RESEARCH PROGRESS REPORT SUMMARY

Grant 02787-E: 2020 Clinician-Scientist Fellowship - North Carolina State University

Principal Investigator: Edward Breitschwerdt, DVM

Research Institution: North Carolina State University

Grant Amount: $12,000

Start Date: 1/1/2020 **End Date:** 6/30/2022

Progress Report: Mid-Year 2

Report Due: 6/30/2021 **Report Received:** 6/30/2021

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:

Mr. Neupane's research focuses on the development and validation of more sensitive and specific immunodiagnostics for canine and human bartonellosis, a disease caused by infection with intracellular bacteria from the genus *Bartonella.* Mr. Neupane's ultimate goal is to develop point of care diagnostics for this insidious infection. He will also contribute to canine hemangiosarcoma research that will better define the potential association between *Bartonella spp*. and hemangiosarcoma in dogs.

This fellowship is generously sponsored by the American German Shepherd Dog Charitable Foundation, Inc. and Briard Club of America Health and Education Trust.

Publications: None at this time.

Presentations: None at this time.

Report to Grant Sponsor from Investigator:

Due to poor sensitivity of currently available PCR, culture, and serological based assays for canine bartonelloses, a reliable serodiagnostic assay is of clinical importance. To evaluate diagnostic utility of *Bartonella henselae* immunodominant proteins for serodiagnosis of *Bartonella* infection in dogs, we have cloned, expressed, and purified five *Bartonella henselae* immunodominant recombinant proteins

(ATP-β, GroEL, LemA, SucB, and VirB5) and two chimera proteins (Bart1 and Bart2) using Escherichia coli expression system. We are currently evaluating sensitivity and specificity of these five *B. henselae* immunodominant recombinant proteins and two chimera proteins by ELISA using sera from Group I (n=30; *B. henselae* IFA-positive dogs) and Group II (n=30; *Bartonella* spp. IFA-negative and PCR-negative dogs). Since Pap31, a bacteriophage associated outer membrane protein of *Bartonella henselae*, is an important virulence factor that mediates host-pathogen interactions and promotes the establishment of *B. henselae* infection in the host, we hypothesize that Pap31/Pap31 fragments can be used as potential diagnostic makers for canine bartonelloses. Evaluation of sensitivity and specificity of purified recombinant Pap31 and Pap31 fragments (N-terminal region, middle region, and C-terminal region of Pap31) by ELISA using sera from *B. henselae* IFA-positive dogs (n=30) and Bartonella spp. IFA-negative and PCR-negative dogs (n=30) is in-progress.

We initially planned to test sera from *B. henselae* IFA-positive dogs (n=55) and Bartonella spp. IFA-negative and PCR negative dogs (n=55). Unfortunately, some *of B. henselae* recombinant proteins expressed poorly in *E. coli* expression system. Despite our continued efforts and strategies to optimize protein expression in *E. coli*, there was no change in the expression of poorly expressed proteins, which resulted in a poor yield of purified proteins. In addition, we were unable to obtain sera from specific pathogen free dogs for Group II as we have planned. Therefore, we are currently evaluating sensitivity and specificity of *B. henselae* immunodominant recombinant proteins and two chimera proteins by ELISA using sera from Group I (n=30; *B. henselae* IFA-positive dogs) and Group II (n=30; *Bartonella* spp. IFA-negative and PCR-negative dogs) than we previously stated for this study.

To identify and characterize *B. henselae* in vivo induced antigens for their potential as diagnostic markers for canine bartonelloses, we are currently employing an immunoscreening-based genetic approach referred to as in vivo induced antigen technology to screen inducible genomic libraries of *B. henselae* San Antonio (SA2) using pooled adsorbed sera from dogs naturally infected with *B. henselae*. We are also currently performing immunoproteomic analysis to identify novel B-cell epitope(s) of *Bartonella henselae* immunodominant protein antigens. Findings from these studies will aid in development of a reliable *Bartonella henselae* protein/peptide(s) based ELISA for diagnosis of canine bartonelloses, which should reduce the diagnostic costs and turn-around time for serological testing and can help clinicians make treatment decision in timely manner compared to tedious and time-consuming traditional IFA testing.