RESEARCH PROGRESS REPORT SUMMARY

Grant 02819: Identification of *Bartonella henselae* In Vivo Induced Antigens for Development of a Reliable Serodiagnostic Assay for Canine Bartonelloses

Principal Investigator: Edward Breitschwerdt, DVM

Research Institution: North Carolina State University

Grant Amount: $52,317

Start Date: 1/1/2021 **End Date:** 12/31/2022

Progress Report: End-Year 1

Report Due: 12/31/2021 **Report Received:** 1/20/2022

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Original Project Description:

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

Publications: None at this time.

Presentations: None at this time.

Report to Grant Sponsor from Investigator:

During the past 4 years, with support from the American Kennel Club Canine Health Foundation, our research group has documented that current serological assays lack sensitivity, specificity, or both for assessing exposure to *Bartonella* spp. in dogs. Thus, when used diagnostically for an individual dog or epidemiologically for the detection of Bartonella spp. antibodies in dog populations, results would be inaccurate due to false-negative (poor sensitivity) or false positive (poor specificity) IFA testing. Importantly, the current "gold standard for *Bartonella* serodiagnosis in dogs and humans is the indirect immunofluorescent antibody assay or IFA test. Although IFA testing proved to be very specific, the assay was sensitive for detecting antibodies directed against *Bartonella* species in healthy or sick dogs. In fact, IFA failed to detect antibodies in nearly all dogs diagnosed with hemangiosarcoma that had documented *Bartonella* species infection, based upon tissue PCR positivity and DNA sequence confirmation. Our initial efforts to improve IFA sensitivity focused on increasing the number of test *Bartonella* species, where each serum sample was independently tested against eight different species/strains. That approach resulted in a minimal increase in IFA sensitivity and was not economically or technically practical for widespread testing by diagnostic laboratories around the world.

We next investigated a serological technique called Western immunoblotting (WB), which resulted in minor improvement in sensitivity over IFA, but the interpretation of WB patterns among naturally infected dogs varied to the extent that specificity was questionable. We next purified specific proteins that we identified during our WB study to assess our ability to document a serological response in dogs with known *Bartonella* infections. Five specific immunodominant proteins, as well as peptides from these proteins were purified and evaluated using individual enzyme linked immunabsorbent assays (ELISA). Compared to IFA or WB, two of these protein targets had markedly improved and diagnostically acceptable sensitivity and specificity indices to warrant additional evaluation.

Concurrently, we used a novel immunoscreening-based genetic approach referred to as in vivo induced antigen technology (IVIAT) to identify *Bartonella in vivo* induced antigens that were not identified using our prior research approaches. The IVIAT approach utilizes pooled immune sera from known infected dogs, adsorbed using the in vitro-grown pathogen (*Bartonella henselae*) to screen inducible recombinant genomic DNA libraries prepared from the cognate pathogen. This is a fancy way of stating that we used a more targeted "genetic" approach to determine which specific *Bartonella* proteins were recognized by known infected dogs. This approach yielded two additional diagnostic candidates (proteins), one of which is a protein that has not been identified in other bacteria. To achieve optimal sensitivity and specificity results, additional research is necessary to generate a specific ELISA assay targeting one or a combination of our candidate peptides. We will then screen large populations of healthy and sick dogs with the optimized ELISA assay. We are in communication with IDEXX Laboratories, a company we have worked with on SNAP assay development for decades, to determine if the company is willing to collaborate with us on the development of a rapid assay that can be used by veterinarians as a point of care test to determine if a dog has been exposed to a *Bartonella* species.