breeds. The mechanism of inheritance is unknown, but does not appear to be simple Mendelian. Numerous studies to determine the mode of inheritance and locate the causative gene(s) have thus far failed. The investigators will use a unique modified twin study approach in an effort to determine the mode of inheritance and locate the causative gene(s). Full-sibling littermates will be identified, where one puppy has normal hearing and one is deaf. Like human twins, full siblings should have very similar DNA, which will reduce the variability of the DNA samples when compared to studies of unrelated dogs. Identifying candidate deafness genes will be an important breakthrough to understanding deafness in dogs and people, with a goal to establish a genetic test to reduce or eliminate deafness in these canine populations.

### **02157-MOU: Genomics of Deafness in the Dalmatian**

Principal Investigator: Claire Wade, PhD; University of Sydney

Total Grant Amount: \$120,960; Grant Period: 1/1/2015 - 12/31/2017

Congenital deafness is a health issue that has higher prevalence in certain breeds, including the Dalmatian. Other studies in this breed have found the trait to be inherited in a complex rather than simple Mendelian manner. Using a large number of samples from animals that have been tested for hearing status, Dr. Wade will employ the latest genomic technologies and computational analyses to conduct this study. The ultimate goal is to identify mutations underlying the trait of congenital deafness in the Dalmatian breed and work towards a genetic testing solution for the Dalmatian breeding community.

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

### **02172-MOU: Understanding Hereditary Deafness in Dogs**

Principal Investigator: George Strain, PhD; Louisiana State University

Total Grant Amount: \$120,015.00; Grant Period: 11/1/2015 - 10/31/2017

Hereditary deafness associated with white pigmentation occurs in numerous dog breeds, including Dalmatian (Dal, 22% unilaterally deaf, 8% bilaterally deaf) and Australian cattle dogs (ACD, 11.4% and 3%). The mechanism of inheritance is unknown, and previous studies to determine the mode of



inheritance and locate the causative gene(s) have been unsuccessful. Using a modified twin study approach, full-sibling littermates will be clinically and genetically evaluated. Using the Illumina CanineHD Beadchip, which contains 172,115 DNA markers (SNPs) spread uniformly across the canine chromosomes, markers will be compared between the sibling pairs, and differences between siblings at individual markers will thus be identified. Using this approach candidate deafness genes can be identified and will advance the current understanding of this disorder.

Funding for the research is provided through the efforts and generosity of the Australian Cattle Dog Health, Education, and Welfare, Australian Cattle Dog Club of America, Dalmatian Club of America, and the Dalmatian Club of America Foundation. The AKC Canine Health Foundation supports this effort and will oversee administration of funds and scientific progress reports.

### **02162-MOU: Defining The Genetic Foundations of Chiari-Like Malformation and Syringomyelia as a Tool to Better Treat Neuropathic Pain in the Dog**

Principal Investigator: Natasha Olby, VetMB PhD; North Carolina State University

Total Grant Amount: \$78,786; Grant Period: 1/1/2015 - 6/30/2018

Chiari-like malformations and syringomyelia (CM/SM) are a problem in Cavalier King Charles Spaniels (CKCS) causing severe neuropathic pain. The development of clinical signs and syringomyelia has been correlated to reduced caudal fossa to cranial cavity volume ratios and stenosis of the jugular foramen respectively. There is evidence this disorder is a complex hereditary trait. Humans with CM report increased sensitivity to touch and temperature. During case phenotyping for the genetic study, Dr. Olby will investigate sensory thresholds in affected and normal CKCS to improve the ability to document and treat pain in these patients. The goals of this project are to define the genetic etiology of this disease with the long-term aim of developing genetic tests, and to quantify the sensory dysfunction experienced by these dogs to facilitate objective therapeutic trials.

Funding for the research is provided through the efforts and generosity of the American Cavalier King Charles Spaniel Club Charitable Trust. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.

### **02165-MOU: Identification of Biomarkers and Therapeutic Targets for Canine Degenerative Myelopathy: The Search for A Cure**

Principal Investigator: Joan Coates, DVM; University of Missouri, Columbia

Total Grant Amount: \$154,077; Grant Period: 1/1/2015 - 12/31/2017

Degenerative myelopathy (DM) is an adult onset disease of the spinal cord causing progressive weakness and paralysis of the hind limbs and eventually all limbs. Mutations in an enzyme called superoxide dismutase 1 (SOD1), have been linked to DM and amyotrophic lateral sclerosis (ALS-Lou Gehrig's disease). DM is associated with degenerative loss of axons, which transmit signals from the brain and spinal cord to muscle. Currently no diagnostic test exists that would allow for repeated measurements with minimal invasiveness. The investigator will develop a test to assay the blood and



cerebrospinal fluid (CSF) for proteins that are exclusively found in axons under non-disease conditions, referred to as neurofilament proteins, to correlate the concentrations of neurofilament proteins in CSF and blood with disease stage, and anticipate that neurofilament protein concentration in blood and CSF will increase as disease progresses. Such a test will allow for minimally invasive monitoring of disease, and could be used to measure the success of therapy. The investigator will complement the test for neurofilament proteins with other studies to measure disease progression.

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 administration of funds and scientific progress reports.

### **02210: Gene Therapy for Canine Degenerative Myelopathy**

Principal Investigator: Kathrin Meyer, PhD; The Research Institute at Nationwide Children's Hospital  
Total Grant Amount: \$50,000; Grant Period: 1/1/2016 - 12/31/2018

Degenerative myelopathy (DM) is a devastating neurodegenerative disease that affects multiple dog breeds. DM is an adult-onset disease that manifests at the later stages of life, characterized by progressive weakness and inability to control the hind limbs, ultimately leading to involvement of forelimbs and complete paralysis. There are no current treatments available. Recent studies have identified mutation in the Superoxide dismutase 1 (SOD1) gene as a high risk factor associated with canine DM. In humans, mutations in the same SOD1 gene cause Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disorder very similar to canine DM. Reduction of mutant SOD1 in ALS mouse models provides beneficial effects. Hence, therapeutic approaches to reduce the expression of mutant SOD1 in DM-affected dogs may improve survival and preserve neurologic function. In this study, a viral-based gene therapy approach to treat DM will be evaluated, utilizing Adeno-associated Virus 9 (AAV9) mediated delivery of shRNA to reduce the mutant SOD1 in DM affected dogs. If successful, this one-time treatment with AAV9 SOD1 shRNA will result in improved quality of life, and significantly extend the survival of dogs affected with this disease.

### **02290-MOU: Further Studies to Identify the Mutation Responsible for DUNGd**

Principal Investigator: Dennis O'Brien, DVM, PhD; University of Missouri, Columbia  
Total Grant Amount: \$14,904; Grant Period: 7/1/2016 - 12/31/2017

A hereditary disease that breeders called DUNGd was recognized in Gordon Setters in the early 1990s and reported in the veterinary literature in 2000. Affected pups develop normally until 3-4 weeks of age when they show progressive behavioral changes, gait abnormalities and weakness. By 5-6 weeks of age, they are recumbent and must be euthanized. The investigators will utilize next-generation whole genome sequencing and gene mapping to identify genes associated with the disease. If a mutation that appears to cause the disease is found, they will develop a DNA test to identify carriers of the mutation, and thus permit breeders to avoid producing affected pups in the future.





