ABCF MESSENGER

Official Newsletter of the American Boxer Charitable Foundation

May 2021

PRESIDENT'S REPORT

2020 proved to have been a challenging year for all of us with the covid-19 epidemic. Nevertheless, the ABCF is grateful and thankful, to all who supported the foundation with their generous donations through membership and or other financial support.

The foundation is engaged with numerous investigators that to date have submitted promising research progress reports to include,
---Disease-Defining Autoantibodies in Canine Addison's Disease---
----The Prevalence of Bartonella Infection in dogs with cardiac and Splenic Hemangiosarcomas within and between Geographic Locations
---The Prevention of Hemangiosarcoma: Lifetime Follow-up. This study is a 4 year project that was initiated August 1 2020.
---Trial of Procaspase-3 Activator (PAC-1) in Combination with Hydroxyurea for Treatment of Canine Meningioma.
---The Role of Bartonella spp. Exposure and Cardiac Genetic Variation on the Clinical expression of Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer dog.
And ----Characterization of Renal Disease in American Boxer Dogs

In collaboration with the AKC/CHF the ABCF maintains a rigorous scientific review process before, during, and after grants are awarded. This ensures that we invest in quality research with the most potential to advance the health of our Boxers. Although, the yearly fundraiser will be restricted this year, I encourage all to participate. Boxer health research depends on your continuous support.

Bill Truesdale

FROM THE TREASURER

The Treasurer's Corner

I'm beginning to think I've become a broken record in a year of unknowns. What a year and what an experience! Our Worldwide Pandemic has altered not only the way we go about our everyday living, but it has affected all the special events we look forward to each year. The upside is we are turning the corner. With many getting the vaccine and with hospitalizations on the decrease there appears to be a light at the end of the tunnel. Those things we always look forward to are on the horizon. We're holding our National Specialty. The Top 20 will take place and we will have an opportunity to again hold our Foundation's major donation events. With fingers crossed, things will only improve from here on out.

Throughout this year with all the "downs" there have been many "ups." It has been a joy to interact with the wonderful people that make up this Foundation through membership and memorial donations. It never ceases to amaze me how far and wide the love of our Breed reaches and how caring our members are.

Now to the question always asked of the Treasurer, "How are we financially doing this year as a Foundation?" Again, I'm happy to say we are not only meeting our financial obligations, but we are financially healthy. We are meeting our obligations. We have over $70,000.00 in our daily operating checking and savings accounts. We have a Certificate of Deposit for $60,000.00 and our Endowment investments now exceed $1,107,000.00. Even during this crazy year, due to the work of our Investment Committee and with Charles Schwab's advice, our investments have gained in excess of 10% up from last year.

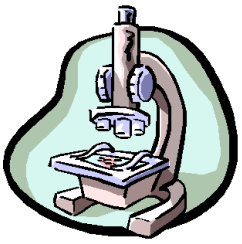
With that being said, we are also in the process of transferring a major stock donation that will greatly increase our Endowment Fund. While we are still in the transfer process things are on the move. Once we have procession of the stocks we can liquidate and reinvest the proceeds. This is a major contribution that mirrors the donation that allowed us to begin our investment portfolio. There isn't much else I can saw at present, but things are looking very good for our Foundation.

As we continue our work in finding cures for those illnesses that effect our beloved Boxers remember it is you, each of you, individually, that makes our work possible. So, keep that in mind as you speak to other Boxer lovers. Let them know of our Foundation's work and encourage them to join our effort. It is your membership fees and donations that are the base, the foundation, of who we are and what we can do.

Thank you to all of you for enabling me to have this wonderful experience as your Treasurer. Get vaccinated. Stay safe and healthy and always remember, our Boxers love you.

Gary Ryan

Research Updates- Your ABCF Dollars at work!



Grant 02437-MOU: Characterization of Renal Disease in American Boxer Dogs

Principal Investigator: Jessica Hokamp,DVM,PhD

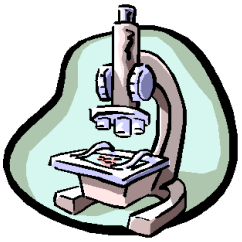
Original Project Description: Chronic kidney disease (CKD) is often a progressive and fatal disease in dogs. Boxer dogs appear to have a predisposition for development of CKD,suggesting that kidney disease in this breed might be heritable. Studies in Europe report an increased frequency of Boxers with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

Update: Boxer dogs have a predisposition for development of chronic kidney disease.There continues to be a concern that renal disease in Boxers might have a genetic basis; however, a genetic link between Boxers with kidney disease has not been identified. We have continued to identify and characterize the predominant type(s) of kidney disease in American Boxer dogs which will help us determine if there is a hereditary link between Boxers with specific types of kidney disease.

