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

Research Institution: North Carolina State University Office of Sponsored Programs

Grant Amount: $219,026.00

Start Date: 2/1/2018 **End Date:** 1/31/2023

Progress Report: FINAL

Report Due: 1/31/2023 **Report Received:** 4/26/2023

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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

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.

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.

Maggi R, Breitschwerdt EB, Qurollo B, Miller JC.Pathogens. Development of a Multiplex Droplet Digital PCR Assay for the Detection of Babesia, Bartonella, and Borrelia Species Pathogens 2021;10:1462. doi: 10.3390/pathogens10111462

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. To be presented at the 12th Biennial AKC Canine Health Foundation National Parent Club Canine Health Conference in St. Louis, MO August 9-11, 2019.

Per email to CHF 10/27/21: I just agreed to give a lecture to veterinary oncologists at next year's ACVIM Forum in Texas. Exact focus is yet to be determined, but will clearly include our work with Bartonella and HSA.

Report to Grant Sponsor from Investigator:

We gained substantial and seemingly important information relative to the aims of 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. Subsequently, the COVID epidemic and loss of research personal resulted in repeated interruptions in our research efforts. After completing all Year I study aims, we continued to work on

improvements in immunohistochemistry and FISH localization of Bartonella organisms within various cell types. Both of these study aims proved to be substantially more challenging than anticipated. One unanticipated complication arose when we found out that the mouse monoclonal antibody was no longer being made and sold commercially. Thus, we initiated work with a commercial entity to generate a new monoclonal antibody for future IHC evaluation of HSA cases and testing of other tissues where Bartonella infection is suspected or confirmed by DNA testing. B. henselae specific FISH probes were designed, after which optimization of the FISH probes resulted in our ability to visualize the bacteria in controlled cell culture infection studies. As we had earlier documented that detection of Bartonella spp. antibodies using current IFA and WB serology assays was very insensitive in dogs with hemangiosarcoma, we subsequently confirmed that molecular diagnostic testing targeting both tumor and non-tumor tissues was needed to assess the prevalence of Bartonella spp. infection. These findings are of relevance to individual dogs and epidemiological studies of hemangiosarcoma in dogs.

We have published several manuscripts as a result of this research funding. One manuscript in the Journal of Clinical Microbiology, representing focused research funding from our AKC-CHF Grant #02287, allowed us to define the Western Blotting (WB) criteria for serodiagnosis of bartonellosis in dogs. That work required a substantial amount of time and research effort to validate WB testing criteria. Although WB marginally improved diagnostic sensitivity, it, like IFA serology, resulted in numerous false negative results in dogs with hemangiosarcoma, dogs with PCR confirmed Bartonella infection. In contrast, we are very excited by the qPCR and ddPCR results obtained from the fresh frozen hemangiosarcoma tissues provided by the NIH-CCOGC. Those results strongly support a role for Bartonella spp. in the etiopathogenesis of hemangiosarcoma in dogs. The extent to which infection with Bartonella spp. is causative or only an association with hemangiosarcoma awaits the results of multiple future studies. The regional study PCR results were disappointing as formalin fixation negatively affected both qPCR and ddPCR amplification of Bartonella spp. DNA. All three of the regions identified, collected and shipped all necessary samples from their region. Those samples were tested by ddPCR, which required months of validation. Validation of the ddPCR methods were published in the Journal of Microbiological Methods and in the journal Pathogens. To assist clinicians and researchers with information related to diagnostic test selection, we published a manuscript in PLOS One describing the comparative sensitivity, specificity and diagnostic utility of IFA and WB serology, as compared to qPCR and ddPCR DNA amplification from blood and tissues of dogs with hemangiosarcoma.