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

## **Oncology Research Program Area**

### **02405-A: Transcriptome Based Diagnostics in Canine Soft Tissue Sarcoma**

Principal Investigator: Andrew Miller, DVM; Cornell University

Total Grant Amount: \$14,880; Grant Period: 10/1/2017 - 9/30/2018

Sarcomas are malignant cancers that can arise in any part of the body; however, in the dog, a subset referred to as soft tissue sarcomas account for 10-15% of all skin and subcutaneous cancers. 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). Currently, histologic grade is 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. Unfortunately, despite how common soft tissue sarcomas are in the dog, veterinarians still rely solely on the histologic grade, which is subjective at best, and does not incorporate any genetic data into the diagnostic plan. The investigators will develop a validated test to analyze the genes present in canine soft tissue sarcoma. This will allow for future prospective studies in which genetic analysis will be used to guide treatment in dogs afflicted with this cancer, lead to more refined care, and ultimately improve patient outcome.

### **01889-G: Innovations in Prevention, Diagnosis, and Treatment of Cancer - Goldens Lead the Way**

Principal Investigator: Jaime Modiano, VMD, PhD; University of Minnesota; Elinor Karlsson, PhD; Broad Institute; Matthew Breen, PhD; North Carolina State University

Total Grant Amount: \$360,933; Grant Period: 1/1/2014 - 12/31/2017

Lymphoma and hemangiosarcoma are major health problems in Golden Retrievers. Through ongoing collaboration, the investigative team has identified several regions of the genome that contain genetic heritable risk factors for lymphoma and hemangiosarcoma in Golden Retrievers. Tumor-specific mutations occur recurrently in both cancers, some linked to duration of remission when treated with standard of care. Their results indicate that a few heritable genetic risk factors may account for as much as 50% of the risk for these cancers. The investigators hope to develop DNA tests that can predict risk for individual dogs, and to manage risk across the whole population. Both the inherited risk factors and tumor mutations point to pathways that have been implicated in the pathogenesis of lymphoma and hemangiosarcoma, and thus should inform the development of targeted therapies. The



investigators propose to find precise mutations for heritable genetic risk factors and to validate markers (mutations) used to determine risk in a population of Golden Retrievers from the United States and Europe in order to develop robust risk prediction tools and an accompanying DNA test. Further, they will identify and characterize tumor mutations and study their relationship to heritable risk factors, tumor pathogenetic mechanisms, and disease outcome.

### **02171-MOU: Histiocytic Sarcoma in Bernese Mountain Dogs: Novel Approaches To Treatment**

Principal Investigator: Vilma Yuzbasiyan-Gurkan, PhD; Michigan State University

Total Grant Amount: \$43,661; Grant Period: 7/1/2015 - 12/31/2017

Canine histiocytic sarcoma (HS) is an aggressive cancer that affects Bernese Mountain Dogs (BMD) with a prevalence that ranges from 15 to 25% of the population. Current treatment options for HS are conventional chemotherapeutic drugs, to which dogs respond poorly and only for a short period of time. The investigators will evaluate a modality of treatment for HS using small molecule inhibitors of key cancer pathways. They will also focus on the gene expression associated with the response to treatment to better understand the events leading to the development of HS in BMD, and therefore, develop better therapeutic strategies.

Funding for the research is provided through the efforts and generosity of the Bernese Mountain Dog Club of America. The AKC Canine Health Foundation supports this effort and will oversee administration of funds and scientific progress reports.

### **02204: Using Enhanced Imaging to Evaluate Tumor Margins for Canine Mammary Cancer and Soft Tissue Sarcoma**

Principal Investigator: Laura Selmic, BVetMed; University of Illinois

Total Grant Amount: \$46,358; Grant Period: 1/1/2016 - 12/31/2017

Surgery is the primary treatment for many common tumors affecting dogs including mammary tumors and soft tissue sarcomas (STS). For these tumors, the best chance of cure is offered if the surgeon can fully remove both visible and microscopic traces of the tumor. Unfortunately, to do this, surgeons must rely on indirect and crude methods to assess the extent of the tumor during surgery. The success of the procedure will not be known until several days later, following sample assessment by the pathologist. After surgery, decisions regarding the necessity of further treatment and the patient's prognosis are often determined from the pathology results. For malignant tumors, if the disease is minimally or incompletely removed, further surgery or radiation therapy is often required. Additional treatments such as these can result in further risk and discomfort for the patient as well as present emotional and financial costs for owners. Optical coherence tomography (OCT) 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 two important canine cancers - mammary tumors and STS. If successful, this technology can



be used to assess for residual cancer during surgery to benefit patients by guiding accurate treatment recommendations and attempting to reduce the need for additional treatments or surgery, and thus advancing the standard of care for canine patients.

### **02237-A: Capturing Tumor Cells in Canine Blood**

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

Total Grant Amount: \$10,239; Grant Period: 6/1/2016 - 11/30/2017

Like their human owners, many dogs suffer from cancer, which is often malignant, spreading through the body via blood. Once tumors have spread, the usual result is a poor outcome. Tumor cells in circulation (CTCs) can be counted in the blood of people with cancer using immunocapture devices, and can tell the clinician how aggressive the tumor is, its potential to spread, and how long a patient might survive. There is currently no such way of detecting CTCs in dogs. Development of an assay for counting CTCs in canine blood would be of benefit to canine patients because, from a simple blood test, we could detect hidden tumors and gather information on tumor severity and the likelihood of metastasis. The investigators will test a novel immunocapture microdevice - the GEDI - for counting tumor cells in canine blood. In this pilot study, blood samples from healthy dogs will be manipulated to test the ability to count how many added tumor cells are captured by the GEDI device. If the GEDI does capture the tumor cells, the next step will be to determine if the device can capture CTCs from the blood of dogs with cancer, paving a path to early detection of canine cancer.

### **02321: Clinical Trial of Procaspace-3 Activator (PAC-1) in Combination with Hydroxyurea for Treatment of Canine Meningioma**

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

Total Grant Amount: \$51,191; Grant Period: 2/1/2017 - 1/31/2019

Primary brain tumors are a significant cause of illness and death in pet dogs, with meningioma accounting for approximately half of the cases seen by veterinary neurologists and oncologists. Although surgery remains the best treatment for dogs with meningioma, some dogs are not good candidates for this approach based on their tumor size and/or location. Dogs also may experience tumor regrowth after an attempt is made to surgically remove the tumor. In these situations, effective treatment options are limited. Thus, new treatments that are both safe and effective are needed for dogs with meningioma. A team of investigators from the National Cancer Institute's Comparative Oncology Program (NCI-COP) and selected veterinary academic centers will work together using state-of-the-art imaging and a novel therapeutic approach for dogs with meningioma that are good surgical candidates. Dogs enrolled in this study will receive an investigational combination of chemotherapy agents (PAC-1 + hydroxyurea) and will be monitored with magnetic resonance and non-invasive molecular imaging techniques. Dogs will then undergo tumor removal and tissue analysis. This approach is the first to validate and advance a new therapy that is directly applicable to dogs, and potentially also to humans, with advanced, locally-recurrent, and/or non-resectable meningioma.



