



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: \$120,983.00
Start Date: 6/1/2018 **End Date:** 5/31/2020
Progress Report: Mid-Year 1
Report Due: 11/30/2018 **Report Received:** 11/30/2018

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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 CHF-funded study #02292.

Publications:

Vasconcelos E.J.R., Geiger J., Oakley B., Diniz P.P.V.P. Best practices for applying Next-Generation Sequencing to vector-borne disease diagnostics. *Veterinary Microbiology or BMC Veterinary Research*. Manuscript in preparation.

Persico E., Quorollo 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). *Journal of Veterinary Internal Medicine*. Manuscript in preparation.



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., Quorollo 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).

International Conference on Emerging Infectious Diseases (ICEID) August 27th, 2018 Atlanta, GA (Omni Hotel at CNN Center) [Poster]

The 15th International Conference on Lyme Borreliosis and other tick-borne diseases (ICLB) September 13th, 2018 Atlanta, GA (Emory University/CDC campus) [Poster]

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:

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 diseases (VBDs) 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. 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. NGS is capable of generating millions of individual sequencing reads from each sample, allowing for the unbiased identification and characterization of multiple organisms from a single sample. We are pioneering this strategy in the Veterinary Medicine VBD diagnostics field, and important results were already achieved from our previous AKC-CHF grant (#02292). Since we are dealing with a cutting-edge technology, our work is under continuous and systematic adjustments, aiming enhancements in the platform in order to accurately detect infected dogs and precisely determine which bacteria are responsible for disease. In this current first report of grant #02528, we describe our bioinformatics efforts on comparing the 'state-of-the-art' computational tools using dog blood samples as input, as well as provide a comprehensive standard operating procedure (SOP) for best practices of microbiome analyses applied to VBD diagnostics, as part of the specific aim 3 (SA#3). In parallel, as part of SA#2, we have also started a search for a new marker gene (other than 16S rRNA) through computational screening of whole genomes in order to achieve a better discriminatory power on the taxonomic classification of VBD-causing bacteria, thus



increasing the diagnostics capability of detecting species and strains. Regarding improvements on benchtop microbial DNA isolation techniques (SA#1), we have performed initial tests, which have demonstrated to reduce host DNA concentration in infected dog blood samples confirmed by quantitative PCR assay. Our results are in line with the proposed timeline. We truly believe that our ongoing AKC-CHF research will support the development of better diagnostic tools that will simultaneously advance both canine and human health.