The primary goal of this study is to diagnose the underlying causes of kidney disease in purebred Boxers with kidney disease. We have completed approximately 2/3 of this objective through re-evaluation of kidney samples that were previously archived at two universities and through prospective evaluation of kidney tissues,urine, and blood from Boxers with suspected kidney

disease. As with all breeds of dogs, there are multiple renal diseases that can affect Boxers. However, 2 predominant forms of kidney disease occur most commonly, including a form that causes juvenile onset of kidney disease. This form of juvenile kidney disease is the most concerning for a possible heritable link, but as mentioned above, we still have not identified a hereditary link between Boxers with this disease.

We are continuing to collect kidney tissue, serum, and urine of purebred Boxers with evidence of kidney disease to further strengthen our data, and we strongly encourage those with purebred Boxers with renal disease to consider study enrollment. We also encourage owners to allow kidney biopsies to be obtained from their Boxers with recently diagnosed kidney disease so that we can try to detect the early stages of disease, however, we also accept autopsied kidney samples. Urine and blood samples can also be submitted from Boxers that cannot be biopsied and from dogs related to those affected by kidney disease, whose owners would be willing to consent to sample collection. These samples will help us to create an extensive pedigree of affected and unaffected Boxers. If one or more of the most common types of kidney disease in Boxers demonstrates a pattern of inheritance, then we will select the best candidate dogs for genetic sequencing. This last step will be done in conjunction with analysis of the pedigree and the guidance of the geneticist for our study. This type of informed approach will ensure that we are examining DNA from Boxers that are affected by a similar disease process.



Grant 02806: Strategic Prevention of Canine Hemangiosarcoma: Lifetime Follow-Up

Principal Investigator: Jaime Modiano, VMD,PhD

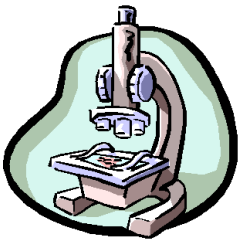
Original Project Description: The Shine On project is designed to utilize complementary technologies to reduce the impact of hemangiosarcoma in companion dogs. This novel, potentially disruptive approach is the first of its kind where artificial intelligence applied to the results of a blood test will be used to assign dogs to a risk category for the development of hemangiosarcoma. The test, called the Shine On Suspicion (SOS) Test is designed to detect hemangiosarcoma at its earliest stages of development before it becomes a clinically-detectable disease. Dogs that are considered to be at high risk based on the SOS Test results will be eligible to receive the drug eBAT for strategic prevention; that is, to eliminate emergent hemangiosarcoma tumors before they form. eBAT is a rationally designed drug developed in the laboratory to attack the cells that initiate and maintain the cancer, as well as to make the environment inhospitable for their growth. For the initial phase of the Shine On project, investigators developed and refined the SOS Test and the artificial intelligence methods to assign dogs to specific diagnostic categories and started to establish the utility of the test in early detection in a group of

209 presumably healthy, pedigreed Golden Retrievers, Boxers, and Portuguese Water Dogs, 6 years of age or older. In this continuation phase of the Shine On project, this group of dogs that had the 505 Test will be followed for their lifetimes to identify any diagnosis of cancer or another chronic disease, the cause of death, and date of death. In addition, a subset of dogs determined to be at high risk using the SOS Test will receive eBAT in the setting of prevention and also followed over their lifetime to establish their outcomes. This project expects to develop firm proof of concept to support larger clinical trials, and eventual deployment of this approach to the veterinary community setting for all dogs at risk of developing hemangiosarcoma.

Update: The goal for this project is to develop a reliable, accessible, and actionable test to identify dogs at risk for hemangiosarcoma during the earliest stages *of* disease and to use a strategic, rationally designed approach to prevent its occurrence in these high-risk dogs before it becomes clinically detrimental and life-threatening. The study has two objectives. The first is to determine the most reasonable duration of an SOS test result. In other words, how long can a low-risk SOS test result be trusted and how much time might elapse between a high-risk SO$ test result and the development of hemangiosarcoma. The second aim is to continue periodic testing for dogs previously enrolled in the Shine On study whose test result would have placed them in a high-risk category for development of hemangiosarcoma, and to provide eBAT as a strategy for prevention in 12 of these dogs.