## Oncology - Hemangiosarcoma Research Program Area

### **02217: A Novel Mechanism to Regulate the Growth of Canine Hemangiosarcoma**

Principal Investigator: Erin Dickerson, PhD; University of Minnesota

Total Grant Amount: \$86,206; Grant Period: 1/1/2016 - 12/31/2017

Hemangiosarcoma (HSA) is an extremely aggressive cancer that is rapidly fatal in dogs. While the lifetime risk is alarmingly high for some breeds such as Golden Retrievers and German Shepherd Dogs, the disease does not discriminate, and it can strike any dog at any time. The outcome for dogs with HSA has changed very little over the past few decades. Recent evidence provides essential clues into how these tumors grow, generating new ideas for treatment approaches. Such new evidence suggests that HSA cells rely on the metabolism of lipids or fatty acids to supply energy to the tumor. To obtain these lipids, HSAs may take over the metabolic machinery of neighboring cells, forcing them to produce nutrients for the tumor. This study will verify that tumor cells rely on lipid metabolism for growth, and determine if tumor cells alter the metabolism of fat cells to obtain cellular nutrients and accelerate tumor cell lipid metabolism. Identifying and exploiting a novel mechanism that may disrupt this process by inhibiting the interactions between tumor cells and cells in the tumor environment may speed clinical investigations and lead to improved outcomes for dogs.

### **02234-MOU: A Novel Approach for Prevention of Canine Hemangiosarcoma**

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

Total Grant Amount: \$432,000; Grant Period: 3/1/2016 - 2/28/2019

Hemangiosarcoma is an aggressive form of cancer in many dogs, with Golden Retrievers, Portuguese Water Dogs and Boxers at an especially high risk. Hemangiosarcoma is incurable partly because the cancer is detected at a very advanced stage when it is resistant to conventional therapies. To improve outcomes for hemangiosarcoma patients will involve effective methods for early detection and disease prevention. The investigators will use two novel technologies consisting of a patented test to detect hemangiosarcoma cells in blood samples, and a treatment that attacks the cells that establish and maintain the disease. Three milestones will be met: first, to expand understanding of the performance and utility of the blood test for cancer in dogs with active disease; second, to confirm utility of the test to predict disease progression in treated dogs, third will be to establish the performance of the test in the "early detection" setting (dogs at high risk without evidence of active cancer), and thus measure hemangiosarcoma prevention through eradication of the tumor initiating cells with the targeted, investigational drug. This project has an ultimate goal for disease prevention in all dogs.

Funding for the research is provided through the collaborative efforts and generosity of the American Boxer Charitable Foundation, Golden Retriever Foundation, and Portuguese Water Dog Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.



## Oncology - Lymphoma Research Program Area

### **01418: Harnessing a Dog's Own Immune System to Kill Lymphoma Tumor Cells**

Principal Investigator: Heather Wilson, DVM; Texas A&M Research Foundation

Total Grant Amount: \$150,000; Grant Period: 1/1/2011 - 6/30/2018

Lymphoma is the most common malignancy of dogs representing up to 25% of diagnosed cancers. Dogs often develop an aggressive form of lymphoma that is rarely curable, with most unfortunately succumbing to disease within 12 months of diagnosis despite best-available chemotherapies. Dr. Wilson will develop a new treatment to re-train the dog's own immune system to attack the most common type of canine lymphoma, B-cell lymphoma. In order to accomplish this they will obtain a small number of circulating white blood cells, called T cells, from the blood of affected dogs and insert a gene that will cause the T cell to express a receptor which recognizes the tumor "fingerprint". After docking with the lymphoma, the T cell will be triggered to mount an immune response against the tumor cells with the specific fingerprint. This therapy could be used alone or in combination with chemotherapy. Their preliminary data demonstrate that it is possible to genetically modify T cells. Further, they have been able to successfully harvest and grow T cells in the laboratory and return them safely to the dog. These infused cells can be found in the blood and tumor weeks after infusion, showing that it is possible for these cells to survive in the dog. If successful this study will be the first to develop an "in-dog" T-cell therapy targeting a tumor that has historically thought to be untreatable.

### **01918-G: Discovery of Biomarkers to Detect Lymphoma Risk, Classify for Treatment, and Predict Outcome in Golden Retrievers**

Principal Investigator: Jeffery Bryan, DVM, PhD; University of Missouri, Columbia

Total Grant Amount: \$404,813; Grant Period: 7/1/2013 - 12/31/2017

Lymphoma strikes 1 in 8 Golden Retrievers, approximately one-third of the cases being B-cell. The investigative team will focus their efforts on an area of emerging importance in cancer: epigenetics, defined as stable and heritable patterns of gene expression that do not entail any alterations to the original DNA sequence. Epigenetic DNA methylation changes clearly underlie development of lymphoma in humans, but have been evaluated minimally in dogs. These investigators will use flow cytometry paired with biopsy to characterize B-cell lymphomas of Golden Retrievers. They will identify DNA methylation changes in lymphoma cells not present in normal cells to develop biomarkers of each class of lymphoma, and identify new therapy targets for affected Golden Retrievers. Because DNA methylation changes occur so early in the process of cancer formation, these could serve as biomarkers of risk, allowing medicine or diet to prevent lymphoma in Golden Retrievers before it develops. Finally, they will identify tumor initiating cells (TIC) in lymphoma biopsies to characterize stem-like cells by surface markers and DNA methylation changes to aid therapeutic strategy development and to advance the prevention and management of lymphoma in Golden Retrievers.



## **02304-A: Investigating a Biomarker and Novel Therapeutic Target for Canine Diffuse Large B Cell Lymphoma**

Principal Investigator: Jennifer Luff, VMD, PhD; North Carolina State University

Total Grant Amount: \$14,509; Grant Period: 12/1/2016 - 11/30/2017

Canine diffuse large B-cell lymphoma (DLBCL) is a common, aggressive cancer in dogs. The average survival time after initial diagnosis is one year. Unfortunately, the diagnosis of DLBCL is often made late in disease when the cancer is advanced, which negatively impacts the survival of the dog. Therefore, there is a need to 1) develop non-invasive screening methods for early diagnosis, and 2) identify novel therapies to treat this cancer. In human oncology, the discovery of a new type of gene called long non-coding RNA (lncRNA) has led to the development of non-invasive screening methods for certain cancers. These lncRNAs are also being explored for their use as new cancer targets for drug development, which are expected to have fewer side effects than current treatments. Since these lncRNA can be detected in blood of cancer patients, they can be used in non-invasive, early detection assays for some cancers. Recently, human DLBCLs were shown to express high levels of the lncRNA HOX transcript antisense RNA (HOTAIR), and its expression was predictive of a poor prognosis; human HOTAIR is also being explored as a new target for cancer therapy. The investigators will study canine lncRNA HOTAIR to determine if it is expressed in canine DLBCL and can be detected in the blood of cancer patients. If successful, this research will open new lines of research improving the detection and treatment of a common and devastating cancer of dogs.

