RESEARCH PROGRESS REPORT SUMMARY

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

Principal Investigator: Edward Breitschwerdt, DVM and Matthew Breen, PhD

Research Institution: North Carolina State University

Grant Amount: $219,026

Start Date: 2/1/2018 **End Date:** 1/31/2022

Progress Report: Mid-Year 4

Report Due: 7/31/2021 **Report Received:** 9/22/2021

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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.

Publications:

Lashnits E, Neupane P, Bradley JM, Richardson T, Thomas R, Linder KE, Breen M, Maggi RG, Breitschwerdt EB. Molecular prevalence of *Bartonella, Babesia*, and hemotropic *Mycoplasma* species in dogs with hemangiosarcoma. PLoS One. 15(1): e0227234. doi:10.1371/journal.pone.0227234 [doi].

Maggi RG, Richardson T, Breitschwerdt EB, Miller JC. Development and validation of a droplet digital PCR assay for the detection and quantification of 2 *Bartonella* species within human clinical samples. J Microbiol Methods. J Microbiol Methods 2020;176:106022 https://pubmed.ncbi.nlm.nih.gov/32795640/

Neupane P, Sindhura S, Balakrishnan N, Marr H, Wilson J, Maggi R, Birkenheuer A, Lappin M, Chomel B, Brietschwerdt EB. Validation of *Bartonella henselae* Western immunoblotting for serodiagnosis of bartonellosis in dogs. J Clin Microbiol 2020;58:e01335-19. https://pubmed.ncbi.nlm.nih.gov/31941695/

Lashnits, E, Neupane P, Bradley JM, Richardson T, Maggi RG, Breitschwerdt EB. Comparison of serological and molecular assays for *Bartonella* species in dogs with hemangiosarcoma. Pathogens. 2021;10:794. doi: 10.3390/pathogens10070794.

Presentations:

Lashnits E, Abstract oral presentation: Molecular prevalence of *Bartonella, Babesia*, and hemotropic *Mycoplasma* species in dogs with hemangiosarcoma. American College of Veterinary Medicine Annual Forum, Phoenix, AZ, June 12-15, 2018.

Breitschwerdt EB. The genus *Bartonella* and vasoproliferative cancers in dogs and humans. Presented at the 12th Biennial AKC Canine Health Foundation National Parent Club Canine Health Conference in St. Louis, MO August 9-11, 2019.

Report to Grant Sponsor from Investigator:

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. Unfortunately the regional study did not provide additional insight as to the issue of potential causation, most likely due to the denaturing of DNA from formalin. All three of the regions were 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. Due to the lack of a commercially available monoclonal antibody, we identified a target and have commissioned a commercial laboratory to generate a monoclonal antibody ($6000) to be able to move forward with improved imaging. We also now have a Keyence immunofluorescent microscope in our laboratory and are in the process of hiring a pathologist from our newest Cohen Foundation grant (3 years of funding). We are also assisting University of Georgia pathologist (Morris Animal collaborative grant that was just funded) to generate a more sensitive DNA probe in insitu hybridization. These factors will allow us to move visualization of *Bartonella* ahead in our laboratory in the future.