To complete the first objective, we are conducting surveys to determine the health status of every dog enrolled in Shine On phase-3 (the early detection phase) at 6-month intervals. This effort will continue throughout the duration of the study.

To complete the second objective, we have finalized the analysis of the data from Shine On phase-1 (used as the "training set" for SOS test) and applied those results to dogs from phase-3 to select candidates for continued, periodic testing. We expect to send out notifications to owners of eligible dogs and begin active testing during the first quarter of 2021 if the COVID-19 pandemic abates and conditions across the country are adequate and safe for individuals to take their dog to the veterinarian for blood draws, as well as for travel to St. Paul, MN for eBAT prevention.

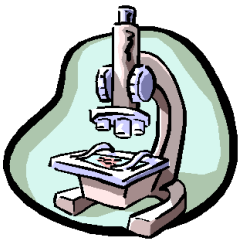


Grant 02550: The Role of Bartonella spp. Exposure and Cardiac Genetic Variation on the Clinical Expression of Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer Dog

Principal Investigator: Edward Breitschwerdt, DVM

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

Update: We are on track to accomplish all of our aims for this study, though have increased the study period to reach our enrollment goals and have continued to experience delays in test results and recruitment because of the SARS-CoV2 pandemic. We have thus far enrolled nearly half of our proposed study population. Of the currently enrolled dogs, nearly one third have died or been euthanized and we have collected tissues from over half of those. Based on the preliminary results from the Boxers tested so far, detection of Bartonella spp. using routine methods (blood tests) in Boxers with ARVC is unlikely. However, multiple dogs have had evidence of *Bartonella henselae* infection when alternative samples were collected, including cheek swabs and skin biopsies obtained post-mortem.



Grant 02519: Prevalence of *Bartonella* spp. Infection in Dogs with Cardiac and Splenic Hemangiosarcomas Within and Between Geographic Locations

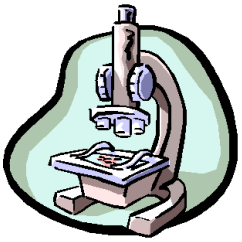
Principal Investigator: Edward Breitschwerdt, DVM

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

Update: We are on track to accomplish all of our aims for this study. We were able to obtain the initial set of samples on April 26, 2018 so we had a short delay in starting this study. We have now completed all Year I study aims, with the exception of immunohistochemistry and FISH localization of *Bartonella* organisms within various cell types. An unanticipated complication arose that the mouse monoclonal antibody was no longer being made commercially. *B. henselae* specific FISH probes have been designed and validation of FISH probes are in-progress. IHC is also in-progress. All qPCR and ddPCR have been completed at this time and samples are waiting for FISH and IHC analysis.

We have published a manuscript to the *Journal of Clinical Microbiology,* representing additional research from our AKC-CHF study #02287, which allowed us to define the Western Blotting (WB) criteria for serodiagnosis of bartonellosis in dogs. That work required additional time and research effort to validate WB testing. We are very excited with the qPCR and ddPCR results obtained from the fresh frozen hemangiosarcoma tissues provided by the NIH-CCOGC. The results strongly support a role for *Bartonella*

spp. in the etiopathogenesis of hemangiosarcoma in dogs. The regional study should provide additional insight as to the issue of potential causation. All three of the regions have identified, collected and shipped all necessary samples from their region. These samples have been tested by ddPCR, which has required months of validation. Validation of the ddPCR methods have been published in the *Journal of Microbiological Methods.* Because of the limitations on research activities at NCSU during the SARS-CoV2 pandemic, the testing and analysis of the study samples have been delayed. The NCSU College of Veterinary Medicine and the Intracellular Pathogens Research Laboratory have had suspended operations since March 2020. As of July 1, research operations are at limited capacity. Samples will continue to be processed, tested and analyzed as soon as possible given the constraints remaining in place to ensure staff safety.