## **02309: Targeting the Cancer Epigenome: The Effect of Specific Histone Lysine Methyltransferase Inhibition in Canine B-Cell Lymphoma**

Principal Investigator: Angela McCleary-Wheeler, DVM, PhD; Cornell University

Total Grant Amount: \$78,069; Grant Period: 1/1/2017 - 12/31/2018

While often treatable, canine lymphoma can rarely be cured. A continued understanding of the mechanisms causing lymphoma in dogs and identification of novel therapies are needed to improve survival. Research that has been actively explored and provided exciting breakthroughs for human lymphoma is epigenetics, or alterations in how genes are turned on and off independent of the DNA sequence. One way this occurs is through modifications of proteins that interact with DNA called histones. Modifications to these histones can result in genes being turned on or off, leading to the development of cancer. One enzyme that modifies histones, EZH2, has been found to play a role in some human lymphomas. Given the striking similarities between human and canine lymphoma, the objective of this study is to characterize the function and role of EZH2 in canine lymphoma. The investigators will utilize an EZH2 inhibitor to study EZH2 in canine lymphoma cells, and help guide the future development of this targeted inhibitor for use as a novel therapy for canine lymphoma.



## **02315-A: Discovering Peptide Targets for Development of Adoptive Cell Therapy for Peripheral T-Cell Lymphoma**

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

Total Grant Amount: \$14,990; Grant Period: 12/1/2016 - 11/30/2017

T cells (a type of white blood cell known as a lymphocyte) constitute the immune system's most potent weapons against cancer, but growing malignant cells can quickly outpace and overwhelm these defenses. In the most advanced form of cancer immunotherapy, these T cells can be isolated from the body, reinvigorated and expanded in the test tube, and then given back to patients -- in some cases, leading to years-long remissions of advanced cancers, usually melanomas, resistant to other treatments. There is tremendous enthusiasm to extend this approach to lymphoma and other incurable malignancies, which has proved difficult because T cells also recognize and attack normal tissues, causing patient death. Because the therapy involves living cells, and is personalized -- T cells typically target a marker unique to a specific patient's tumor -- the complexity and cost is enormous. Efforts to make this immunotherapy safer and readily available are being pursued, focused on finding cancer proteins recognized by T cells that are 1) shared between patients with the same cancer, and 2) not expressed by normal tissues, sparing them from inappropriate attack. The investigators recently discovered a protein expressed by lymphoma cells across multiple canine patients; importantly, normal tissue expression appears minimal. This study's goal is to identify the correct tiny fragment (peptide) of this protein that T cells directly recognize, which then will be used to extract these T cells from patients for development of immunotherapy for dogs.

## **02316: Genetic Risk Factors for Canine T zone Lymphoma**

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

Total Grant Amount: \$52,894; Grant Period: 1/1/2017 - 12/31/2017

The Golden Retriever is a breed that develops a variety of cancers at a high frequency. One type of cancer, T zone lymphoma, is so common that 40% of cases are seen in Golden Retrievers. This observation suggests a genetic predisposition. The investigators have completed an environmental risk factor study and the first phase of a genetic risk factor study to better understand the causes of this disease. Two key findings from this work are, 1) The same genetic region associated with risk for mast cell tumors was identified as conferring risk for T zone lymphoma, and 2) The presence of hypothyroidism was protective for T zone lymphoma, and genes associated with thyroid function are also found in the risk regions. The goal of this study is to complete the genetic risk factor study by identifying specific genetic mutations associated with the disease, to improve understanding of the mechanisms that lead to T zone lymphoma as well as mast cell tumors in dogs.



## **02317: The Role of Complex Translocations Associated with TP53 Somatic Mutations for Aiding Prognosis of Canine Diffuse Large B cell Lymphoma**

Principal Investigator: Matthew Breen, PhD; North Carolina State University

Total Grant Amount: \$177,327; Grant Period: 1/1/2017 - 12/31/2018

Lymphoma accounts for up to 24% of all cancers diagnosed in pet dogs; diffuse large B-cell lymphoma (DLBCL) is the most common subtype. The response to treatment for canine lymphoma remains highly variable with no reliable means to predict response. Studies of lymphoma in people have identified characteristic genome changes that have both diagnostic and prognostic significance. In human DLBCL, mutations in the TP53 gene, and genome rearrangements involving the MYC, BCL2 and BCL6 genes have been shown to confer particularly poor prognosis in cases treated with standard of care multi-agent (CHOP-based) chemotherapy. The investigator's previous CHF-funded studies have shown that canine cancers, including lymphoma, exhibit genomic changes that are conserved with those observed in the corresponding human cancers, and have identified MYC and BCL2 rearrangements and a high frequency of TP53 mutation in canine DLBCL. This research will screen a well-defined collection of over 450 pre-treatment, canine DLBCL samples to determine accurate frequencies of these genome changes. The researchers will investigate the correlation of these target aberrations with duration of first remission, and identify key genomic signatures that may aid prognosis of prospective canine lymphoma cases. The data generated should assist owners and veterinarians with decisions regarding treatment, and patients with signatures predictive of poor response to CHOP chemotherapy may benefit from more aggressive treatment to improve outcomes.

## **02318: Genetic and Environmental Risk for Lymphoma in Boxer Dogs**

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

Total Grant Amount: \$112,861; Grant Period: 1/1/2017 - 12/31/2018

Lymphoma is a fatal cancer that can occur in any dog. Lymphoma is more common in Boxers, Golden Retrievers, and several other purebreds, which suggests involvement of inherited genes. Recent research has focused on gene mutations in the tumors of dogs with lymphoma. However, we do not understand why these mutations accumulate in certain dogs, and this understanding is essential for disease prevention. Canine lymphoma resembles Non-Hodgkin lymphoma (NHL) in humans, which is more common in industrialized countries and is associated with chemicals found in tobacco smoke, certain household products, pesticides, herbicides, and fungicides. Glutathione-S-transferases (GSTs) are enzymes that can break down toxic chemicals in the body and prevent tumor mutations. Inherited gene defects in the 3 major GST enzymes, GST-theta, GST-pi and GST-mu, each increase NHL risk. Simultaneous defects in more than one enzyme further increase NHL risk. The investigators have characterized two GST-theta enzymes in dogs, and both have defective gene variants. Findings suggest one variant is a risk factor for lymphoma in dogs of varying breeds. This research will determine whether defective GST genes along with certain household and yard chemicals are associated with lymphoma in dogs, with a focus on the high-risk Boxer breed. The overall goal of this study is to identify combinations of genes and environmental chemicals that contribute to the development of lymphoma in dogs, so that better cancer prevention strategies can be developed.





