RESEARCH PROGRESS REPORT SUMMARY

Grant 02528: Developing a Next Generation Sequencing Diagnostic Platform for Tick-Borne Diseases

Principal Investigator: Pedro Diniz, DVM, PhD

Research Institution: Western University of Health Sciences

Grant Amount: $120,983

Start Date: 6/1/2018   **End Date:** 5/31/2021

Progress Report:

Report Due: 11/30/2020   **Report Received:** 11/17/2020

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

Original Project Description:

Diagnostic tests based on the detection of DNA from harmful organisms in clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several vector-borne organisms are available through universities and private labs in the USA and abroad. However, the vast majority of results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. These panels are based on the detection of previously known DNA sequences of each pathogen, which limits their ability to detect novel organisms. In this study, the investigators will adapt high-throughput next-generation sequencing (NGS) to the detection of tick-borne bacteria in dog blood in an effort to overcome the limitations of current diagnostics for tick-borne diseases. If successful, increasing the capabilities of NGS to detect infected dogs and to accurately determine which bacteria are responsible for disease will support the development of a better diagnostic tool to simultaneously advance canine and human health. This work expands on Dr. Diniz's previous AKC CHF-funded study #02292.

Publications:

Under review:
- o Comprehensive workflow for Next-Generation Sequencing applied to vector-borne disease diagnostics Elton J. R. Vasconcelos, Chayan Roy, Joseph A. Geiger, Kristina Oney, Brian B. Oakley, Pedro Paulo Vissotto de Paiva Diniz.
   BMC Veterinary Research

Under co-author review prior submission:

AKC Canine Health Foundation, Inc. • 8051 Arco Corporate Drive, Suite 300 • Raleigh, NC 27617 • 888-682-9696 • www.akcchf.org

Page 1 of 3

o Evaluation of a commercial microbial enrichment kit used prior DNA extraction to improve the molecular detection of vector-borne pathogens from naturally infected dogs. Kristina Oney, Melody Koo, Chayan Roy, Songyang Ren, Barbara Qurollo, Nicholas Juhaz, Elton J. R. Vasconcelos, Brian Oakley, Pedro P. V. P. Diniz
Journal of Microbiological Methods

In preparation:
o Detection of selected piroplasms of veterinary importance using 18S rRNA next-generation sequencing. Melody Koo, Kristina Oney, Chayan Roy, Songyang Ren, Brittany Thomas, Barbara Qurollo, Brian Oakley, Pedro Diniz.
Vector-Borne and Zoonotic Diseases Journal.

Presentations:

• Vasconcelos E.J.R., Oakley B.B., Diniz P.P. Utilizing Omics Approaches to Better Understand and Diagnose Vector-Borne Pathogens. Lecture for undergrad students in Biological Sciences at the Harvey Mudd College, Claremont, CA. Invited by Dr. Eliot Bush (Associate Professor at HMC), Oct 3rd, 2018.
• Persico E., Qurollo B., Thomas B., Hegarty B., Breitschwerdt E., Diniz P.P.V.P. Molecular prevalence of selected canine vector-borne pathogens in the United States (2008-2015).
Poster presentation at:
• International Conference on Emerging Infectious Diseases (ICEID) August 27th, 2018 Atlanta, GA (Omni Hotel at CNN Center)
• The 15th International Conference on Lyme Borreliosis and other tick-borne diseases (ICLB) September 13th, 2018 Atlanta, GA (Emory University/CDC campus)
Oral Presentation at the American Public Health Association Annual Meeting (APHA) November 12th, 2018 San Diego, CA (San Diego Convention Center)

Report to Grant Sponsor from Investigator:

Despite the wide availability of tick-borne disease panels at laboratories in the USA and abroad, most results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. Using an innovative approach, our study proposes the adaptation of high-throughput next-generation sequencing (NGS) to the detection of tick-borne bacteria in dog blood to overcome the limitations of the current diagnostics. Our previous results showed the potential of NGS in detecting known and potentially novel tick-borne bacteria. Recently, we have tested a commercially available kit designed to concentrate microbial DNA from blood to facilitate the detection of bacteria in circulation. However, even when 24 times more blood volume was used, no evidence of the concentration of blood microbiome or tick-borne organisms was detected using two distinct molecular

AKC Canine Health Foundation, Inc. • 8051 Arco Corporate Drive, Suite 300 • Raleigh, NC 27617 • 888-682-9696 • www.akcchf.org

Page 2 of 3

assays. As microbial concentration remains the next logical step in improving the detection of tick-borne organisms, we continue to investigate other potential approaches. In parallel, we have focused on finding new and better "DNA fingerprints" of these harmful bacteria, so we can precisely detect them from blood samples of naturally-infected dogs. Unfortunately, the COVID-19 pandemic forced us to stop all benchtop activities related to this effort. With new biosecurity measures implemented in our campus, we plan to resume our efforts, and tests in the lab the DNA fingerprint candidates that we previously identified using large-scale bioinformatic analyses. Collectively, our efforts may yield a novel diagnostic platform for the broad detection of tick- and blood-borne organisms in dogs. The AKC-CHF support has been instrumental in the development of such diagnostic tools in veterinary medicine.

AKC Canine Health Foundation, Inc. • 8051 Arco Corporate Drive, Suite 300 • Raleigh, NC 27617 • 888-682-9696 • www.akcchf.org

Page 3 of 3