Grant 02428: Identifying the Disease-Defining Autoantibodies in Canine Addison's Disease

Principal Investigator: Steven Friedenberg, DVM, PhD

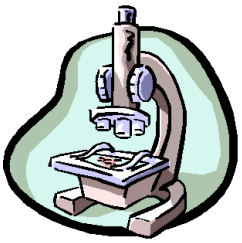
Original Project Description: Addison's disease is a common and life-threatening disorder in dogs in which the body's immune system destroys the outer layer of the adrenal glands. The adrenal glands produce hormones that are critical for energy metabolism, immune system function, intestinal health, and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

Update: The goal of this project is to identify autoantibodies that are present in the blood of dogs who are newly diagnosed with Addison's disease in three breeds: Standard Poodles, Portuguese Water Dogs, and English Cocker Spaniels. To accomplish these goals, we have been focusing on (1) collecting blood samples from dogs across all three target breeds, and (2) employing methods that allow us to detect these autoantibodies.

In terms of collecting blood samples, during the first 2.5 years of this project we have collected all the samples required from Standard Poodles and Portuguese Water Dogs, and most of the samples required for English Cocker Spaniels. We are continuing to actively recruit newly diagnosed dogs across all three breeds through many online channels.

Over the past 1.5 years, we have these samples to detect the presence of autoantibodies in newly diagnosed dogs using a technique called two-dimensional Western blotting. Our findings show that there are autoantibodies that are consistently present against adrenocortical proteins in dogs with a new diagnosis of Addison's disease. We have also shown that the specific proteins targeted by these autoantibodies may be different by breed or by dog.

Currently, we are focused on the next phase of our work which is to identify which specific proteins are targeted by these autoantibodies. We are genetically engineering three candidate proteins in our laboratory at present. Once we have made and isolated these proteins, we will test the serum from affected and unaffected dogs for reactivity against these proteins. We hope that these tests of reactivity will help us determine the predominant target of autoantibodies in canine Addison's disease for each breed. This will then set us on a path to developing a robust immunologic test to predict which dogs are at highest risk of developing the disease.



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

Principal Investigator: Timothy Fan, DVM, PhD

Original Project Description: 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.

Update: To date, we have been successful in enrolling and completing the treatment of 4 pet dogs with intracranial tumors, which marks a 66% estimated completion (we plan on recruiting a total of 6 dogs). However, upon histologic review of resected tumors, only 2 of the 4 patients have confirmed meningioma, while the other 2 patients have other types of tumors (gliosarcoma and histiocytic sarcoma). If we persist with the target of 6 dogs with meningioma, there is the requirement for the prospective identification and recruitment of another 4 patients. The principal investigators do not believe that this goal will be achievable given the historical patient recruitment across 3 sites.

From the 4 dogs completing the clinical trial, we can make several preliminary statements regarding the tolerability and activity of this novel combination of oral drugs for managing canine intracranial tumors. First, the combination of PAC-1 and hydroxyurea is safe and does not cause any unacceptable toxicity to typical body parts including the bone marrow and gastrointestinal tract. This is an important finding because whenever 2 new drugs are combined, it is necessary to ensure that drugs do not interact with one another to produce more severe side effects. Second, given that PAC-1 can penetrate the brain, we were uncertain if PAC-1 would cause worsening of neurologic status in dogs with preexisting brain diseases. Based upon our preliminary findings from this study, we are much more confident that PAC-1 is safe to use in dogs with brain cancer, and these findings are similar to what is observed in human beings with the most aggressive form of brain cancer (glioblastoma multiforme) who also are being treated with PAC-1 combined with another chemotherapeutic agent temozolomide, with a human Phase 1 component 2 trial has been initiated (NCT03332355).

However, we are cognizant of the observed therapeutic limitations of combining PAC-1 and hydroxyurea, as we have only been able to achieve stable disease in 3 of our patients, while the remaining patient demonstrated progressive disease. We were hopeful that the combination of PAC-1 and hydroxyurea would exert greater cytoreductive activity, but this does not appear to be the case based upon the responses we have documented so far in the existing 4 patients enrolled.

Despite the limited number of dogs meeting all inclusion criteria outlined by the study, we have still been able to gather important and valuable data from the support provided by the AKC Canine Health Foundation. This data has been combined with a larger overarching R01 initiative, and has matured to the submission of a manuscript to Neuro-Oncology entitled, "Evaluation of a procaspase-3 activator with hydroxyurea or temozolomide against high-grade meningioma in cell culture and canine cancer patients".

Thank you to all the current 2021 ABCF Members!!
And a reminder to complete your membership for 2021!

Ann Anderson
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Bob and Bobbi Compton
Frances Davidson
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Jacqueline Fabrizzio
Glenn Glass
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