## Oncology - Osteosarcoma Research Program Area

### **01660: 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 - 12/31/2017

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.

### **02215: A Cancer Vaccine for Canine Osteosarcoma**

Principal Investigator: Rowan Milner, BVSc; University of Florida  
Total Grant Amount: \$80,974; Grant Period: 1/1/2016 - 12/31/2017

Osteosarcoma is a malignant cancer that carries a very poor prognosis in most large breeds of dogs. The standard of care treatment for osteosarcoma is surgery followed by chemotherapy. A large number of osteosarcomas undergo early metastasis (spread) even with early surgical intervention and chemotherapy. Infections of the surgery site, especially when limb-sparing surgery is used, have been known to stimulate the immune system post-operatively in dogs, resulting in improved survival. Developing an osteosarcoma cancer vaccine holds promise as an adjunct treatment to surgery and chemotherapy. In a previous study of 400 dogs with melanoma the investigators showed that a vaccine containing the ganglioside (GD3) causes a measurable immune response in normal dogs and dogs with melanoma, and prolonged survival. In this study, dogs with osteosarcoma will be randomly assigned to two treatment groups with the outcomes of dogs receiving the vaccine plus standard of care will be compared to dogs who receive standard of care without vaccination. Vaccines will be administered monthly for 4 treatments and the dogs monitored for life. The outcome of this study will help us understand the immune process associated with cancer vaccines for osteosarcoma and with an ultimate goal to improve survival for dogs.



## Ophthalmology Research Program Area

### **02332-A: Identification of Mutations for Primary Lens Luxation in Multiple Dog Breeds**

Principal Investigator: Cathryn Mellersh, PhD; Animal Health Trust

Total Grant Amount: \$14,812; Grant Period: 5/1/2017 - 4/30/2018

Primary lens luxation (PLL) is a painful inherited disease that affects many breeds of dog. A mutation in the gene ADAMTS17 has been identified that causes PLL in at least 20 breeds and DNA tests are available for these breeds. Different mutations in ADAMTS17 are also known to cause a different disease, primary open angle glaucoma (POAG), in a small number of additional breeds and POAG in two more breeds is known to be caused by mutations in the closely related gene ADAMTS10. POAG is characterized by increased pressure within the eye that is due to abnormalities deep within the part of the eye known as the ciliary cleft that disrupt the normal drainage of fluid within the eye. Although PLL and POAG are different diseases, they are both caused by abnormalities in the part of the eye known as the ciliary body or in nearby tissues. There are currently several breeds of dog that are affected by PLL but for which mutations are currently unknown. The investigators will investigate both ADAMTS10 and ADAMTS17 for novel mutations that explain PLL in five breeds of dog. The DNA sequence data can also be used to facilitate future studies of other inherited disorders in dogs, beyond the scope of this study.

### **02336: 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 - 4/30/2018

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 also be translatable to the human condition.



## **02340: Clinical and Genetic Background of Progressive Retinal Atrophy in Miniature Schnauzers**

Principal Investigator: Hannes Lohi, PhD; University of Helsinki and the Folkhälsan Institute of Genetics  
Total Grant Amount: \$46,224; Grant Period: 12/1/2017 - 11/30/2018

Dogs may be affected with hereditary eye disorders, which cause severe vision impairment, and sometimes progress to complete blindness. One hereditary condition is progressive retinal atrophy (PRA), in which the light-sensing receptors in the retina are lost, leading to complete blindness. Currently there are no treatment options for this disease. The development of genetic testing would be an important breakthrough for veterinary medicine. The identification of a causative gene would also enable a study of the molecular background of the disease for improved treatment plans. The investigators have established a large pedigree and clinically-investigated sample cohort in Miniature Schnauzers with PRA to identify its genetic cause, and have already identified the chromosomal region suspected to harbor the causative gene. Through this study, the researchers hope to identify a PRA gene and mutation, leading to a genetic test for the eradication of this disorder from the Miniature Schnauzer breed.

## **02403-MOU: 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 - 10/31/2019

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.



## **02061: Emergence of Pigmentary Uveitis as a Potential Cause of Cataracts and Glaucoma**

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

Total Grant Amount: \$74,070; Grant Period: 1/1/2014 - 12/31/2017

Pigmentary uveitis affects 10% of senior Golden Retrievers and frequently results in blindness due to cataracts and/or glaucoma. The pain of glaucoma often leads to removal of the eye. Currently there is no prevention or effective treatment for pigmentary uveitis. Evidence strongly suggests pigmentary uveitis is an inherited disease in the Golden Retriever breed, and family members (parents/offspring, full- and half-siblings) can be affected. Complicating the phenotype is the fact that most dogs are 8 years or older before developing clinical signs. Therefore, affected dogs may be used extensively in a breeding program before being diagnosed. This has frustrated conscientious breeders in their efforts to decrease the prevalence of pigmentary uveitis. The investigators will perform a genome-wide association study (GWAS) to identify a chromosomal region associated with Golden Retriever pigmentary uveitis, and use high-throughput DNA sequencing to allow identification of the causative mutation. Identification of the gene responsible for pigmentary uveitis would permit development of a genetic test to inform breeding decisions. Knowing the molecular basis underlying pigmentary uveitis may allow researchers to develop more effective treatments for dogs to possibly prevent the blindness, cataracts, and glaucoma caused by pigmentary uveitis.

## **02105-A: The Genetics of Keratoconjunctivitis Sicca in West Highland White Terriers**

Principal Investigator: Christopher Murphy, DVM, PhD; University of California, Davis

Total Grant Amount: \$5,000; Grant Period: 6/1/2015 - 11/30/2017

Dry eye disease or keratoconjunctivitis sicca (KCS) is a devastating disease in dogs and humans where inadequate tear production can result in ocular pain, corneal ulceration and even blindness. The most common cause for KCS in dogs is immune-mediated. A variety of treatments for KCS exist including immunomodulators, tear replacements, and surgical interventions, but are often incompletely effective in dogs and humans. Several dog breeds including West Highland White Terriers are seen more commonly for KCS in comparison to other breeds, suggesting a genetic component. The investigators hope to identify the region of the dog genome associated with KCS in the West Highland White Terrier. The entire canine genome will be evaluated for an association with KCS to identify the gene(s) responsible for this condition in West Highland White Terriers and help us understand KCS better in dogs and humans. The ultimate goal will be to develop a genetic test for KCS in West Highland White Terriers and possibly other breeds with an increased risk of KCS.



