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: End-Year 2

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

(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: Neupane, P., Maggi, R. G., Basnet, M., Lashnits, E., Andrews, G. P., & Breitschwerdt, E. B. (2022). *Bartonella henselae* Recombinant Pap31 for the Diagnosis of Canine and Human Bartonelloses. Pathogens, 11(2), 182. https://doi.org/10.3390/pathogens11020182

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 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 evaluated sensitivity and specificity of these five *B. henselae* immunodominant recombinant proteins and two chimera proteins by ELISA using sera from Group I (n=36; *B. henselae* IFA-positive dogs) and Group II (n=34; *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. We evaluated 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=36) and *Bartonella* spp. IFA-negative and PCR-negative dogs (n=34).

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.