## **02164-MOU: Determining the Genetic Contribution to Boxer Corneal Ulcers**

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

Total Grant Amount: \$68,053; Grant Period: 1/1/2015 - 12/31/2017

Spontaneous chronic corneal epithelial defects (SCCEDs) are commonly seen in Boxers. . The predilection for Boxers suggests that SCCEDs is inherited in this breed. Affected dogs develop spontaneous corneal ulcers that are often exceptionally painful and persist for weeks to months. Most dogs require surgical therapy and experience corneal scarring as a result. The impact on the quality of life for dogs during episodes of ulceration has led to increased interest in disease prevention. However, since SCCED is an adult onset disease, many dogs are selected for breeding before they are diagnosed. Early testing could identify affected animals and greatly decrease the prevalence of SCCEDs. In a previous study funded by the AKC CHF, the investigators performed a genome wide association study in Boxers. They will now perform whole genome sequencing on a subset of affected and unaffected dogs, using data from the GWAS. Variants of interest will be used to determine the gene and ultimately the causative genetic mutation. The identification of a genetic cause for SCCEDs in the Boxer can be used to reduce the prevalence of this disease in this breed and others.

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 administration of funds and scientific progress reports.

## **02243-A: Genomic Profiling of Canine Corneal Endothelial Dystrophy**

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

Total Grant Amount: \$12,960; Grant Period: 1/1/2016 - 12/31/2017

Corneal endothelial dystrophy (CED) is a disease in dogs that can result in blindness and ocular pain. The endothelial cells comprise the most inner aspect of the cornea and are responsible for maintaining a proper fluid balance and thus corneal transparency. In dogs with CED, the endothelial cells degenerate prematurely until the remaining cells no longer function properly, resulting in corneal swelling, secondary vision compromise and corneal ulceration. The only definitive treatment for CED is a corneal transplant. Unfortunately, corneal transplants are rarely performed in canine patients with CED due to the expense of the surgery and follow-up care, high risk of complications, and lack of appropriate donor tissue. Several dog breeds including Boston Terriers, German Shorthaired Pointers and German Wirehaired Pointers are seen more commonly for CED compared to other breeds, thus this disease may have a genetic basis. A similar condition called Fuchs endothelial corneal dystrophy (FECD) occurs in humans and several genes associated with FECD have been identified. This project will investigate the genetics of CED in dogs, evaluating the entire canine genome for an association with CED to identify the gene(s) responsible for this condition in these 3 breeds, and to develop a genetic test for CED.



## Renal Disease Research Program Area

### **01844: Treatment of Urinary Incontinence with Multipotent Muscle Cells: A Regenerative Medicine Approach to a Common Canine Health Problem**

Principal Investigator: Shelly Vaden, DVM, PhD; North Carolina State University

Total Grant Amount: \$116,184; Grant Period: 1/1/2013 - 12/31/2017

Urinary incontinence affects more than 20% of spayed female dogs, with medium and large breeds more commonly affected. In the majority of cases, urinary incontinence is caused by dysfunction of the muscles controlling the urethral sphincter. This results in uncontrolled loss of urine and can lead to serious bladder and kidney infections, in addition to irritation and/or ulceration of the skin. Treatment can include hormone therapy, drugs designed to strengthen the muscle tone of the urethral sphincter, collagen injections, or surgery. The investigator has reported that injection of muscle progenitor cells into damaged urethral sphincters can restore normal function in dogs. This project will extend those observations and examine the usefulness of cultured muscle cells for the restoration of function of the urethral sphincter in dogs with naturally occurring urinary incontinence.

### **02066: Identification of Novel Biomarkers and Therapeutic Targets for Chronic Kidney Disease in Dogs**

Principal Investigator: Mary Nability, DVM, PhD; Texas A&M AgriLife Research

Total Grant Amount: \$108,243; Grant Period: 1/1/2014 - 6/30/2018

Chronic kidney disease is a significant cause of illness and death in dogs. Early treatment can prolong the lives of dogs with chronic kidney disease, but timely detection can be difficult. Improvements in tests to detect kidney damage at an earlier stage would allow veterinarians to provide dogs with treatments in a more timely fashion to slow disease progression and improve quality and length of life. Further, better treatments are needed to prevent disease progression. MicroRNAs (miRNAs) are small molecules that can regulate gene expression. Many studies have found that increases or decreases in miRNAs can serve as biomarkers of diseases, including human chronic kidney disease. They also contribute to the development of diseases. The investigator will evaluate miRNAs in the serum and urine of dogs with chronic kidney disease to determine their use as biomarkers of kidney injury and their potential as targets for future therapeutics. Gene and protein targets of altered miRNAs will also be evaluated to learn more about the mechanisms that contribute to the development of chronic kidney disease in dogs.



## **02152: Translation of MicroRNA into an Early Diagnostic Test for Chronic Kidney Disease**

Principal Investigator: Mary Nabity, DVM, PhD; Texas A&M AgriLife Research

Total Grant Amount: \$26,988; Grant Period: 1/1/2015 - 12/31/2017

Chronic kidney disease (CKD) is a significant cause of illness and death in dogs and is often due to glomerular diseases. Dogs with glomerular disease often have poor outcomes with standard therapy, and specific treatment recommendations are difficult without performing a kidney biopsy. Tests to non-invasively diagnose the type of glomerular disease would help veterinarians more appropriately treat these patients and provide insight into the mechanisms that cause disease. This could lead to better therapies that slow disease progression and improve quality and length of life in dogs with CKD. One area of emerging importance in CKD is the role of microRNAs (miRNAs) in disease pathogenesis and progression. The goal of this study is to identify miRNAs in serum and urine of dogs that are specific for the three major causes of canine glomerular disease. They also aim to identify miRNAs associated with disease progression. Successful completion of these goals will support the translation of miRNAs into diagnostic tests and viable targets for future drug development.

## **02263-MOU: Characterization of Kidney Disease in Dalmatians**

Principal Investigator: Rachel Cianciolo, VMD, PhD; Ohio State University

Total Grant Amount: \$31,434; Grant Period: 5/1/2016 - 4/30/2018

Chronic kidney disease is a significant progressive problem in dogs. Two different hereditary diseases of the urinary system are being studied in Dalmatian dogs: urinary stone formation (urolithiasis) and glomerular disease. These diseases cause distinct clinical signs: urolithiasis leads to urinary tract obstruction while glomerular disease results in protein loss into the urine (proteinuria). The genetic cause of urolithiasis is known while the genetic cause of glomerular disease has not yet been identified. Preliminary investigations indicate that there may be multiple causes of proteinuria in Dalmatians. Evaluation of kidney tissue by the International Veterinary Renal Pathology Service has revealed diverse types of glomerular diseases in Dalmatians, at least 4 of which might be hereditary. Therefore, the most common disease type is unknown and must be identified and characterized. A detailed review of necropsy and biopsy sample archives previously obtained from Dalmatians with proteinuria will be performed. Next, prospective examination of select kidney samples using advanced techniques (electron microscopy and immunofluorescence) will ensure an accurate diagnosis of the glomerular disease. Ultimately, genetic analyses performed on related dogs could demonstrate similar glomerular lesions to identify candidate genes.

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



## Reproductive Conditions and Disease Research Program Area

### **02264-A: Role of E. Coli Biofilm in Canine Pyometra**

Principal Investigator: Marco Coutinho da Silva, DVM, PhD; Ohio State University

Total Grant Amount: \$14,731; Grant Period: 5/1/2016 - 12/31/2017

Pyometra is a potentially life-threatening infection of the canine uterus by bacteria, most commonly *Escherichia coli* (*E. coli*). In humans with recurrent infections, *E. coli* produces a biofilm, a layer of polysaccharide that protects the organism from the host immune system as well as antibiotic agents, decreasing treatment efficacy. The investigators postulate that biofilm production by *E. coli* within the endometrium of the bitch may be responsible for perpetuating the disease and making treatment difficult. In this pilot study, the potential of *E. coli* obtained from clinical cases of canine pyometra to produce biofilm will be evaluated *in vitro* and *in vivo*. Endometrial samples from clinical cases of pyometra will be evaluated for the presence of biofilm *in situ*, as well as the ability of the isolated bacteria to produce biofilm *in vitro*. If successful, demonstration of the presence of biofilm in the endometrium of bitches affected by pyometra could lead to development of new therapeutics targeted to disrupt the biofilm, resulting in improved treatment for canine pyometra.

### **02267-A: An Epidemiological Study of *Brucella canis***

Principal Investigator: Tory Whitten, MPH; Minnesota Department of Health

Total Grant Amount: \$14,985.65; Grant Period: 11/1/2016 - 12/31/2017

Canine brucellosis is a reproductive disease caused by the bacterium *Brucella canis* (*B. canis*) that can cause infertility, abortion and severe spinal infections in dogs. Though well understood in the context of canine breeding operations, this disease is an under-recognized public health issue in the canine rescue and shelter populations, and may constitute a source of infection to dog and human populations. In 2015 there was an increase in the number of rescue dogs identified with canine brucellosis in Minnesota where, prior to 2015, there had been no cases of canine brucellosis identified in a dog not used in a breeding program. This study will measure exposure to this disease in rescue and shelter dogs entering Minnesota, as a first step to understanding prevalence of this important reproductive disease. The results of this study will be used to determine prevalence and raise awareness of this disease in rescue and shelter dog populations, help identify risk factors for canine brucellosis, and develop a diagnostic PCR test for canine brucellosis. An important outcome of this study will be to create prevention and control measures applicable to dogs.





## Tick-Borne Disease Research Program Area

### **02383: Identifying Cellular Mechanisms of Inflammation During Canine Tick-Borne Diseases**

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

Total Grant Amount: \$207,526; Grant Period: 9/1/2017 - 8/31/2019

Tick-borne diseases are found in all 50 states of the United States and are the most common vector-borne disease diagnosed in people in the US. The predominant disease is Lyme disease, caused by *Borrelia burgdorferi* and related species (*sensu lato*). Other important canine tick-borne diseases include those caused by *Anaplasma platys*, *Anaplasma phagocytophilum* (Anaplasmosis), *Babesia canis*, *Babesia conradae* and *Babesia gibsonii* (Babesiosis), and *Ehrlichia canis*, *Ehrlichia chaffiensis* and *Ehrlichia ewingii* (Ehrlichiosis). Many of these diseases also affect people. Dogs can serve as sentinel species for human disease and there are many areas where the immune responses and disease outcomes are very similar in people and dogs, meaning that important lessons can be learned by sharing information between human and animal health (One Health). The researchers will further investigate the dog's immune system to determine which immune cells are responsible for the cure or creation of canine tick-borne disease. Through understanding which cells are responsible for causing disease, the goal is to then specifically target the molecules they produce using immunotherapy or immune modulation to improve treatment of tick-borne diseases in all dogs.

### **02386-A: Surveillance of *Hepatozoon americanum* In Populations of the Gulf Coast Tick Vector**

Principal Investigator: Andrea Varela-Stokes, DVM, PhD; Mississippi State University

Total Grant Amount: \$12,960; Grant Period: 12/1/2017 - 11/30/2019

American Canine Hepatozoonosis is a debilitating tick-borne disease with poor prognosis and limited treatment options. Affected dogs usually experience fever, muscle pain, and body wasting. Some dogs may have a thickening of their long bones. While most tick-borne diseases occur after transmission of the disease agent during tick feeding, in American Canine Hepatozoonosis, dogs are infected by eating the tick vector carrying the disease agent. *Hepatozoon americanum* is the agent that causes American Canine Hepatozoonosis. It is a protozoan parasite carried by the tick species, *Amblyomma maculatum*, also known as the Gulf Coast tick. The percentage of Gulf Coast ticks carrying *H. americanum* is unknown. The investigators will use an optimized test to perform active surveillance on Gulf Coast ticks collected in Mississippi during the summer seasons of 2018 and 2019 when adult Gulf Coast tick stages are active. Veterinary summer research students will participate in the research each year. By involving veterinary students and obtaining active surveillance data on tick populations, the researchers will fill an important gap in our knowledge of American Canine Hepatozoonosis, and increase veterinary and public awareness of potential risk in canine patients.



## **02284-A: Lyme Disease in Dogs: Prevalence, Clinical Illness, and Prognosis**

Principal Investigator: Jason Stull, VMD, PhD; Ohio State University  
Total Grant Amount: \$14,148; Grant Period: 7/1/2016 - 6/30/2018

Lyme disease (or Borreliosis) is a bacterial disease of dogs and humans that is transmitted by tick bites. While most common in the northeastern coastal states and the upper Midwest, Lyme disease is moving into other regions of the U.S. and Canada. Dogs infected with Lyme disease rarely show signs of illness (typically lameness), but can be severe (e.g., kidney disease). Diagnosis, treatment and prevention of Lyme disease in dogs are complicated by limited research and conflicting professional guidance. Current practices may unnecessarily place dogs at risk for illness and negative outcomes. The investigators will follow a large group of dogs from different regions of the U.S. and Canada to determine how often healthy dogs test positive for Lyme disease (meaning they have been bitten by an infected tick) and identify how often they later develop a Lyme-related illness. The risks and benefits of management strategies for Lyme-positive dogs and obstacles to effective tick prevention will be determined to help clarify unmet pet owner education needs. Collectively, this research will allow us to identify, define and improve upon best practices for prevention and control of Lyme disease in areas with different Lyme risks, ultimately improving the health of dogs and people.

## **02287: Enhanced Testing for the Diagnosis of Bartonellosis in Dogs**

Principal Investigator: Edward Breitschwerdt, DVM; North Carolina State University  
Total Grant Amount: \$103,013; Grant Period: 8/1/2016 - 7/31/2018

Bartonellosis, a zoonotic bacterial disease of worldwide distribution, is caused by approximately 10 different Bartonella species. Bartonella are transmitted to canines and humans by ticks, fleas, lice, mites, and sand flies. Bartonella species have been associated with an expanding spectrum of important disease manifestations including anemia, endocarditis, hepatitis, lymphadenitis, myocarditis, thrombocytopenia and vascular tumor-like lesions. Infections can be life-threatening. Due to a lack of sensitive and reliable diagnostic assays, definitive diagnosis of bartonellosis in dogs remains a significant problem. Because these bacteria invade cells and infect tissues throughout the body, this chronic intracellular infection is difficult to cure with currently used antibiotic regimens. This study will develop improved serodiagnostic tests for bartonellosis in dogs. These assays can also be used for world-wide sero-epidemiological prevalence studies, and to establish early and accurate diagnosis. Dr. Breitschwerdt's research group has described concurrent infection in dogs, their owners and veterinary workers; this allows for a One Health approach to this important emerging infectious disease.

## **02292: Broad-Range Detection of Canine Tick-Borne Disease and Improved Diagnostics Using Next-Generation Sequencing**

Principal Investigator: Pedro Diniz, DVM, PhD; Western University of Health Sciences  
Total Grant Amount: \$60,717; Grant Period: 9/1/2016 - 2/28/2018

Diagnostic tests based on the detection of DNA of infectious organisms from clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several tick-



borne organisms are available through universities and private laboratories in the USA and abroad. However, the vast majority of results from clinically ill dogs are negative for tick-borne diseases, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis and improve treatment options. These panels are based on the detection of previously known DNA sequences of each pathogen, with little room for detecting new organisms. Using an innovative approach, the investigators will employ next-generation sequencing (NGS) to overcome the limitations of current diagnostic technology and generate millions of individual gene sequencing reads from each clinical sample, allowing for the identification and characterization of multiple organisms from a single sample. Testing samples from dogs naturally exposed to tick-borne diseases, NGS will detect not only new organisms but also characterize genetic differences among known organisms. The resulting dataset of a large number of DNA sequences of known tick-borne organisms and previously undetected organisms in naturally-infected dogs will support the development of diagnostic tools to simultaneously advance canine and human health.

### **01780: Defining the Mechanism by Which Ticks Locate Dogs in Order to Better Prevent Disease Transmission**

Principal Investigator: Emma Weeks, PhD; University of Florida

Total Grant Amount: \$104,867; Grant Period: 3/1/2013 - 2/28/2018

The brown dog tick (BDT) is common across the U.S. and is the most widely distributed tick in the world. BDT's carry and transmit the pathogens that cause debilitating diseases such as canine ehrlichiosis and babesiosis. Prevention of these diseases is accomplished through tick control. BDT's can complete their entire life cycle indoors, making management difficult. Records of infestations are increasing and unpublished data indicates that a high level of pesticide resistance is present in domestic populations. Consequently once introduced, these ticks are particularly hard to eradicate and as one female tick may lay 5,000 eggs, the problem soon gets out-of-hand. Pesticide resistance leads to aggressive treatment regimes, which in turn, lead to increased exposure of humans and pets to chemical residues. Alternatives to pesticides are needed. Studies have shown that BDT's are attracted to dog odor, a blend of volatile chemicals used by ticks to find a blood meal. In this study, Dr. Weeks will identify the chemicals BDT's use to locate a dog. This will enable manipulation of tick behavior thereby facilitating management and reducing the need for extensive use of pesticides. Improved tick control without the need for increased environmental pesticide applications will improve the quality of life for dogs and their owners.

### **02295-A: The Role of Lymphocytes in Canine Monocytic Ehrlichiosis**

Principal Investigator: Mary Anna Thrall, DVM, MS; Ross University School of Veterinary Medicine

Total Grant Amount: \$15,000; Grant Period: 7/1/2016 - 12/31/2017

Canine monocytic ehrlichiosis (CME) is a serious disease of dogs, caused by the intracellular bacteria *Ehrlichia canis* that is transmitted by a tick bite. There is no vaccine for CME, and the pathophysiology of why the disease is more serious in some dogs is not understood. CME is very common in St. Kitts, home to Ross University School of Veterinary Medicine. The large numbers of affected dogs are a



valuable resource for studies of this important disease. Lymphocytes (a type of white blood cell) appear to be related to the pathophysiology of CME. The investigators will study the types of lymphocytes present in dogs with both mild and severe disease and compare them to non-affected dogs. Lymphocytes will be identified by type as B or T cells using antibody markers for lymphocytes and flow cytometry. The investigators will determine if an increase in lymphocyte counts (lymphocytosis) is associated with severity of disease, and whether clonality (having a large number of the exact same type of lymphocyte) is associated with severity of disease. Fifty Ehrlichia-positive dogs admitted to Ross University will be evaluated for their number of lymphocytes by blood cell counts, by flow cytometry to determine their lymphocyte subsets, and by PCR and antibody testing for the presence of tick-borne disease. These dogs will be compared to healthy control dogs. The researchers will also evaluate 50 dogs presenting with persistent lymphocytosis and determine the percentage of those dogs that are Ehrlichia positive. The findings of this study will advance understanding of the pathophysiology and diagnosis of ehrlichiosis and lymphocytosis.

## **EDUCATIONAL GRANTS PROGRAMS**

### **2018 Clinician-Scientist Fellowship Research Program Area**

To sustain future advancements in canine health, the AKC Canine Health Foundation encourages and supports the next generation of canine health researchers, understanding the impact of present fiscal restraints on research and development. To help diminish this impact, the AKC Canine Health Foundation Clinician-Scientist Fellowship Program supports young scientists. Through this effort the Foundation's mission to prevent, treat and cure canine disease will endure for years to come.

*Four new grants will be announced in late October 2017; support can be directed to this research program area.*

### **Theriogenology Residency Training Research Program Area**

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. 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. Beginning in 2018, these training programs are also required to include training in clinical genetics and genetic counseling.

*Support can be directed to this research program area.*



**02281-E: 2016 Theriogenology Residency - North Carolina State University**

Principal Investigator: Scott Bailey, DVM, MS; North Carolina State University

Total Grant Amount: \$100,000; Grant Period: 7/1/2016 - 6/30/2019

**02282-E: 2016 Theriogenology Residency - Auburn University**

Principal Investigator: Robin Wilborn, DVM, MS; Auburn University

Total Grant Amount: \$100,000; Grant Period: 7/1/2016 - 6/30/2019

**02294-E: 2016 Theriogenology Residency - Ohio State University**

Principal Investigator: Marco Coutinho da Silva, DVM, PhD; Ohio State University

Total Grant Amount: \$100,000; Grant Period: 7/1/2016 - 6/30/2018

**02283-E: 2016 Theriogenology Residency - University of Pennsylvania**

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

Total Grant Amount: \$100,000; Grant Period: 7/1/2016 - 6/30/2018